

Clinical Pathology: White blood cell counts were decreased about 62-69% (compared to the sham control) in all males from both strains treated with vehicle and test articles, and about 30-35% in female vehicle control groups and high dose C57BL/6 females. However, the decrease in WBC was not consistently dose-dependent and WBC in mid-dose females was even slightly higher than the sham control female p53^{+/-} mice. All the values of WBC were within the reference range. The changes in other parameters including red blood cell counts, hemoglobin and hematocrit, MCH, MCHC and %RDW, reticulocytes, potassium, sodium, albumin, and globulin, etc. were slight in magnitude (<20%) and/or not consistently in a dose-dependent manner, or only observed in single gender/strain, hence, were not considered toxicologically significant.

Gross pathology: In positive control animals, the gross lesion was observed in urinary bladder and kidneys as expected.

For p53^{+/-} and C57BL/6 animals, incidence of the lesions at site of injection (SOI) is listed in the tables below (excerpted from text table 1 of Vol. 19, page 537 and text table 2 of Vol. 19, page 538). Non-tumorous lesions were equivalent between vehicle control and treated groups. The incidence in mass finding (tumorous lesion) was higher in mid- and high dose males than in vehicle control males. There was no difference in incidence rate of mass finding (tumorous lesion) between female vehicle control and female treated groups. Both incidences in non tumorous lesions and masses were higher in vehicle and test article-treated groups than the sham-control groups. The corresponding histopathologic findings were observed (see below in the section of Histopathology).

TEXT TABLE 1: GROSS LESIONS AT THE SOI (p53^{+/-} ANIMALS)

MALES						
Skin, SOI Lesion	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6
Non-tumorous lesions	1	24	NA	25	24	23
Masses	0	3	NA	5*	9**	8*
FEMALES						
Skin, SOI Lesion	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6
Non-tumorous lesions	1	25	NA	25	25	21
Masses	0	6*	NA	2	5*	1

Note: The one gross lesion noted at the SOI in Sham Control (Group 1) for both males and females are considered to be incidental findings. Table represents number of animals within group with finding. Number of animals examined in Groups 1, 2, 4, 5 and 6 = 25. Group 3 (positive control animals) were dosed orally and did not have SOI tissue for analysis.

* p<0.05 (Fisher's Exact Test) when compared to Group 1 (Sham Control).

** p<0.05 (Fisher's Exact Test) when compared to Group 1 (Sham Control) and to Group 2 (Vehicle Control).

Nominal Dose: Group 1 - Sham Control Group 2 - Vehicle Control Group 3 - 400 mg/kg Positive Control
 Group 4 - 3 mg/kg/day (b) (4) Group 5 - 10 mg/kg/day (b) (4)
 Group 6 - 30 mg/kg/day

TEXT TABLE 2: GROSS LESIONS AT THE SOI (C57BL/6 ANIMALS)

MALES			
Skin, SOI Lesion	Gp 1	Gp 2	Gp 6
Non-tumorous lesions	0	24	21
FEMALES			
Skin, SOI Lesion	Gp 1	Gp 2	Gp 6
Non-tumorous lesions	0	23	24

Nominal Dose: Group 1 - Sham Control Group 2 - Vehicle Control

Group 6 - 30 mg/kg/day

(b) (4)

Table represents number of animals within group with finding. Number of animals examined in Groups 1, 2 and 6 = 25.

For C57BL/6 mice, no mass were observed in all groups. Non-tumorous lesions were observed in vehicle and high dose groups with comparable incidence.

Organ Weights: There were no significant difference in absolute and relative organ weights between sham, vehicle and treated groups.

Histopathology:

Non-neoplastic:

In the positive control animals, an increased incidence of minimal to moderate nephrosis (23/25 males, 24/25 females) that included degenerative changes in the tubules, papillary necrosis and chronic inflammatory changes (secondary to degeneration) was observed in the kidneys. The above lesions noted in the positive control animals were not observed in the sham and vehicle control p53^{+/-} animals. The other less significant non-neoplastic findings included acute nasal cavity inflammation (marked, 2/5), liver diffuse/multifocal hypertrophy (marked/moderate, 4/6), liver necrosis (marked, 1/6), liver lipid infiltration (marked, 1/6), bile duct proliferation (marked, 1/6), acute liver inflammation (marked, 1/6), seminal vesicle dilation (marked, 1/6) and acute trachea inflammation (marked, 2/5) and trachea necrotizing and serosa (marked, 1/5).

In test article-treated p53^{+/-} mice, the noteworthy findings are listed in the tables below. The major findings at site of injection (SOI, including at the last site and not at the last site or generalized SOI) were epidermal hyperplasia, subcutaneous hemorrhages and subcutaneous chronic inflammatory lesions. Most chronic inflammatory lesions were marked in severity and epidermal hyperplasia findings were minimal to mild. Evidently, the incidence of these two findings in the treated group were significantly higher compared to the sham-control group but comparable to the vehicle groups. The incidence of hemorrhage was higher in treated groups than that in vehicle control group but the increase in the incidence lacked dose-dependency and less noticeable in females. The lipid infiltration and histiocytosis observed in multiple organs as listed were also significant compared to the sham control but were similar cross vehicle and test article treated groups. These findings were considered vehicle-related. There were no other significant non-neoplastic histopathologic findings.

