Dose/Route/Duration: 0, 100, 300 and 1000 µg/5 ml/kg, iv, qd x 13 weeks (After approximately 1 month of study, the dosing route was changed to ip because of difficulty with dosing in the tail vein.)

Animals: CD-1®(ICR)BR mice, 8 weeks of age, weighing 28-32 g for ♂ and 23-26 g for ♀, 15/sex/group

Study Location: [redacted]

GLP/QAU: Yes

Study Date: 11/26/96-12/2/98

Study Design: Mice (15/sex/group) were given 0.1, 0.3 or 1.0 mg/kg AL06221 or vehicle control via iv (first month) and ip (second and third months) administration qd for 13 weeks. Toxicity was assessed as shown below.

- Mortality – Twice daily
- Clinical Signs – 5 times daily
- Body Weights – Weekly
- Food Consumption – Weekly
- Ophthalmoscopic Examination – pretest and during the last week of study
- Clinical Pathology – Blood and urine samples were collected from 10 animals/sex/group at study termination
- Necropsy (Including Organ Weights & Histopathology) – Gross necropsy examination was performed on all animals. All tissues listed in the following table from the animals of control and high dose groups, and animals that died during the treatment period were processed and microscopically examined. In addition, all gross lesions from all animals were microscopically examined. Organs denoted with * from all animals were weighed at the scheduled necropsy.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Mandibular Lymph Nodes	Spinal Cord
Bone (sternum and femur)	Rectum	Mammary Gland	Spleen
Bone Marrow	Testes*	Ovaries*	
Brain*	Heart*	Pancreas	Thymus
		Seminal vesicle	Tissue mass
Eyes with Optic Nerve and Adnexa	Kidneys*	Sciatic Nerve	Thyroid/Parathyroid*
Esophagus	Injection site	Pituitary	Trachea
Stomach	Liver*	Prostate	Urinary Bladder
Ileum	Epididymis	Skin	Uterus and cervix
Jejunum	Lungs	Salivary Glands	Gross Lesions
Duodenum	Gall bladder	Harderian gland	Vagina

Results:

- Mortality – Five male mice were found dead and one female mouse was sacrificed in extremis between day 31 and day 73 of the study (see table below). No dose-relationship to these mortalities was noted. The cause of the deaths for the males was unknown while the female mouse had a large abscess in the thymus. Mortality did not occur in the control animals.

Incidence of mortality in mice treated with AL-6221

Dosage (mg/kg)	Vehicle	0.1	0.3	1.0
Males	0	3 (Days 31, 33 and 73)	0	2 (Days 33 and 72)
Females	0	0	0	1 (Day 34)

- Clinical Signs - During the study period, no remarkable clinical signs attributable to the treatment were noted. Decreased activity and decreased defecation were occasionally noted in a few mice (1♂ and 1♀ at 0.1 mg/kg, 2♀ at 1.0 mg/kg) for a few days, which were not considered to be biologically relevant events.

- Body Weights – No treatment-related differences in body weights were noted.
- Food Consumption – No toxicologically significant, treatment-related changes in food consumption were noted.
- Ophthalmoscopic Examination – No toxicologically significant findings were noted.
- Clinical Pathology – No drug-related changes in clinical chemistry, hematology and urinalysis were observed.
- Necropsy - No treatment-related gross changes were identified.
- Organ Weights – No biologically relevant, toxicologically significant changes in organ weights were noted.
- Histopathology – There were no AL-6221-induced microscopic findings in the mice that either died during the study or were euthanized at the end of the treatment. Several findings, including granulomatous inflammation in mesentery, subacute and chronic inflammation in the liver, ileum and seminal vesicle, were found in control and treated mice and were considered to be secondary to manipulation during the ip injection. Severe abscess and bacterial colonies were noted in the thymus gland of the female mouse that was moribund on day 34. No cause for the deaths in male mice was noted.

In summary, CD-1®(ICR)BR mice were treated with AL-6221 at 0.1, 0.3 and 1.0 mg/kg for 3 months (iv qd for the 1st month and ip qd for the last 2 months). Six mortalities (five males and one female) occurred on the study, with no apparent relationship to dose. The cause of the deaths for the males was not established while the female mouse had a large abscess in the thymus. Mortality did not occur in the control animals. No treatment related changes in pharmacotoxicologic signs, body weights, food consumption, ophthalmic parameters, hematology, serum chemistry or urinalysis examinations, macroscopic or organ weight data were observed. Histopathologic evaluations demonstrated no significant effects, including bone morphology.

3.2.3 CHRONIC TOXICITY

3.2.3.1 Six Month Subcutaneous Toxicity Study in Rats with AL-6221, TR No. 052:30:0300, MPI 298-028 (Vol. 2.33-2.34)

Study №: 298-028
Report №: 052:30:0300
Study Aim: To determine the potential toxicity of AL06221 when administered subcutaneously to rats for 6 months
Compound: AL06221 (Lot#: AL06221-009, purity = 96.69%)
Vehicle Control: [redacted]
Dose/Route/Duration: 0, 10, 30 and 100 µg/ml/kg, subcutaneous injection, qd x 6 months
Animals: Crl:CD®BR VAF/Plus rats, 6 weeks of age, weighing 117-160 g for ♂ and 107-144 g for ♀, 25/sex/group
Study Location: [redacted]
GLP/QAU: Yes
Study Date: 9/19/97-2/23/00
Study Design: Rats (25/sex/group) were given 10, 30 and 100 µg/ml/kg AL06221 or vehicle control via subcutaneous administration qd for 6 months. Toxicity was assessed as shown below.

- Mortality – Twice daily
- Clinical Signs – Twice daily. A detailed clinical examination was performed on each animal weekly.

- Body Weights – Weekly
- Food Consumption – Weekly
- Ophthalmoscopic Examination – pretest and during the 3rd and 6th months of study
- Bone Density – Using Lunar® DPX α Dual Energy X0Ray Densitometery System, 5 animals/sex/dose, control and high dose groups: Weeks 12 and 23; low and mid dose groups: Week 24.
- Clinical Pathology – Blood and urine samples were collected from 10 animals/sex/group at 3 and 6 months of the study.
- Necropsy (Including Organ Weights & Histopathology) – Gross necropsy examination was performed on all animals. All tissues listed in the following table from the animals of control and high dose groups, and animals that died during the treatment period, and gross lesions from all animals were processed and microscopically examined. In addition, the bone with bone marrow (femur and sternum), liver and spleen from all animals were microscopically examined. Organs denoted with * from all animals were weighed at the scheduled necropsy.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Bone (sternum and femur)	Colon	Mandibular Lymph Nodes	Spinal Cord
Bone Marrow	Rectum	Mammary Gland	Spleen
Brain*	Testes*	Ovaries*	Tongue
Eyes with Optic Nerve and Adnexa	Heart*	Pancreas	Thymus
Esophagus	Kidneys*	Sciatic Nerve	Thyroid/Parathyroid*
Stomach	Injection site	Pituitary	Trachea
Ileum	Liver*	Prostate	Urinary Bladder
Jejunum	Epididymis	Skin	Uterus and cervix
Duodenum	Lungs	Salivary Glands	Gross Lesions
Tissue masses	Harderian gland	Seminal vesicle	Vagina

Results:

- Mortality – Five male rats died at 100 µg/kg/day and one female died at 10 µg/kg/day (see table below). The deaths occurred on days 93 and 183 were following the scheduled blood collection. The cause of death in these animals was not established, however, the deaths in the 100 µg/kg group could be treatment-related except for the single animal that died following blood collection.