Findings Dose (mg/kg/day)	Males					Females				
	Sham	Veh	3	10	30	Sham	Veh	3	10	30
Total animals with hyperplasia in SOI (last, not last or generalized sites)	0	25	25	25	25	0	25	25	25	25
Total animals with chronic inflammatory lesions at SOI (last, not last or generalized sites)	0	25	25	25	25	0	25	25	25	25
Total animals with hemorrhage at SOI (last, not last or generalized site)	0	7	13	5	14	0	0	1	3	2
Subcutaneous chronic inflammation at non-SOI skin	0	25	25	24	25	0	23	24	21	25
Adrenal, lipid infiltration	0	25	25	25	24	0	25	25	25	25
Bone Marrow, femur, histiocytosis	0	24	25	25	25	0	25	25	25	25
Bone Marrow, sternum histiocytosis	0	24	25	25	25	0	25	25	24	25
Brain, lipid infiltration	0	24	25	25	25	0	25	25	24	25
Epididymides, histiocytosis	0	23	25	25	25	NA	NA	NA	NA	NA
Liver, lipid infiltration	0	24	25	25	24	0	25	25	25	25
Lung, histiocytosis	0	0	0	2	2	0	2	1	0	3
Mesenteric LN, histiocytosis	0	24	25	25	25	0	25	25	25	25
Ovaries, lipid infiltration	NA	NA	NA	NA	NA	0	25	25	25	25
Parathyroid gland, lipid infiltration	0	22	23	25	22	0	19	23	22	23
Pituitary gland, lipid infiltration	0	24	25	25	24	0	25	25	24	24
Salivary gland, lipid infiltration	0	22	25	25	23	0	24	24	24	25
Submandibular LN, histiocytosis	0	24	25	25	25	0	25	24	25	25
Spleen, histiocytosis	0	24	25	25	24	0	25	25	25	25
Testes, histiocytosis	0	24	24	25	25	NA	NA	NA	NA	NA
Uteri, lipid infiltration	NA	NA	NA	NA	NA	0	25	25	25	25

The number in the table indicates the incidence of N=25 examined animals. Sham = sham control group, Veh. = vehicle control group

In C57BL/6 mice, the non-neoplastic skin lesions at SOI (epidermal hyperplasia and chronic inflammation) and the lipid infiltration and histiocytosis in multiple organs were similar to the findings observed in p53^{+/-} mice. These findings were also considered vehicle-related. There were no other significant non-neoplastic histopathologic findings.

Neoplastic:

In the positive control animals, increased incidences of transitional cell hyperplasia and squamous metaplasia in the females (3/25) as well as papilloma and carcinoma in both males (23/25) and females (21/25) were observed in the urinary bladder, which were not observed in the sham and vehicle control p53^{+/-} mice. Leukemia (granulocytic) in lungs was observed in one female.

In test article-treated p53^{+/-} mice, the neoplastic findings are listed below. The incidence of sarcoma observed at injection site skin in the treated and vehicle control groups was significantly higher than the sham control. Although the incidence in male mid-dose group was higher than that in male vehicle group, the increase in incidence was not dose-dependent, not observed in females groups. It was indicated that the subcutaneous/skin sarcomas were the most common spontaneous tumor in the p53^{+/-} mice (Youssef, Borellini, Jacobson-Kram and Fort, 2001). In rats and mice, sarcoma often occurs in response to materials that injected into the subcutaneous tissue, regardless of the chemical composition of the injected material. The process that results in the development of these sarcomas is related to the physical nature of the active component and the local biological response to the material and/or its method of implementation. The sarcomas develop as a result of neoplastic transformation in the fibrous connective tissue that proliferates around the embedded foreign material. This response called "solid state carcinogenesis" has been demonstrated in rodents. The sponsor stated that the process "solid state carcinogenesis" can occur with some normal innocuous vehicles when the repeated injury and the connective tissue response to subcutaneous injection are combined (p53^{+/-} 6-month Carcinogenicity Studies, Study Director Communication regarding proprietary studies). Youssef et al (2001) indicated that repeated needle-induced trauma, change in redox status and/or acidic pH of the vehicle interfered with cellular proliferation mechanisms which resulted in the site of injection sarcoma. Additionally, repeated subcutaneous injection of one vehicle component (povidone) has previously been shown to result in the development of sarcoma in rodent carcinogenicity studies (Nair, B., 1998). The povidone has been removed from the current clinical formulation. The inactive ingredients in the current clinical formulation which were included in the vehicle are commonly used in intranasal products. The sarcomas in this study appeared to be a vehicle-related, repeated trauma from subcutaneous injection and unlikely to be test article-related.

The leukemia in multiple organs was not considered significant in terms of its low incidence and comparable incidence rate in both vehicle control and treated groups. Granulocytic leukemia is the most common type of leukemia recorded in p53^{+/-} mice with an approximate incidence of <1% (Storer, 2001).

Findings	Males					Females				
	Sham	Veh	3	10	30	Sham	Veh	3	10	30
Dose (mg/kg/day)										
Skin, SOI (last site), sarcoma	0	2	3	6	0	0	1	0	0	0
Skin, SOI (not last*), sarcoma	0	1	1	4	6	0	5	1	4	0
Skin, SOI (generalized), sarcoma	0	1	0	0	0	0	0	0	1	1
Total animal with skin sarcoma at SOI	0	3	4	9	6	0	6	1	5	1
Skin, untreated, non-SOI, sarcoma	0	1	1	2*	0	0	2	0	0	0
Total animal with skin sarcoma	0	4	5	11	6	0	7	1	5	1
Bone marrow, Leukemia,	0	0	0	0	0	0	0	1	0	0

granulocytic										
Bone marrow, Leukemia, myelomonocytic	0	0	0	0	0	0	1	0	0	0
Liver, leukemia, granulocytic	0	0	0	0	1	0	0	1	0	0
Liver, leukemia, monocytic	0	0	0	0	0	0	1	0	0	0
Spleen, leukemia, granulocytic	0	0	0	0	0	0	0	1	0	0
Spleen, leukemia, monocytic	0	0	0	0	0	0	1	0	1	0
Spleen, leukemia, myelomonocytic	0	0	0	0	1	0	1	0	2	0
Total animals with leukemia	0	0	0	0	1	0	2	1	3	1