Incidence of mortality in rats treated with AL-6221

Dosage (µg/kg)	Vehicle	10	30	100
Males	0	0	0	5 (Days 62, 119, 135, 178 and 183)
Females	0	1 (Day 93)	0	0

- Clinical Signs - During the study period, no remarkable clinical signs attributable to the treatment were noted.
- Body Weights – A decrease in body weight gain was noted in high dose male animals (see table below). No treatment-related differences in females were noted.

Body weight changes in male animals treated with travoprost (g)

Dosage (µg/kg)	Vehicle	10	30	100
Pre-dose	136±10.8	136±10.8	136±10.8	136±10.5
Week 26	666±83.3	671±78.7	636±59.3	612±62.4
% of control	100	100	95.5	91.9
Body weight gain	530	535	500	476
% of control	100	100	94.3	89.8

- Food Consumption – Average food consumption was comparable between control and treated animals.
- Ophthalmoscopic Examination – No toxicologically significant findings were noted.

- Bone Density – Bone density examination did not show any consistent, meaning differences between control and treated animals.
- Hematology – Slight decreases in RBC counts, platelet counts, HB and hematocrit levels were noted in female animals at 30 and 100 µg/kg/day (see table below). In male rats treated at 100 µg/kg, a decrease in platelet count (↓16-19%) was noted in both 3 month and 6 month examinations. These changes might be related to the bone marrow cavities changes observed in the histopathological examination (endosteal thickening and reduction in bone marrow cavities).

Hematology changes in female rats treated with AL-6221

	Month 3				Month 6			
Dosage (µg/kg)	Vehicle	10	30	100	Vehicle	10	30	100
RBC (10^6/µl)	8.00±0.46	7.76±0.29	7.59±0.39	7.49±0.27	7.80±0.44	7.30±0.43	7.18±0.86	6.96±0.60
Hb (g/dl)	15.0±0.65	15.2±0.48	14.7±0.47	14.8±0.22	14.9±0.59	14.4±0.81	14.1±1.55	14.0±1.06
Hematocrit (%)	45.7±2.23	45.6±1.09	44.1±1.89	44.2±0.91	44.9±2.16	42.8±2.27	42.0±5.01	41.5±3.50
Platelet (10^3/µl)	1093±141.1	1118±143.8	971±109.6	919±115.0	978±3.9	1015±111.5	928±164.3	790±143.2

- Clinical Chenistry and Urinalysis – No treatment-related, toxicologically significant findings were noted. A slight decrease in urine pH (see table below) was not considered biologically relevant since no histological findings in the kidneys were noted.

Urine pH changes in rats treated with AL-6221

	Males				Females			
Dosage (µg/kg)	Vehicle	10	30	100	Vehicle	10	30	100
Month 3	7.0±0.47	7.0±0.41	7.0±0.41	6.4±0.52	6.6±0.55	6.4±0.39	6.8±0.42	6.3±0.26
Month 6	6.8±0.26	6.7±0.48	6.5±0.41	6.5±0.50	6.8±0.42	6.6±0.80	6.4±0.52	6.3±0.42

- Necropsy - No treatment-related gross changes were identified.
- Organ weights – No biologically relevant, toxicologically significant changes in organ weights were noted. In females treated at 30 and 100 µg/kg, an increase adrenal weights (94-99 mg vs. control's 87 mg) was noted. Since there were no corresponding histopathological findings, these changes were not considered to be biologically relevant.
- Histopathology – Treatment-related microscopic changes are summarized in the table below. The bones were considered as the primary target organs evidenced by hyperostosis and a few cases of endosteal fibrosis. Endosteal fibrosis was characterized by laying down a thin layer of fibrous tissue to the endosteal surface. Hyperostosis was characterized by thickening of cancellus and compact bone by formation of new bone on the endosteal surface. These changes resulted in partial obliteration of the marrow cavity bringing about a reduction in bone marrow. Hematopoiesis seen in the spleen and liver was considered to be secondary to the narrowing of the bone marrow cavities. The bone marrow was histologically normal.

Microscopic examination in rats treated with AL-6221

Dosage (µg/kg)		Vehicle		10		30		100	
		♂	♀	♂	♀	♂	♀	♂	♀
N		25	25	25	25	25	25	25	25
Bone, femur: Fibrosis, endosteal	Total					1		4	
	Trace					1			
Bone, femur: Hyperostosis	Total					23	20	25	21
	Trace					17	18		
	Mild					6	2	25	21
Bone, sternum: Fibrosis, endosteal	Total							4	
	Mild							4	
Bone, sternum: Hyperostosis	Total					25	22	24	25
	Trace					21	21		
	Mild					4	1	24	25

Dosage (µg/kg)		Vehicle		10		30		100	
		♂	♀	♂	♀	♂	♀	♂	♀
N		25	25	25	25	25	25	25	25
Liver: hematopoiesis, extramedullary	Trace	4	1	2	3	5	13	15	20
Spleen: hematopoiesis, extramedullary, increased	Trace	1		4	1				
	Mild	6	12	8	13	15	18	20	22

In summary, Crl:CD®BR VAF/Plus rats were subcutaneously treated (qd) with AL-6221 at 10, 30 and 100 µg/kg for 6 months. Five males of the high dose group and one female of the low dose group died during the study. Mortalities were observed between Days 62 and 183, and, with two exceptions that were associated with blood collection procedures, were considered to be drug-related. Body weight gain for high dose males was lower (-10%) than controls. A slight reduction in RBC parameters was observed in the mid dose and high dose female groups. In both males and females at 100 mg/kg, platelet counts were decreased. Histologically, trace-to-mild hyperostosis and endosteal fibrosis were observed in femur and sternum at the mid and high dose levels for both males and females, but this was not observed at the low dose for either sex. The hematologic changes might be associated with the effect of histologic changes in bone on hematopoietic capacity in the affected bone marrow. The NOAEL was considered to be 10 µg/kg/day for both males and females, based on microscopic findings observed in bone.

3.3 CARCINOGENICITY

No study data were submitted.

3.4 REPRODUCTIVE TOXICITY

3.4.1.3 Study of Fertility and Early Embryonic Development to Implantation (Segment I) in Rats With AL-6221, TR No. 082:30:0400, [redacted] Study 298-035 (Vol. 35-36)

Study №: 298-035
Report №: 082:30:0400
Study Aim: To determine the toxic effects of AL06221 on female estrous cycle, tubal transport, implantation and development of peri-implantation stages of the embryo and detection of functional effects of male fertility
Compound: AL06221 (Lot#: AL06221-009, purity = 96.69%)
Vehicle Control: [redacted]
Dose/Route/Duration: 0, 1, 3 and 10 µg/2 ml/kg, subcutaneous injection, qd, ♂: 28 days prior to mating and continued until euthanasia (approximately 71 days), ♀: 14 days prior to mating and continued through Day 7 of gestation
Animals: Sprague Dawley rats, ♂: 7-week old, ♀: 10-week old, 26/sex/group
Study Location: [redacted]
GLP/QAU: Yes
Study Date: 1/10/98-4/2/00
Study Design: Rats (26/sex/group) were given 1, 3 and 10 µg/2 ml/kg AL06221 or vehicle control via daily subcutaneous administration. Administration of travoprost to males began 4 weeks prior to cohabitation and to females two weeks prior to cohabitation. Dosing was continued through gestation Day 7 for females and through completion of evaluations (approximately 71 days) for males. Cohabitation was on a 1:1 ratio and continued until evidence of mating (copulatory plug or sperm in the estrus smear) was observed. The day on which evidence of copulation was observed was designated as Day 0 of gestation. On Day 13 of gestation each female was

euthanatized and subject to laparohysterectomy. Toxicity was assessed as shown below.