The number in the table indicates the incidence of N=25 examined animals. Sham = sham control group, Veh. = vehicle control group; *animal #7720 sarcoma noted in spinal cord and abdominal activity, animal#7721 sarcoma noted in spinal cord;

In C57BL/6 mice, there was no sarcoma observed at SOI and no leukemia in multiple organs was noted.

Toxicokinetics: The toxicokinetics were evaluated in C57BL/6 mice. The parameters are summarized in the table below (excerpted from Vol 20, page 963). All vehicle control samples were found to be below the limit of quantitation (< 500 ng/mL).

Table 4.1.-1: Mean (± S.D.) Plasma AUC (0 to 6 hours) (ng*h/mL) in Study N-04-189

Sampling Day	Dose (mg/kg/day)	Males	Females
91	3	39000 ± 2000	20500 ± 3700
	10	174000 ± 6000	98800 ± 9400
	30	382000 ± 37000	373000 ± 16000
181	3	34500 ± 5600	26300 ± 5400
	10	175000 ± 2000	128000 ± 9000
	30	350000 ± 45000	322000 ± 35000

Table 4.1.-2: Mean (± S.D.) Plasma Cmax (ng/mL) in Study N-04-189

Sampling Day	Dose (mg/kg/day)	Males	Females
91	3	10400 ± 1600	7140 ± 2560
	10	54700 ± 6300	25500 ± 7200
	30	95200 ± 26600	115000 ± 18000
181	3	11800 ± 1300	7500 ± 180
	10	41900 ± 300	32700 ± 4800
	30	90400 ± 27900	82100 ± 37900

Study title: 26-Week Repeated Subcutaneous Dose Carcinogenicity Study In p53^{+/−} Mice with A Toxicokinetic Study in C57BL/6 Mice with (b) (4)

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:
The study protocol was not evaluated by ECAC prior to the study initiation.

The dose selection was based on the 28-day repeated dose toxicity study of (b) (4) in C57BL/6 mice at dose levels of 5, 12.5, and 20.0 mg/kg/day. Mortality was observed in 2/12 mid-dose and 1/12 high dose females, and moribund sacrifice was reported in 1/12 mid dose TK male. Unresolved toxicity including post-dose comatose followed by lethargy and hyperactivity were observed in mid- and high dose animals during the last two weeks of dosing. The dose of 12.5 mg/kg/day was selected as the MTD for 26-week carcinogenicity study. In the 26-week carcinogenicity study, the test article-related mortality was observed at high dose and the high dose was changed to 8 mg/kg/day. Therefore, the high dose selected was considered adequate.

The positive control, p-cresidine in corn oil, at dose of 400 mg/kg, produced expected neoplastic and hyperplastic lesions in the urinary bladder and degenerative lesions in the kidneys.

The genotoxic potential of (b) (4) was evaluated in several in vitro and in vivo assays including Ames bacterial mutation assay, mouse lymphoma assay (MLA), Syrian hamster embryo assay (SHE) and in vivo mouse micronucleus assay. The positive results were observed in SHE and MLA assays.

Evaluation of tumor findings:

The incidence of sarcoma observed at injection site skin in the treated and vehicle control groups was significant higher than the sham control. The incidence in high dose females was higher than that in female vehicle-control group but was comparable to the incidence in male vehicle control. The sarcomas in this study appeared to be a vehicle-related, repeated trauma from subcutaneous injection and unlikely to be test article-related.

The test article, as a degradant of olopatadine which is intended to be intranasally administered to human, is not considered to be carcinogenic.

Study no.: AA69DN.7S8P.BTL (Alcon Document No. TDOC-0002455, version 4.0)

Volume #, and page #: Module 4, Vol. 13, Page 1 to Vol. 15, Page 1110 (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: December 06, 2004

GLP compliance: GLP compliant

QA report: yes (X) no ()

Drug, lot #, and % purity: (b) (4) Lot No. 11421:010, purity: (b) (4)
dried basis

CAC concurrence: Study protocol and dose selection were not submitted for ECAC evaluation prior to study initiation. The study report evaluation by ECAC will be added as an addendum to this review.

Methods

Doses: (b) (4) was administered at doses of 1, 5 and 8 mg/kg/day (the high dose was 12.5 mg/kg/day from day 1 and changed to 8 mg/kg/day from day 62)

and day 61 for main and TK males, from day 56 and day 54 from main and TK females) for 26 weeks.

See the study design in the table below (excerpted from Vol. 13, page 44).

Treatment Group Number	Treatment Group	Number of Animals					
		p53+/- (Main Study)		C57BL/6 (Main Study)		C57BL/6 (TK Study)	
		Male	Female	Male	Female	Male	Female
1	Sham Control	25	25	25	25	-	-
2	Vehicle Control	25	25	25	25	12	12
3	Positive Control, p-cresidine 400 mg/kg	25	25	-	-	-	-
4	Low Dose, 1 mg/kg/day	25	25	-	-	20	20
5	Mid Dose, 5 mg/kg/day	25	25	-	-	20	20
6	High Dose, 8.0 mg/kg/day ¹	25	25	25	25	20	20
Total mice		150	150	75	75	72	72

¹ At study initiation (Day 1 for all animals) the high dose administered daily was 12.5 mg/kg/day. On February 7, 2005 (Day 62 and Day 61 for the Main and TK study males, respectively and Day 56 and Day 54 for the Main and TK study females, respectively) the high dose was changed to 8.0 mg/kg/day.