- Clinical Observations – Twice daily
- Body Weights – Weekly (♀ also on Days 0, 4, 7, 10 and 13 of gestation)
- Food Consumption – Weekly
- Estrous Cycle Determination – Daily (from 10 days prior to the treatment initiation to the day on which the evidence of copulation was noted.)
- Uterine and Ovarian Examinations – On Day 13 of gestation, all females were euthanized and viable and nonviable embryos, early resorptions, the number of total implantations and corpora lutea were recorded.
- Postmortem Evaluations – A necropsy examination was performed on all treated male and female animals. For the males euthanized at the study termination, the testes and epididymides were weighted. The concentration, motility and morphology of the sperm were measured.

Results:

- Clinical Signs – During the study period, no remarkable clinical signs attributable to the treatment were noted.
- Body Weight – No treatment-related body weight changes were noted in male and female animals.
- Food Consumption – No drug-related changes in food consumption were noted.
- Cohabitation Data – Mating results are summarized in the table below. No toxicologically significant, treatment-related changes were noted.

Copulation index and fertility index in male and female rats

Dose	Number of females			Copulation index*	Fertility index*
	Females				
(µg/kg/day)	Paired	Inseminated	Pregnant	%	%
0	26	25	24	96.2	96
1	26	26	26	100	100
3	26	26	25	100	96.2
10	26	26	26	100	100
Historical					94.0
	Males				
(µg/kg/day)	Paired	With ♀ inseminated	With ♀ pregnant	%	%
0	26	23	23	92	100
1	26	21	21	80.8	100
3	26	21	20	80.8	95.2
10	26	25	25	96.2	100

* Copulation index = (# inseminated/# paired x 100); Fertility index = (# pregnant/# inseminated x 100)

- Estrous Cycle Determination – The mean estrous cycle length was slightly higher in the treated groups (4.24-4.31 days vs. control's 4.09 days). This change was not considered biologically significant.
- Uterine and Ovarian Examinations – The results are summarized in the table below. In all treated groups, the number of corpora lutea was slightly lower than the controls but was within the historical control range (15.5-19.2/dam). Therefore, it was not considered toxicologically significant. At 10 µg/kg, the number of early resorptions (1.84/dam) was higher than that of the controls (0.91/dam), indicative of embryolethality effects at this dosage.

Uterine and ovarian examination

(µg/kg/ day)	pregnant	Corpora lutea (#/dam)	Implantations (#/dam)	Pre-implantation loss (%/dam)	Early resorptions (#/dam)	Late resorptions (#/dam)	Live fetuses (#/dam)	Dead embryos (#/dam)
0	24	19.9±2.6	17.3±1.3	11.9±10.5	0.91±0.79	0	16.4±1.2	0
1	26	17.9±2.4	16.8±2.1	5.7±4.7	0.95±1.16	0	15.9±.0	0
3	25	18.1±1.9	17.3±1.6	4.5±4.8	1.20±1.20	0	16.1±1.8	0
10	26	17.5±2.7	16.6±2.7	6.7±10.6	1.84±1.46	0	14.8±2.5	0
Historical		17.1(15.5-19.2)	15.7		1.1 (0.5-1.4)	0	14.6	

- Uterine and adjusted body weights – No drug-related changes in uterine, ovary and adjusted body weights were noted.
- Necropsy examinations – No treatment-related changes were noted in male and female treated rats.
- Testes and epididymis weights – No treatment-related differences were noted.
- Sperm analysis – No drug-related abnormal findings were observed.

In summary, Sprague Dawley rats (25/sex/group) were given 1, 3 and 10 µg/kg AL06221 or vehicle control via daily subcutaneous administration. Dosing to males began 4 weeks prior to cohabitation and to females two weeks prior to cohabitation. Dosing was continued through gestation Day 7 for females and through completion of evaluations (approximately 71 days) for males. Cohabitation was on a 1:1 ratio and continued until evidence of mating (copulatory plug or sperm in the estrus smear) was observed. In pregnant animals at 10 µg/kg, AL-6221 induced an increase in early resorptions, indicating embryolethality effect of the drug. No other toxicologically significant abnormal findings were observed in both male and female animals. In conclusion, AL-6221 did not have any effects on fertility in rats in this study. NOAEL was considered as 3 µg/kg.

3.4.1.2 Teratology Study in Rats with AL-6221, TR No. 079:30:0400, [redacted]298-029 (Vol. 37)

Study №: 298-029
Report №: 079:30:0400
Study Aim: To determine the toxic potential of AL06221 on the fetal development of rats when administered to the pregnant dam during the gestation period
Compound: AL06221 (Lot#: AL06221-009, purity = 96.69%)
Vehicle Control: [redacted]
Dose/Route/Duration: 0, 1, 3 and 10 µg/2 ml/kg, intravenous injection, qd, gestation Days 6-17
Animals: Female mated Sprague Dawley rats, 26/group
Study Location: [redacted]
Compliance with GLP/QAU: Yes
Study Date: 10/16/97-3/15/00
Study Design: Rats (26/group) were given 1, 3 and 10 µg/2 ml/kg AL06221 or vehicle control via daily intravenous administration from Day 6 of gestation to Day 7 of gestation. The day on which evidence of copulation was observed was designated as Day 0 of gestation. On Day 20 of gestation each female was euthanatized and immediately subject to a laparohysterectomy. Toxicity was assessed as shown below.

- Clinical Observations – Twice daily
- Body Weights – Days 0, 6, 9, 12, 15, 18 and 20 of gestation
- Food Consumption – Days 0, 6, 9, 12, 15, 18 and 20 of gestation
- Uterine and Ovarian Examinations –Gravid uterine weight, viable and nonviable fetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded.

- Teratologic Evaluations – Fetuses were weighted, sexed and examined for external malformations and variations. One-half of the fetuses were examined for soft tissue defects. The other half of the fetuses were examined for skeletal anomalies.
- Anatomic pathology – A complete necropsy was performed on all dams.

Results:

- Clinical Signs – During the study period, no remarkable clinical signs attributable to the treatment were noted. No drug-related mortality was noted.
- Body Weight – A decrease in body weight and body weight gain was seen in dams at 10 μg/kg (see table below). This change was partially due to the decrease in gravid uterine weight in this group. Adjusted body weight and body weight gain were slightly lower than those of controls.