Basis of dose selection (MTD, MFD, AUC etc.): MTD.

The dose selection was based on the 28-day repeated dose toxicity study of (b) (4) in C57BL/6 mice (Vol. 11, Page 1-Vol. 12, Page 575, Document No. TDOC-0001781). Male and female mice (n=12/sex/group) were administered subcutaneously with vehicle or (b) (4) in the vehicle at dose levels of 5, 12.5, and 20.0 mg/kg/day. Mortality was observed in 2/12 mid-dose and 1/12 high dose females, and moribund sacrifice was reported in 1/12 mid dose TK male. Unresolved toxicity including post-dose comatose followed by lethargy and hyperactivity were observed in mid- and high dose animals during the last two weeks of dosing. The hyperplastic lesions and inflammation at site of injection (SOI) were observed in vehicle and test article treated groups. The severity of the lesions at SOI was higher in mid-dose males, and high dose males and females, but lower in low and mid dose females. The ulcerative change at SOI was observed in the high dose females but not in any other vehicle or treated groups. The dose of 12.5 mg/kg/day was selected as the MTD for 26-week carcinogenicity study. In the 26-week carcinogenicity study, the high dose 12.5 mg/kg/day was changed to 8 mg/kg/day due to the test article-related early deaths observed in high dose groups. Therefore, the high dose selected was considered adequate for carcinogenicity evaluation.

Species/strain: mouse/ C57BL/6TacfBR-[KO]p53(p53^{+/-}) (heterozygous knockout mouse) and C57BL/6NTacfBR (conventional inbred mouse). Both p53^{+/-} and C57BL/6 mice were obtained from (b) (4)

Number/sex/group (main study): n=25/sex/group

Route, formulation, volume: Vehicle (b) (4) Povidone (b) (4) Diabasic sodium phosphate, (b) (4) NaCl, 0.01% Benzalkonium chloride, (b) (4) Disodium EDTA, pH (b) (4) and the test article in vehicle at 0.1, 0.5 and 1.25 or 0.8 mg/mL were administered subcutaneously at dose volume of 10 mL/kg. Sham control animals were not dosed with any materials. The positive control animals were administered by oral gavage at dose volume of 10 mL/kg with p-cresidine in corn oil at dose of 400 mg/kg.

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: n=12/sex for vehicle control group, n=20/sex/group for (b) (4) treated groups

Age: Both p53^{+/-} and C57BL/6 mice were 8-9 weeks at initiation of dosing. Body weight for p53^{+/-} mice ranged 21.2 g to 28.4 g in males and 15.9 g to 22.4 g in females. Body weight for C57BL/6 mice ranged 20.8 g to 26.0 g in males and 15.9 g to 22.0 g in females.

Animal housing: The animals were individually housed during the treatment of the study in polycarbonate cages containing (b) (4) Hardwood bedding. The animals were provided Harlan TEDLAD Global Diet #2018C in meal form and *ad libitum* access to drinking water.

Restriction paradigm for dietary restriction studies: No.

Drug stability/homogeneity: The analysis of the benzalkonium chloride (BAC) content of the vehicle and the analysis of the test article, (b) (4) were performed by HPLC. All dosing formulations met the acceptance criterion of ≤ 10% of the target concentration. The result showed (b) (4) in Olopatadine Hydrochloride Nasal Spray Vehicle was stable for 19 days when stored at 2-8 °C. Each dose formulation was (b) (4). The formulation analysis showed that the filter had no effect on the (b) (4) content of the dosing formulation. There were no samples taken from top, middle and bottom of the dosing formulation for homogeneity check in this study. However, the homogeneity of the same formulation at concentrations of 0.625 mg/mL and 10 mg/mL was checked in the 28-day repeated dose study and was found acceptable.

Dual controls employed: No

Interim sacrifices: No

Deviations from original study protocol: There were no significant deviations from the study protocol that had significant impact on the outcome of the study.

Observation times

Mortality: All animals were observed twice daily for moribundity and mortality.

Clinical signs: Main study animals were observed once weekly for post dose clinical signs of toxicity. Detailed hands-on examinations were performed for main study animals from Day 1 and weekly thereafter, in addition to the cageside observations.

Body weights: All animal body weights were measured once weekly from Day 1 through Week 13 and biweekly thereafter, with a pre-fasting body weight taken on Day 182 and a terminal fasting body weight taken at the day of scheduled sacrifice (for main study animal only).

Food consumption: Food consumption of main study animals was recorded weekly through the duration of the study with the exception of the final week when food consumption was recorded on Day 182. No food consumption was recorded for TK animals.

Clinical pathology: Samples were taken from all survival main animals (10/sex/group) at the termination.

Histopathology: All main study animals that died during study were necropsied as soon as possible after being found. A scheduled necropsy was performed on all survived main study animals. The specified organs as listed in the Histopathology Table below were weighed at the scheduled necropsy. The tissues/organs from all necropsied main study animals and 5/sex positive control animals, as listed in the Histopathology Table below, were preserved in 10% neutral-buffered formalin unless specified otherwise. The urinary bladder and kidneys were collected from all necropsied positive control animals. All collected tissues/organs from the necropsied animals were stained with hematoxylin and eosin and examined for histopathological effects.

Peer review: yes (), no (x)

Toxicokinetics: Blood samples from TK animals (n=3/sex/time point) were taken at the following timepoints: For vehicle group only, pre-dose on Day 1 and at pre-dose and 0.5 hour post dose on Days 91 and 181 (males) /180 (females);

For other treated groups: pre-dose, and at 0.5, and 3 hours post dose on Days 91 and 181 (males) /180 (females). Additional blood samples were taken from high dose animals at 0.5 hour post dose on Day 51 (females) and Day 58 (males) due to the change in dose level to the high dose group.

Results

Mortality: The incidence of the mortality in main study p53^{+/-} animals and C57BL/6 animals is listed in the table below.

Mortality	Males						Females					
	Sham	Veh	1	5	12.5/8	PC	Sham	Veh	1	5	12.5/8	PC
p53^{+/-} mice total	0/25	1/25	2/25	2/25	10/25	1/25	1/25	0/25	2/25	5/25	9/25	0/25
Days 1-62M /1-56F	0	0	0	0	4	0	0	0	0	1	4	0
Days 62-91M /56-91F	0	0	1	1	2	0	0	0	0	0	0	0
Days 91-185	0	1	1	1	4	1	1	0	2	4	5	0
C57BL/6 mice total	0/25	1/37	1/20	2/20	11/45	NA	0/25	1/37	0/20	1/20	5/45	NA
Days 1-62M /1-56F	0	0	0	1	6	NA	0	0	0	1	2	NA
Days 62-91M /56-91F	0	1	1	0	1	NA	0	0	0	0	0	NA
Days 91-185	0	0	0	1	4	NA	0	1	0	0	3	NA

Sham—Sham control group; Veh---Vehicle control group; PC---positive control group

In the p53^{+/-} mice, the incidence of early death (including found dead and moribund sacrifice) was markedly higher in the high dose males and females, and mid dose females than the vehicle control group and the sham control group (not reach statistical significance when compared with mid-dose females), indicating a test article-related toxic finding. For the C57BL/6 mice, the incidence of early death was also higher in the high dose group than the sham and vehicle control groups, though the incidence in high dose females did not reach statistical significance when compared to the both controls. More than 50% death in the high dose groups occurred after the dose level was lowered to 8 mg/kg/day. Therefore, the early death is considered a test article-related toxic finding. The cause of deaths is unclear. There were no related histopathologic findings reported.

Clinical signs: The incidence of significant clinical signs was listed in the table below.

Clinical signs	p53 ^{+/-} Males					p53 ^{+/-} Females				
	Dose (mg/kg/day)	Sham	Veh	1	5	12.5/8	Sham	Veh	1	5
Coma (reversed)	0	0	0	4	8	0	0	0	19	25
Lethargy (reversed)	0	0	25	23	18	0	0	24	13	11
Prostration	0	0	0	17	24	0	0	0	0	0
Hyperactivity	0	0	0	25	22	0	0	0	24	25
Hyperreactivity/excitability	0	0	0	25	19	0	0	0	21	22
Seizure	0	0	0	23	25	0	0	0	23	21
Thin appearance	0	0	1	3	5	2	3	2	2	6
Skin abnormality at SOI	0	3	15	25	21	0	4	15	23	21
Alopecia	2	25	24	20	21	7	25	25	24	21
Ulcer at SOI	0	0	1	2	8	0	0	2	3	10
Mass at SOI	0	6	6	1	9	0	4	2	5	12
Palpable Mass	0	5	2	3	2	0	0	2	1	3

Sham—Sham control group; Veh—Vehicle control group;

In the p53^{+/-} mice, the test article-related toxic signs observed in the mid and high dose included coma, lethargy, prostration, hyperactive, hyperreactivity/excitability, seizure and skin abnormality at injection sites. The findings observed in the high dose group including thin appearance and ulcer at SOI were considered test article-related toxic. The significant high incidence of lethargy was also observed in the low dose group. However, it was not accompanied with other observations, reversed after day 64 (males) and day 8 (females), and was monitorable, hence, it is not considered a dose-limiting toxicity. The skin abnormality would be evaluated along with the gross and microscopic evaluations (see section of histopathology evaluation). The high incidence of the mass at SOI in high dose group was considered an effect resulted from subcutaneous injection with vehicle and vehicle combined with test article (see section of histopathologic evaluation). Alopecia was observed in both vehicle and test article treated groups with significant

higher incidence when compared to the sham control and it was considered to be related to the vehicle treatment.

In the C57BL/6 mice, the clinical signs of toxicity similar to the findings of p53^{+/-} mice, as listed in the table below. The test article-related toxic findings included hyperactivity, hyperreactivity/excitability, thin appearance, skin abnormality, and ulcer at SOI. Alopecia was considered vehicle-related.

Clinical signs	C57BL/6 Males			C57BL/6 Females		
	Sham	Veh	12.5/8	Sham	Veh	12.5/8
Hyperactivity	0	0	21	1	2	22
Hyperreactivity/excitability	0	0	19	0	2	24
Thin appearance	0	1	8	0	0	1
Skin abnormality at SOI	0	7	22	0	1	23
Alopecia	0	24	22	1	25	23
Ulcer at SOI	0	0	4	0	0	6
Mass at ventral side of the body	0	0	0	0	0	1

Sham—Sham control group; Veh---Vehicle control group;

In positive control p53^{+/-} mice, lethargic was observed in 13/25 males and 9/25 females, indicating a clinical signs of toxicity. There were no other significant clinical observations reported.