Body weight changes in rats treated with AL-6221 (g)

Dose (μg/kg)	Day of gestation	Control	1	3	10
Body weights	0	188±11.0	188±12.0	187±10.0	189±9.2
	18	319±14.6	319±20.8	323±17.2	305±17.8
	20	360±16.4	357±20.9	364±20.9	335±25.9
% control		100	99.2	100	93.1
Body weight gain	0-20	173±11.3	170±15.9	177±16.2	146±23.2
% control		100	100	100	84.4
Gravid uterine weight	20	73.2	74.3	76.9	55.0
% control		100	100	100	75.1
Adjusted body weight	20	287.2	283.0	287.3	279.6
Adjusted body weight gain	0-20	99.4	95.3	100.0	90.5
% control		100	100	100	91

- Food Consumption – Correlated with the reduction in body weight gain in dams at 10 μg/kg, food consumption was reduced from Day 9 of gestation (see table below).

Food consumption changes in rats treated with AL-6221 (g/animal/day)

Dose (μg/kg)	Control	1	3	10
Gestation days 9-12	24.0±1.6	24.1±4.0	23.2±1.5	22.0±2.0
Gestation days 12-15	25.0±1.7	24.4±2.0	24.5±1.7	23.4±2.7
Gestation days 15-18	27.6±1.6	26.9±.9	27.3±2.5	25.4±2.3
Gestation days 18-20	26.5±2.2	25.8±2.2	26.8±1.9	23.1±3.2

- Necropsy Findings – No abnormal findings were noted.
- Uterine Examinations – Maternal and developmental observations at laparohysterectomy are summarized in the following table. No treatment-related effects were noted in dams at 1 and 3 μg/kg. At 10 μg/kg, five dams had total litter resorptions and one dam delivered early (within 24 hr of termination). The number of post-implantation loss was increased, and the number of live fetuses per litter and fetal body weight was reduced. All these findings were considered treatment-related.

Summary of maternal and developmental observations at laparohysterectomy

Dose (μg/kg)	Control	1	3	10	Historical
Number of dams assigned to study	26	26	26	26	
Pregnant	24	25	24	24	
Dams with total litter resorptions	0	0	0	5	
Dams with viable fetuses	24	25	24	19 (1 early delivery)	
Corpora lutes/dam	15.6±2.3	14.8±2.1	15.4±2.4	13.8±.5	15.3(14.3-16.0)
Implantation/dam	13.0±1.2	13.2±1.4	13.6±1.5	12.2±2.2	13.1
Pre-implantation loss/dam	15.5±11.0	9.4±9.3	10.5±9.2	10.3±10.7	
Early resorptions/dam	0.74±0.69	0.76±1.09	1.04±0.62	4.83±5.05	0.7 (0.6-1.0)
Late resorption/dam	0	0.04±0.20	0	0.17±0.49	0

Dose (μg/kg)	Control	1	3	10	Historical
Number of dams assigned to study	26	26	26	26	
Live fetuses/dam	12.3±1.1	12.4±1.2	12.6±1.5	7.2±5.4	12.4 (12.1-12.8)
Fetal sex ratio (♂/♀)	52.3/47.7	51.4/48.6	48.6/51.4	50/50	48.9/51.1
Fetal weight (g)	4.0±0.3	4.0±0.3	4.1±0.2	3.4±0.6	4.0

- Fetal examinations – Positive external, visceral and skeletal findings are summarized in the table below. No historical control data were provided; however, the sponsor indicated that at 10 μg/kg, the findings such as domed head, hydrocephaly, vertebral malformation, vertebral malformation with associated rib malformation, fused sternebrae and incomplete ossification of vertebral arches were all above historical control range, and were considered drug-related.

Summary of fetus/litter incidence of external, visceral and skeletal effects

Dose (μg/kg)	Class	Control	1	3	10
External exam: Number of fetuses/litters		**282/23**	**311/25**	**302/24**	**166/18**
Domed head	Malformation	0/0	0/0	0/0	2/2
Microphthalmia	Malformation	0/0	0/0	0/0	1/1
Cleft palate	Malformation	0/0	0/0	0/0	1/1
Total malformations		0/0	0/0	0/0	3/3
Total variations		0/0	0/0	0/0	3/3
Visceral exam: Number of fetuses/litters		**141/23**	**155/25**	**150/24**	**86/17**
Hydrocephaly, internal	Malformation	0/0	0/0	0/0	2/2
Renal agenesis	Malformation	0/0	0/0	0/0	2/2
Folded retina	Malformation	1/1	0/0	3/3	1/1
Diaphragmatic hernia	Malformation	0/0	0/0	0/0	1/1
Ureters distended	Variation	0/0	0/0	2/2	9/7
Total malformations		1/1	0/0	3/3	4/3
Total variations		0/0	0/0	2/2	9/7
Skeletal exam: Number of fetuses/litters		**141/23**	**156/25**	**152/24**	**81/17**
Vertebral malformation	Malformation	0/0	0/0	0/0	4/3
Vertebral with associated rib malformation	Malformation	0/0	1/1	0/0	2/2
Sternebrae fused	Malformation	0/0	0/0	0/0	8/4
Malformed skull bones	Malformation	0/0	0/0	0/0	1/1
Malformed basioccipital	Malformation	0/0	1/1	0/0	0/0
Articulating rib head absent	Malformation	0/0	0/0	0/0	1/1
Forelimb, micromelia	Malformation	0/0	0/0	0/0	1/1
Ectrodactyly	Malformation	0/0	0/0	0/0	1/1
Absent phalanges	Malformation	0/0	0/0	0/0	1/1
Vertebral arches, incomplete ossification	Variation	0/0	0/0	0/0	7/5
Sternebrae unossified	Variation	23/11	24/12	7/7	23/12
Rudimentary rib	Variation	11/8	24/12	35/13	21/11
Total malformations		0/0	1/1	0/0	14/7
Total variations		48/18	53/19	51/16	47/16

In summary, pregnant Sprague Dawley rats (26/group) were given 1, 3 and 10 μg/kg AL06221 or vehicle control via daily intravenous administration from gestation days 6-17. Slight decreases in body weight gain and food consumption were noted in dams at 10 μg/kg. At the same dose, AL-6221 induced an increase in post-implantation loss, and a decrease in the number of viable fetuses per dam. The fetal body weights were also reduced. Teratologic examinations showed treatment-related malformations and variations at 10 μg/kg that included domed head, hydrocephaly, vertebral malformation, vertebral malformation with associated rib malformation, fused sternebrae and incomplete ossification of vertebral arches. In conclusion, travoprost was considered to be teratogenic at 10 μg/kg/day. NOAEL was considered as 3 μg/kg for both dams and fetuses.

3.4.1.3 Second Teratology Study in Mice with AL-6221, TR No. 099:30:0400, [redacted]298-047 (Vol. 38)

Study №: 298-047
Report №: 099:30:0400
Study Aim: To determine the developmental toxicity, including the teratogenic potential, of AL06221 in the mouse
Compound: AL06221 (Lot#: P165.15/97002, purity = 97.7%)
Vehicle Control: [redacted]
Dose/Route/Duration: 0, 0.1, 0.3 and 1.0 µg/2 ml/kg, subsutaneous injection, qd, gestation Days 6-16
Animals: Female mated Crl:CD-1®(ICR)BR mice, 30/group, 9-week old at start of mating
Study Location: [redacted]
Compliance with GLP/QAU: Yes
Study Date: 2/18/99-3/6/00
Study Design: Mated mice (30/group) were given 0.1, 0.3 and 1.0 µg/2 ml/kg AL06221 or vehicle control via daily subcutaneous administration from Day 6 of gestation to Day 16 of gestation. The day on which the presence of a plug was noted in the animals was designated as Day 0 of gestation. On Day 18 of gestation each female was euthanized and immediately subject to a laparohysterectomy. Toxicity was assessed as shown below.

- Clinical Observations and Mortality – At least twice daily
- Body Weights – Days 0, 6, 9, 12, 14, 17 and 18 of gestation
- Food Consumption – Days 0, 6, 9, 12, 15, 18 and 20 of gestation
- Uterine Examinations – Gravid uterine weight, viable and nonviable fetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded.
- Teratologic Evaluations – Fetuses were weighted, sexed and examined for external malformations and variations. One-half of the fetuses were examined for soft tissue defects. The other half of the fetuses were examined for skeletal anomalies.
- Anatomic Pathology – A complete necropsy was performed on all dams.

Results:

- Mortality and Clinical Signs – One animal at 1.0 µg/kg was found dead on gestation day 13. No abnormal findings were noted in this animal in necropsy examination. The sponsor indicated that this death was not considered treatment-related. During the study period, no remarkable clinical signs attributable to the treatment were noted.
- Body Weight – No drug-related changes were noted. The gravid uterine weight and adjusted body weight in different groups were also comparable.
- Food Consumption – There were no treatment-related abnormal findings.
- Maternal Necropsy findings – No treatment-related abnormal findings were noted.
- Uterine Examinations – Maternal and developmental observations at laparohysterectomy are summarized in the following table. No treatment-related effects were noted in dams at 0.1 and 0.3 µg/kg. At 1.0 µg/kg, there was a treatment-related increase in early deliveries (delivery within 24 hr of scheduled euthanasia on Day 18 of gestation), abortions (24 hr before scheduled euthanasia on Day 18 of gestation) and total litter resorptions. The number of post-implantation loss was increased, and the number of live fetuses per litter was reduced. All these findings were considered treatment-related. No treatment-related changes in the mean number of corpora lutea, implantation and preimplantation loss were noted. [**Reviewer's Comment**: No historical control data were provided.]

Summary of maternal and developmental observations at laparohysterectomy

Dose (µg/kg)	Control	0.1	0.3	1.0	Historical
Number of dams assigned to study	30	30	30	30	
Pregnant	29	27	27	30	
Dams aborting	0	0	0	3	
Dams with total litter resorptions	0	1	2	7	
Dams delivering early	2	1	2	14	
Dams with viable fetuses	27	25	23	5	
Corpora lutes/dam	14.11±1.45	14.08±1.73	15.30±1.18	14.60±1.82	
Implantation/dam	13.04±1.48	13.04±1.54	13.44±1.23	13.00±1.65	
Pre-implantation loss/dam	7.49±6.32	6.75±7.05	10.75±7.66	10.62±4.74	
Early resorptions/dam	0.81±0.79	1.12±2.39	1.64±3.11	7.75±6.68	
Late resorption/dam	0.07±0.27	0.04±0.20	0.12±0.44	0.08±0.29	
Live fetuses/dam	12.15±1.59	11.85±3.02	11.64±3.70	5.17±6.48	
Fetal sex ratio (♂/♀)	50/50	51/49	49.1/50.9	49/51	
Fetal weight (g)	1.35±0.09	1.37±0.08	1.38±0.08	1.41±0.06	

- Fetal examinations – Fetal external, visceral and skeletal examinations showed no treatment-related effects. Several malformations were seen in skeletal observations (see table below). Since the incidence was low, and there was no dose-relationship, these changes were not considered drug-related.

Summary of malformations in skeletal examination

Dose (µg/kg)	Control	0.1	0.3	1.0
Skeletal exam: Number of fetuses/litters	**164/27**	**152/25**	**146/23**	**31/5**
Cervical vertebrae: interrupted ossification of an arch	3/3	3/3	2/1	1/1
Vertebrae: vertebral malformation	8/6	1/1	5/5	0/0
Vertebrae: vertebral with associated rib malformation	0/0	0/0	1/1	0/0
Vertebrae: forked arch	0/0	5/5	0/0	0/0
Sternebrae: fused	10/8	2/2	0/0	0/0
Ribs: interrupted ossification	0/0	1/1	1/1	0/0
Ribs: extra thoracic rib	0/0	1/1	0/0	0/0
Total malformations	**21/14**	**12/10**	**9/7**	**1/1**

In summary, pregnant Crl:CD-1®(ICR)BR mice (30/group) were given 0.1, 0.3 and 1.0 µg/kg AL06221 or vehicle control via daily subcutaneous administration from gestation Days 6-16. Animals were terminated on Day 18 of gestation and examinations on the uterine and fetuses were performed. No effects on body weights and food consumption were noted in any treated groups. Necropsy examination revealed no drug-related abnormalities. No developmental toxicities were noted at 0.1 and 0.3 µg/kg. At 10 µg/kg, AL-6221 induced 14 early deliveries, 3 abortions, and 7 total litter resorptions. An increase in post-implantation loss, and a decrease in the number of viable fetuses per dam were also observed at 10 µg/kg. The fetal body weights and sex distribution were reduced. Teratologic examinations showed no treatment-related external, visceral and skeletal findings. In conclusion, travoprost was not considered to be teratogenic under the conditions of this study. NOAEL was considered as 3 µg/kg for both dams and fetuses.

3.4.1.4 Study of the Effects of AL-6221 on Pre- and Postnatal Development Including Maternal Function in Rats (Segment III), TR No. 085:30:0400, [redacted]298-040 (Vol. 2.39)

Study №: 298-040
Report №: 085:30:0400

Study Aim: To determine the toxicity potential of AL06221 in the pregnant/lactating females and in the development of conceptus and the offspring following exposure of the female from implantation through weaning

Compound: AL06221 (Lot#: 1376, purity = 96.06%)

Vehicle Control: [redacted]

Dose/Route/Duration: 0, 0.12, 0.36 and 0.72 µg/2 ml/kg, subcutaneous injection, qd, from gestation Day 7 to lactation Day 21

Animals: Female mated Sprague Dawley rats, 8 weeks old, 26/group

Study Location: [redacted]

Compliance with GLP/QAU: Yes

Study Date: 10/22/98-3/6/00

Study Design: Rats (26/group) were given 0.12, 0.36 and 0.72 µg/2 ml/kg AL06221 or vehicle control via daily subcutaneous administration from Day 7 of gestation to Day 21 of lactation. The day on which evidence of mating was observed was designated as Day 0 of gestation. Toxicity was assessed as shown below.