Body weights:

The significant decrease in body weight (19- 22% in males and females) was observed in the positive control group. The body weights on Day 182 are listed below. The body weight was decreased up to about 14% in males and 18% in females of both strains treated with vehicle and test article, when compared to the sham control. The decreases in body weight in test article-treated groups were not dose-dependent and in small magnitude when compared to the vehicle control group in both strains, hence, were not considered a test article-related toxicity.

Body Weight Gain	Males						Females						
	Sham	Veh	1	5	12.5/8	PC	Sham	Veh	1	5	12.5/8	PC	
Dose (mg/kg/day)													
BW (g) in p53 mice	32.0	28.9	27.6	29.6	30.9	25.8	29.7	25.5	24.4	26.4	27.0	23.3	
BW (g) in C57BL/6 mice	33.4	29.5	NA	NA	29.5	NA	31.0	26.4	NA	NA	28.8	NA	

Sham—Sham control group; Veh---Vehicle control group; PC---positive control group

Food consumption: There was a significant decrease (17-19%) in total food consumptions of positive control mice. The changes (decrease or increase) in total food consumption of vehicle control groups and test article-treated groups were -5% to 15 % in males, -5% to 11 % in female from both of strains, when compared to the sham control

groups. Therefore, there was no significant test article-related effect on food consumption.

Clinical Pathology: White blood cell counts were decreased up to 69% (compared to the sham control) in males, and up to 57% in females, from both strains treated with vehicle and test article. However, the decrease in WBC was not dose-dependent and was comparable between vehicle control and test article treated groups. All the values of WBC were within the reference range. The changes in other parameters including red blood cell counts, hemoglobin and hematocrit, MCH, MCHC and %RDW, reticulocytes, potassium, sodium, BUN, albumin, and globulin, etc. were small in magnitude and/or not consistently in a dose-dependent manner, or only observed in single gender/strain, hence, were not considered toxicologically significant. The single finding of the increase in AST (66%) of mid-dose p53^{+/-} females was not considered biologically significant due to lack of dose-dependency, parallel finding in ALT and corresponding histopathologic observations.

In positive control groups, the significant increases in AST ALT, BUN, and Creatinine were observed in males and only significant increase in ALT was observed in females.

Gross pathology: In positive control animals, the gross lesion was observed in urinary bladder and kidneys as expected.

For p53^{+/-} and C57BL/6 animals, incidence of the lesions at site of injection (SOI) is listed in the tables below (excerpted from text table 1 of Vol. 14, page 647 and text table 2 of Vol. 14, page 648). Non-tumorous lesions (alopecia, discoloration, pigmentation, etc.) were equivalent between vehicle control and treated groups. The incidence in mass finding (tumorous lesion) was higher in high dose males and females comparing to vehicle control groups. Both incidences in non tumorous lesions and masses were higher in vehicle and test article-treated groups than the sham-control groups. The corresponding histopathologic findings were observed (see below in the section of Histopathology).

TEXT TABLE 1: GROSS LESIONS AT THE SOI (p53^{+/-} ANIMALS)

MALES						
Skin, SOI Lesion	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6
Non-tumorous lesions	2	25	0	23	17	22
Tumorous lesions	0	8	0	6	4	11
FEMALES						
Skin, SOI Lesion	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6
Non-tumorous lesions	0	24	0	24	23	23
Tumorous lesions	0	4	0	3	5	14

Note: Two gross lesions noted at the SOI in Sham Control males (Group 1) are considered to be incidental findings. Table represents number of animals within group with finding. Number of animals examined in Groups 1, 2, 4, 5 and 6 = 25. Number of animals examined in Group 3 = 20.

Nominal Dose: Group 1 – Sham Control Group 2 - Vehicle Control Group 3 – 400 mg/kg Positive Control Group 4 – 1 mg/kg/day (b) (4) Group 5 – 5 mg/kg/day (b) (4) Group 6 – At study initiation (Day 1 for all animals) the high dose administered daily was 12.5 mg/kg/day. On Day 62 (for the males) or Day 56 (for the females) the high dose was changed to 8.0 mg/kg/day.

TEXT TABLE 2: GROSS LESIONS AT THE SOI (C57BL/6 ANIMALS)

MALES			
Skin, SOI Lesion	Gp 1	Gp 2	Gp 6
Non-tumorous lesions	0	23	22
FEMALES			
Skin, SOI Lesion	Gp 1	Gp 2	Gp 6
Non-tumorous lesions	0	25	20

Nominal Dose: Group 1 - Sham Control; Group 2 - Vehicle Control; Group 6 - At study initiation (Day 1 for all animals) the high dose administered daily was 12.5 mg/kg/day. On Day 62 (for the males) or Day 56 (for the females) the high dose was changed to 8.0 mg/kg/day.
Table represents number of animals within group with finding. Number of animals examined in Groups 1, 2, 4, 5 and 6 = 25.

For C57BL/6 mice, no mass were observed in all groups. Non-tumorous lesions were observed in vehicle and high dose groups with comparable incidence.

Organ Weights: There were no significant difference in absolute and relative organ weights between sham, vehicle and treated groups.

Histopathology:

Non-neoplastic:

In the positive control animals, there were increased incidence of minimal to mild degeneration, (24/25 males, 25/25 females), papillary necrosis (24/25 males, 25/25 females) and lymphocytic infiltration (7/25 males, 13/25 females) observed in the kidneys. The above lesions noted in the positive control animals were not observed in the sham and vehicle control p53^{+/+} animals.