- Clinical Observations – At least twice daily
- Body Weights – Days 0, 7, 10, 14 and 20 of gestation and on Days 0, 7, 10, 14, 17 and 21 of lactation
- Food Consumption – Days 0, 7, 10, 14 and 20 of gestation and on Days 0, 7, 10, 14, 17 and 21 of lactation
- F_0 parturition and F_1 litter observations – The pregnant animals were allowed to deliver and pups were examined for viability, sex, body weight and gross abnormalities. Litters were culled to eight pups at Day 4 and remained with their dams until weaning at Day 21. Pup survival and body weight was recorded. Following weaning dams were sacrificed, examined and the number of implantation sites recorded. Dams delivered litters before Day 20 of gestation were considered to have aborted. Delivery of a litter on Day 20 of gestation was considered early delivery. All dams that delivered prematurely were euthanized and subjected to a necropsy.
- F_1 behavioral and developmental indices testing – Behavioral and developmental indices were recorded for offspring during the lactation period including static righting reflex (Day 2), pinna detachment (Day 2), cliff aversion, (Day 11), eye opening (Day 13), air drop righting reflex (Day 16), the Irwin evaluation for neuropharmacologic parameters (Day 21). Auditory response (Day 33), Root-Rod performance (Day 34), Vaginal opening (Day 34), balanopreputial cleavage (Day 40), Active and emotionality (Day 35), and passive avoidance (70 - 85 days of age) were also assessed in the post-weaning growth period.
- F_1 Reproduction Assessment – Selected F_1 (26/sex/group) offspring were mated (1♂ and 1♀) at age of 80 days for assessment of breeding and reproductive effects. The day on which evidence of mating (sperm in smear or plug in female) was designated as Day 0 of gestation. On Day 20 of gestation, F1 dams were euthanized and immediately subject to a laparohysterectomy. Gravid uterine weight, viable and nonviable fetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded.
- F_2 teratologic Examinations – F2 fetuses were weighted, sexed and examined for external malformations and variations.
- Anatomic pathology – A necropsy was performed on all F_0 dams (Day 21 of lactation), weaned F_1 pups that died in study, weaned F_1 pups that exhibited abnormal developmental or behavioral indices.

Results:

- F_0: Maternal Observations – One animal at 0.72 µg/kg had a prolapsed uterus following delivery of its pups, all of which were dead. Since no abnormal findings were noted for this rat at necropsy, it was

not clear if this was treatment-related. No toxicologically significant changes other than abortion or early delivery were noted in the other animals.

- F_0: Maternal Body Weight – No toxicologically significant changes in body weights and body weight gain were observed.
- F_0: Maternal Food Consumption – A decrease in food consumption was noted in all treated groups during lactation Days 0-10 (see table below).

Food consumption changes in rats treated with AL-6221 (g/animal/day) during lactation

Dose (µg/kg)	Control	0.12	0.36	0.72
N	25	20	16	1
Lactation days 0-7	41.7±3.7	37.2±5.2	36.6±3.8	29.0
Lactation days 7-10	59.2±3.9	55.2±7.8	54.4±5.1	52.0
Lactation days 10-14	69.6±5.5	65.0±10.0	64.4±6.3	62.0
Lactation days 14-17	77.0±5.1	73.7±11.4	72.9±7.4	70.0

- F_0: Maternal Necropsy Findings – No abnormal findings were noted.
- F_0: Reproductive Parameters – Reproductive parameters of F_0 dams are summarized in the table below. All treated groups showed a reduction in gestation length. At 0.72 µg/kg, the number of litters with live born pups was decreased, and the number of litters with stillborn pups was increased. At the same dose, 11 abortions were seen.

Summary of reproductive parameters of F_0 dams

Dose (µg/kg)	Control	0.12	0.36	0.72
Number of dams assigned to study	26	26	26	26
Pregnant	25	24	26	26
Number of abortions	0	0	0	11
Dams delivering litters	25	24	26	15
Gestation length (days)	21.56±0.51	21.13±0.34	20.77±0.51	20.20±0.41
Dams with viable fetuses	25	23	25	9
Dams with stillborn pups	0	8	14	13
Dams with all stillborn	0	1	1	6

- F_1: Offspring Viability and Survival – F_1 offspring viability data are summarized in the table below. Treatment-related changes included decreases in the number of pups per litter at birth, the number of live born pups per litter, and the percentage of pups surviving after 1 and 4 days. No pups delivered on gestation Day 20 survived beyond Day 4 of lactation.

Summary of F_1 offspring survival

Dose (µg/kg)	Control	0.12	0.36	0.72	Historical
Number of litters delivered	25	24	26	15	
Number of pups delivered /litter	12.6±2.2	11.1±2.7	12.5±3.2	10.0±4.3	
Number of live pups delivered /litter	12.6±2.2	10.6±3.0	9.6±5.3	4.3±4.8	12.1-12.8
Pups surviving 1 day (%)	99.7	85.0	63.9	17.0	
Pups surviving 4 days (%)	98.0	82.3	57.3	8.9	
Number of litters delivered on Day 20 of gestation	0	0	7 (7)	12 (12)	
Number of litters delivered on Day 21 of gestation	11 (0)	21 (4)	18 (3)	3 (2)	
Number of litters delivered on Day 22 of gestation	14 (0)	3 (0)	1 (0)	0	

() Number of litters died by Day 4 of lactation

- F_1: Offspring Growth – Drug-related effects on growth were noted in all treated groups evidenced by the low body weights (see table below).

Body weight changes in F_1 pups during lactation (g)

Dose (µg/kg)	Control	0.12	0.36	0.72
Lactation Day 0	6.64±0.49	6.16±0.93	5.54±0.97	4.69±0.41
Lactation Day 4	10.72±0.78	9.74±1.39	8.31±1.25	6.91
Lactation Day 7	17.53±1.02	16.00±2.09	14.24±2.26	11.13
Lactation Day 21	57.40±2.85	52.32±4.43	49.28±5.60	47.21

- F_1: Behavioral and Developmental Indices – Drug-related effects on physical development are summarized in the table below). In male F_1 animals of mid and high dose groups, AL-6221 also showed effects on several motor activity parameters including decreases in horizontal activity (↓12-30%), vertical activity (↓17-47%) and total distance (↓10-31%).

Summary of physical development in F_1 pups

Dose (µg/kg)	Control	0.12	0.36	0.72
Static righting reflex (days)	2.66±0.45	2.95±0.65	3.20±0.71	2.69±0.97
Pinna detachment (days)	2.63±0.46	3.19±0.63	3.71±0.64	4.38
Eye opening (days)	14.20±0.53	14.68±0.47	14.98±0.67	15.38
Preputial separation (days)	41.52±1.00	42.20±0.89	43.13±1.31	44.00

- F_1: Parental Observations – There were no treatment-related effects in male and female F_1 animals during premating period, and in females during gestation period.

- F_1: Body Weights – A slight, dose-related decrease in body weights was noted in both males and females in all treated groups (see table below). Body weights and body weight gain in all groups were comparable during gestation period.

Selected F_1 body weight data (g) during premating period

	Males				Females			
Dose (µg/kg)	Control	0.12	0.36	0.72	Control	0.12	0.36	0.72
Premating Day 42	229±10.1	217±15.3	209±23.5	190±15.2	181±9.8	171±14.4	169±17.2	165±4.9
Premating Day 56	360±19.3	349±23.3	336±32.1	311±23.8	240±18.7	229±13.7	229±22.3	223±12.0
Premating Day 70	451±28.6	446±32.8	431±41.9	397±30.8	274±23.2	269±19.6	265±24.6	259±3.4
Premating Day 77	480±33.5	479±37.6	449±49.2	430±34.1	289±24.0	279±19.2	278±27.8	271±10.6

- F_1: Necropsy Observations – No treatment-related, toxicologically significant findings were noted.

- F_1: Cohabitation Data and Reproductive Parameters – The cohabitation data and reproductive parameters are summarized in the table below. Only one litter from the 0.72 mg/kg group survived to mating, and since sibling pairings were avoided, no animals from this group were mated. In the other groups, no treatment-related, toxicologically significant findings were noted.