In test article-treated p53^{+/+} mice, the noteworthy findings are listed in the tables below. The major findings at site of injection (SOI, including at the last site and not at the last site or generalized SOI) were hyperplasia, subcutaneous chronic inflammatory lesions and hemorrhage. Most chronic inflammatory lesions were marked in severity and epidermal hyperplasia findings were minimal to mild. Evidently, the incidence of these two findings in the treated group were significantly higher compared to the sham-control group but comparable to the vehicle groups. The incidence of hemorrhage was higher in treated females than that in vehicle control group but the increase in the incidence lacked dose-dependency and the increase was not observed in males, hence, it is not considered a test article-related toxicological finding. The subcutaneous chronic inflammatory lesions were also observed in the mammary gland adjacent non-SOI skin which was thought to attribute to the spread of the inflammatory process from the primary sites of injection or subcutaneous exposure to injected material from the primary injection sites. The lipid infiltration and histiocytosis observed in multiple organs as listed were also significant compared to the sham control but were similar cross vehicle and test article

treated groups. The incidence of some of these lesions was lower in the high dose groups because more animals in those high dose groups died early before developing these lesions. These findings were considered vehicle-related. There were no other significant non-neoplastic histopathologic findings.

Findings	Males					Females				
	Sham	Veh	1	5	12.5/8	Sham	Veh	1	5	12.5/8
Total animals with hyperplasia in SOI (last, not last or generalized sites)	0	25	25	25	25	0	25	25	25	25
Total animals with chronic inflammatory lesions at SOI (last, not last or generalized sites)	0	25	25	25	25	0	25	25	25	25
Total incidences with hemorrhage at SOI (last, not last or generalized site)	2	13	7	6	9	0	3	11	12	6
Subcutaneous chronic inflammation at non-SOI skin	0	16	24	25	24	0	23	25	25	23
Adrenal, lipid infiltration	0	24	24	25	19	0	25	25	24	21
Bone Marrow, femur, histiocytosis	0	25	25	25	21	0	25	24	24	21
Bone Marrow, sternum histiocytosis	0	25	25	25	21	0	25	24	24	21
Liver, lipid infiltration	0	25	16	23	20	0	24	25	23	21
Mesenteric LN, histiocytosis	0	25	23	25	23	0	25	25	24	21
Submandibular LN, histiocytosis	0	25	24	25	22	0	25	25	24	21
Spleen, histiocytosis	0	24	24	25	20	0	22	25	23	20
Ovaries, lipid infiltration	NA	NA	NA	NA	NA	0	23	25	24	21
Uteri, lipid infiltration	NA	NA	NA	NA	NA	0	22	24	23	20
Epididymides, histiocytosis	0	0	0	0	1	NA	NA	NA	NA	NA

The number in the table indicates the incidence of N=25 examined animals. Sham = sham control group, Veh. = vehicle control group

In C57BL/6 mice, the non-neoplastic skin lesions at SOI (epidermal hyperplasia and chronic inflammation) and non-SOI, and the lipid infiltration and histiocytosis in multiple organs were similar to the findings observed in p53^{+/-} mice. These findings were also considered vehicle-related. There were no other significant non-neoplastic histopathologic findings.

Neoplastic:

In the positive control animals, there were increased incidences of marked transitional cell hyperplasia and squamous metaplasia (4/25 males, 7/25 females) as well as papilloma (12/25 males, 11/25 females) and carcinoma (9/25 males, 7/25 females) observed in the urinary bladder. The findings were not observed in the sham and vehicle control p53^{+/-} mice.

In p53^{+/-} mice, sarcoma at SOI was the only neoplastic lesion as listed in the table below. The incidence of sarcoma observed at injection site skin in the treated and vehicle control

groups was significant higher than the sham control. The incidence in high dose females was higher than that in female vehicle-control group but was comparable to the incidence in male vehicle control.

Findings	Males					Females				
	Sham	Veh	1	5	12.5/8	Sham	Veh	1	5	12.5/8
Dose (mg/kg/day)										
Skin, SOI (last site), sarcoma	0	2	0	0	6	0	1	1	0	0
Skin, SOI (not last*) , sarcoma	0	7	6	4	11	0	5	3	5	12
Skin, SOI (generalized), sarcoma	0	1	0	0	1	0	0	0	0	3
Total animal with skin sarcoma at SOI	0	10	6	4	11	0	6	3	5	12
Skin, untreated, non-SOI, sarcoma	0	0	0	0	0	0	0	0	0	0

The number in the table indicates the incidence of N=25 examined animals. Sham = sham control group, Veh. = vehicle control group;

It was indicated that the subcutaneous/skin sarcomas were the most common spontaneous tumor in the p53^{+/-} mice (Youssef, Borellini, Jacobson-Kram and Fort, 2001). In rats and mice, sarcoma often occurs in response to materials that injected into the subcutaneous tissue, regardless of the chemical composition of the injected material. The process that results in the development of these sarcomas is related to the physical nature of the active component and the local biological response to the material and/or its method of implementation. The sarcomas develop as a result of neoplastic transformation in the fibrous connective tissue that proliferates around the embedded foreign material. This response called "solid state carcinogenesis" has been demonstrated in rodents. The sponsor stated that the process "solid state carcinogenesis" can occur with some normal innocuous vehicles when the repeated injury and the connective tissue response to subcutaneous injection are combined (p53^{+/-} 6-month Carcinogenicity Studies, Study Director Communication regarding proprietary studies). Youssef et al (2001) indicated that repeated needle-induced trauma, change in redox status and/or acidic pH of the vehicle interfered with cellular proliferation mechanisms which resulted in the site of injection sarcoma. Additionally, repeated subcutaneous injection of one vehicle component (povidone) has previously been shown to result in the development of sarcoma in rodent carcinogenicity studies (Nair, B., 1998). The povidone has been removed from the current clinical formulation. The inactive ingredients in the current clinical formulation which were included in the vehicle are commonly used in intranasal products. The sarcomas in this study appeared to be a vehicle-related, repeated trauma from subcutaneous injection and unlikely to be test article-related.

In C57BL/6 mice, there was no sarcoma observed at SOI or non-SOI skin.

Toxicokinetics: The toxicokinetics were evaluated in C57BL/6 mice. The parameters are summarized in the table below (excerpted from Vol 15, page 1066). All vehicle control samples were found to be below the limit of quantitation (< 500 ng/mL).

Table 4.1.-1: Mean (\pm S.D.) Plasma AUC (0 to 3 hours) (ng*h/mL) in Study N-04-178

Sampling Day	Dose (mg/kg/day)	Males	Females
91	1	97.9 \pm 6.6	81.7 \pm 10.8
	5	587 \pm 87	393 \pm 32
	12.5/8	889 \pm 112	476 \pm 52
180/181 ^a	1	68.4 \pm 9.7	50.8 \pm 2.1
	5	362 \pm 64	288 \pm 16
	12.5/8	ND	342 \pm 55

ND: Not determined due to insufficient data resulting from male animal deaths during the study.

^a Female/Male sampling days.

Table 4.1.-2: Mean (\pm S.D.) Plasma Cmax (ng/mL) in Study N-04-178

Sampling Day	Dose (mg/kg/day)	Males	Females
51/58 ^{a,b}	12.5	423 \pm 73	404 \pm 81
91	1	50.7 \pm 7.3	38.5 \pm 9.4
	5	301 \pm 100	207 \pm 34
	12.5/8	449 \pm 57	267 \pm 58
180/181 ^a	1	37.9 \pm 11.1	19.7 \pm 1.9
	5	174 \pm 74	138 \pm 11
	12.5/8	265 \pm 54	169 \pm 63

^a Female/Male sampling days.

^b Both days were 3 days before dose reduction from 12.5 to 8 mg/kg/day.

Histopathology inventory (optional)

Study	26-week, (b) (4)	26-week (b) (4)
Species	p53 ^{+/+} mice	p53 ^{+/+} mice
Adrenals	X	X
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur and sternum)	X	X
Brain	X	X
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder	X	X
Gross lesions	X	X
Harderian gland	X	X
Heart	X	X
Ileum	X	X
Injection site	X	X
Jejunum	X	X

Kidneys	X	X
Lachrymal gland		
Larynx	X	X
Liver	X	X
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mesenteric and submaxillary	X	X
Mammary Gland with adjacent non-SOI skin	X	X
Nasal cavity	X	X
Optic nerves	X	X
Ovaries	X	X
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X	X
Prostate	X	X
Rectum	X	X
Salivary gland (mandibular)	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin (at the last site of injection) ^a	X	X
Spinal cord	X	X
Spleen	X	X
Sternum	X	X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid	X	X
Tongue		
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland	X	X

X, histopathology performed

*, organ weight obtained

a. when the last site of injection (SOI) bore a gross lesion, a representative SOI was taken from the next sequential injection site comparable in appearance to the vehicle control SOI.

SUMMARY

(b) (4) was tested with subcutaneous administration at dose levels of 3, 10 and 30 mg/kg/day in the p53^{+/+} mice carcinogenicity study. The sarcomas at injection site skin

appeared to be a vehicle-related, repeated trauma from subcutaneous injection. (b) (4), as a degradant of olopatadine which is intended for intranasal administration, is not considered carcinogenic to human. The proposed acceptance criteria for (b) (4) is acceptable.

(b) (4) was tested with subcutaneous administration at dose levels of 1, 5 and 8 mg/kg/day in the p53^{+/-} mice carcinogenicity study. The sarcomas at injection site skin appeared to be a vehicle-related, repeated trauma from subcutaneous injection. (b) (4), as a degradant of olopatadine which is intended for intranasal administration, is not considered carcinogenic to human. The proposed acceptance criteria for (b) (4) is acceptable.

The sponsor mentioned that the (b) (4) has not been observed in the current formulation up to date but did not change the acceptance criteria of (b) (4). No carcinogenicity study with (b) (4) is submitted. Based on the recommended olopatadine dosage (4.8 mg/day), the (b) (4) acceptance criteria should be NMT (b) (4) (equivalent to NMT daily intake 1.5 µg/day). Therefore, the acceptance criteria for (b) (4) should be lower to NMT (b) (4) although limit of NMT (b) (4) was required in the previous NA letter.

RECOMMENDATIONS

Pending the ECAC concurrence with the negative carcinogenicity study results, the proposed acceptance criteria, (b) (4) and (b) (4) are considered acceptable.

The sponsor mentioned that the (b) (4) has not been observed in the current formulation up to date but did not change the acceptance criteria of (b) (4). No carcinogenicity study with (b) (4) is submitted. The sponsor should be informed to lower the acceptance criteria to NMT (b) (4)% for (b) (4).

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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

/s/

Jean Wu
2/5/2008 12:16:28 PM
PHARMACOLOGIST

Joseph Sun
2/5/2008 12:43:52 PM
PHARMACOLOGIST
I concur.