Summary of cohabitation data and reproductive parameters of F_1 dams

Dose (µg/kg)	Control	0.12	0.36
Number of males placed with females	26	25	26
Number of males mated	21	21	24
Number of males with females pregnant	20	20	21
Number of females placed with males	26	26	26
Number of females inseminated	25	25	26
Number of females pregnant	24	23	23
Number of corpora lutea/dam	19.19	18.68	18.14
Number of implantation/dam	16.77	16.45	16.38
Preimplantation loss (%)	11.27	11.02	9.36
Number of live fetuses/litter	16.09±2.71	15.59±2.15	15.29±2.97
Post-implantation loss (%)	4.27±4.24	5.24±7.03	7.20±8.69
Gravid uterine weights (g)	93.7	91.3	87.5
Adjusted body weights (g)	387.7±30.6	367.9±23.9	380.6±44.9
Fetal weights (g)	3.83±0.52	3.82±0.41	3.76±0.28

- F_2: Fetal External Observations – Two fetuses from different litters in the 0.12 µg/kg group had microphthalmia and another fetus from a third litter at 0.12 µg/kg had anal atresia and short thread-like tail. Similar findings were noted seen at 0.36 µg/kg, and these changes might not be treatment-related.

In summary, pregnant Sprague Dawley rats (26/group) were given 0.12, 0.36 and 0.72 µg/kg AL06221 or vehicle control via daily subcutaneous administration from gestation Day 7 to lactation Day 21. Dams were allowed to give birth. At lactation Day 4, the litters were culled to eight pups and remained with their dams until weaning at Day 21. Selected F_1 offspring were mated for assessment of breeding and reproductive effects. One dam of the 0.72 µg/kg/day group had a prolapsed uterus following delivery of a stillborn litter. Food consumption was lower than controls for the treated groups during lactation period. No treatment-related findings, except for the one prolapsed uterus, were observed at necropsy of dams. Eleven abortions occurred in 0.72 µg/kg/day group. Gestation length was reduced in a dose-related manner. The number of dams delivering litter and the number of dams with viable pups were greatly reduced at the high dose. The number of litters with stillborn pups was increased for all travoprost treated groups. This pattern carried over into the lactation period, with pup survival reduced in the treated groups up to Day 4. Pup body weights were reduced in a dose-related manner for all treated groups from lactation Day 0 through Day 21. Pup development was affected as demonstrated by delayed static righting reflex, eye opening, pinna detachment and preputial separation at all doses. Following the weaning period mean body weights were reduced, as compared with controls, for all treated groups, but differences tended to diminish over time. F_1 reproductive performance was comparable with controls for all groups, and there were no treatment-related findings upon external examination of F_2 fetuses. On the basis of these findings, an NOAEL could not be established for F_0 maternal effects or F_1 offspring development. The F_2 generation was unaffected by administration of travoprost to F_0 dams during the gestation and lactation periods.

3.4.1.5 Study of the Effects of AL-6221 on Pre- and Postnatal Development Including Maternal Function in Rats (Segment III), TR No. 083:30:0400, [redacted]298-049 (Vol. 40-41)

Study №: 298-049
Report №: 083:30:0400
Study Aim: To determine the possible adverse effects of AL06221 on the pregnant/lactating females and on the development of conceptus and the offspring following exposure of the female from implantation through weaning
Compound: AL06221 (Lot#: P165.15/97002, purity = 97.7%)
Vehicle Control: [redacted]
Dose/Route/Duration: 0, 0.01, 0.03 and 0.1 µg/ml/kg, subcutaneous injection, qd, from gestation Day 7 to lactation Day 21
Animals: Female mated Sprague Dawley rats, 8 weeks old, 152-239 g, 26/group
Study Location: [redacted]
Compliance with GLP/QAU: Yes
Study Date: 4/27/99-4/14/00
Study Design: Rats (26/group) were given 0.12, 0.36 and 0.72 µg/2 ml/kg AL06221 or vehicle control via daily subcutaneous administration from Day 7 of gestation to Day 21 of lactation. The day on which evidence of mating was observed was designated as Day 0 of gestation. Toxicity was assessed as shown below.

- Clinical Observations – At least twice daily.

- Body Weights – Days 0, 7, 10, 14, 17 and 20 of gestation and on Days 0, 7, 10, 14, 17 and 21 of lactation
- Food Consumption – Days 0, 7, 10, 14, 17 and 20 of gestation and on Days 0, 7, 10, 14, 17 and 21 of lactation
- F_0 parturition and F_1 litter observations – The pregnant animals were allowed to deliver and pups were examined for viability, sex, body weight and gross abnormalities. The day on which all pups were delivered was designated as Day 0 of lactation. Litters were culled to eight pups at Day 4 and remained with their dams until weaning at Day 21. Pup survival and body weight was recorded. Following weaning dams were sacrificed, examined and the number of implantation sites recorded.
- F_1 behavioral and developmental indices testing – Behavioral and developmental indices were recorded for offspring during the lactation period included static righting reflex (Day 2), pinna detachment (Day 2), cliff aversion, (Day 11), eye opening (Day 13), air drop righting reflex (Day 16), the Irwin evaluation for neuropharmacologic parameters (Day 21).
- Anatomic pathology – A necropsy was performed on all F_0 dams (Day 21 of lactation). Culled F_1 pups were euthanized and examined for external abnormalities.

Results:

- F_0: Maternal Observations – All dams survived to scheduled euthanasia. No toxicologically significant findings were noted.
- F_0: Maternal Body Weight – No toxicologically significant changes in body weights and body weight gain were observed.
- F_0: Maternal Food Consumption – No drug-related changes in food consumption was noted.
- F_0: Maternal Necropsy Findings – No treatment-related abnormal findings were noted.
- F_0: Reproductive Parameters – Reproductive parameters of F_0 dams are summarized in the table below. Total litter resorption was noted in 1 dam of low dose group. High dose and low dose dams had lower implantation sites relative to the controls, which might not be treatment-related.

Summary of reproductive parameters of F_0 dams

Dose (µg/kg)	Control	0.01	0.03	0.1	Historical
Number of dams assigned to study	26	26	26	26	
Pregnant	26	26	24	25	
Pregnant index (%)	100	100	92.3	96.2	85-100
Dams delivering litters	26	25	24	25	
Gestation length (days)	21.92±0.39	21.88±0.44	22.08±0.41	21.88±0.44	
Dams with viable fetuses	26	25	24	25	
Dams with stillborn pups	1	4	3	1	
Total litter resorption	0	1	0	0	
Implantation sites/litter	13.19±1.55	12.12±2.42	12.75±1.54	11.17±2.81	12.7-13.8

- F_1: Offspring viability and survival – F_1 offspring viability data are summarized in the table below. In low dose and high dose groups, the number of pups delivered and the number of live pups delivered were lower than those in the control group. The sponsor indicated that these changes could be due to lower implantation rate seen in these 2 groups. No other abnormal findings were noted.

Summary of F_1 offspring survival

Dose (µg/kg)	Control	0.01	0.03	0.1	Historical
Number of litters delivered	26	25	24	25	
Number of pups delivered /litter	12.6±1.5	11.3±2.2	12.4±1.7	10.5±2.8	
Number of live pups delivered /litter	12.5±1.5	11.0±2.4	12.3±1.8	10.5±1.8	12.1-12.8
Total stillborn pups	1	7	3	1	
Pups dying, missing and/or cannibalized					
Days 1-4	3	5	3	5	

Dose (µg/kg)	Control	0.01	0.03	0.1
Pups dying, missing and/or cannibalized				
Days 5-7	1	0	2	1
Days 8-14	1	0	1	0
Days 15-21	0	0	0	1
Total	5	5	6	7

- F_1: Offspring Growth – There were no drug-related differences in pup body weights between control and treated groups.
- F_1: Offspring Necropsy Observations – No abnormal findings that could be attributed to the treatment were noted.
- F_1: Behavioral and Developmental Indices – No treatment-related, biologically relevant changes were noted.

In summary, pregnant Sprague Dawley rats (26/group) were given 0.01, 0.03 and 0.1 µg/kg AL06221 or vehicle control via daily subcutaneous administration from gestation Day 7 to lactation Day 21. The pregnant animals were allowed to give birth. At lactation Day 4, the litters were culled to eight pups and remained with their dams until weaning at Day 21. No toxicities in the F_0 animals were observed. The implantation sites in high and low dose groups were lower than those in the control group. However, it was not considered treatment-related since implantation occurred on Days 5 to 6 of gestation, before dosing initiation. As a consequence of the lowered implantation rate, the number of pups or viable pups per dam were reduced at the low and high doses relative to the controls. No toxicities in F_1 offspring's survival, growth and physical and behavioral development were observed. The high dose, 0.1 µg/kg/day, was considered as the NOAEL for both dams and fetuses.

3.5 GENOOXICITY

3.5.1.1 Bacterial Reverse Mutation Assay with an Independent Repeat with AL-6221, TR No. 056:30:0300, [redacted]96CB63.502002 (Vol. 42)

Study №.: G96CB63.502002
Report №.: TR No. 056:30:0300
Study Aim: To evaluate the mutagenic potential of AL-6221 in *S. typhimurium* and *E. coli* in the presence and absence of S9 activation.
Compound: AL-6221 (Lot #s: AL-6221-04 and AL-6221-05, purity = 96.46% and 99.31%)
Concentrations: Without S9: 25, 75, 200, 600, 1800 and 5000 µg/plate; With S9: 75, 200, 600, 1800, and 5000 µg/plate (The doses were selected based on the results of a dose range finding study.)
Vehicle Control: [redacted]
(+) Control:

Compounds	S9	Bacteria Tester Strains	Concentrations (µg/plate)
[redacted]			

Tester Strains: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA.
Incubation Time: 37°C for 48 hr

Study Location: [redacted]
GLP/QAU: Yes
Study Initiation Date: 7/2/1997
Study Design: The study was performed in 2 phases. The first phase, the preliminary assay, was used to establish the dose range for the study. The second phase, the mutagenicity assay including initial and confirmatory trials, was used to evaluate the mutagenic potential of AL-6221. All dose levels of AL-6221, vehicle and positive controls were plated in triplicate.

Results:

In the preliminary assay, precipitation was observed at ≥ 3333 or at 5000 µg/plate, which was described by the sponsor as "non-interfering precipitation". Reduced revertant counts were observed in test strains TA98, TA100 and TA1535 without S9 activation. Based on the findings of the preliminary assay, 5000 µg/plate was selected as the maximum dose in the mutagenic assay. In both initial and confirmatory trials for mutagenicity, AL-6221 at the concentrations up to 5000 µg/plate did not increase the numbers of revertant colonies of *Salmonella typhimurium* (strains TA-1535, TA-1537, TA-98, and TA-100) and *Escherichia coli* (strain WP2uvrA) in the presence or absence of S9 activation. Therefore, AL-6221 was not mutagenic under the present testing conditions.

3.5.1.2 L5178Y TK +/- Mouse Lymphoma Forward Mutation Assay with Confirmatory Assay with AL-6221, TR No. 062:30:0300, [redacted] No. 19682-0-431 ICH (Vol. 42)

Study №.: 19682-0-431 ICH
Report №.: TR No. 062:30:0300
Study Aim: To evaluate the potential of AL-6221 to induce forward mutation at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line in the presence and absence of S9 activation
Compound: AL-6221 (Lot #: AL-6221-10, purity = 98.88%)
Concentrations: Without S9: 5-120 µg/ml and 10-140 µg/ml; With S9: 10-200 µg/ml and 30-150 µg/ml (The doses were selected based on the results of a dose range finding study.)
(+) Control: Methyl methanesulfonate (MMS, 5 and 10 nl/ml) w/o S9 Methylcholanthrene (MCA, 2.0 and 4.0 µg/ml) w/ S9
Vehicle Control: [redacted]
Indicator Cell: Mouse lymphoma L5178Y cell line, clone 3.7.2C
Study Location: [redacted]
GLP/QAU: Yes
Study Initiation Date: 7/13/1998
Study Design: In the initial trial, cells were exposed to the drug for 4 hr with and without S9 activation. In the confirmatory trial, cells were exposed to the drug for 4 hr (with S9) or 24 hr (without S9).

Results:

In a dose range finding assay, cells were treated with AL-6221 at 1.97 µg/ml to 1 mg/ml for 4 hr (with or without S9) or 24 hr (without S9). Without S9 activation, doses lower than 62.5 µg/ml were not cytotoxic. However, higher doses (≥ 125 µg/ml) completely inhibited cell growth (100%). With S9

activation, 60% cell growth inhibition was noted at 125 µg/ml and 100% inhibition at higher concentrations.

In both initial and confirmatory trials, AL-6221 produced cytotoxic effects evidenced by inhibition of cell growth with S9 activation (↓83-85% at 120 µg/ml) or without S9 activation (↓75% at 140 µg/ml). The drug did not increase the mutant frequency with or without S9 activation. Therefore, AL-6221 was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells in this study.

3.5.1.3 *In vitro* Mammalian Cell Gene Mutation Test with an Independent Repeat Assay, TR No. 062:30:0300, [redacted] G96CB63.702001 (Vol. 42)

Study №.: G96CB63.702001
Report №.: TR No. 058:30:0300
Study Aim: To evaluate the potential of AL-6221 to induce forward mutation at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line in the presence and absence of S9 activation
Compound: AL-6221 (Lot #: AL-6221-05, purity = 99.31%)
Concentrations: Without S9: [redacted] (The doses were selected based on the results of a dose range finding study.)
(+) Control: Methyl methanesulfonate (MMS, 10 and 20 µg/ml) w/o S9; 7, 12 Dimethylbenz (a) anthracene (DMBA, 2.5 and 4.0 µg/ml) w/ S9
Vehicle Control: [redacted]
Indicator Cell: Mouse lymphoma L5178Y cell line, clone 3.7.2C
Study Location: [redacted]
Compliance with GLP/QAU: Yes
Study Initiation Date: 11/8/1996
Study Design: The study included a preliminary toxicity assay and 2 mutagenesis assays (one initial assay and one confirmatory assay). In both assays, cells were exposed to the drug for 4 hr with and without S9 activation.
Evaluation of Results:

Positive: A concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequency of ≥ 100 mutants per 10^6 clonable cells over the background level.
Equivocal: The mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level.
Negative: Fewer than 55 mutants per 10^6 clonable cells over the background level.

Results:

In the preliminary toxicity assay, cells were treated with AL-6221 at 0.15 µg/ml to 1 mg/ml with or without S9 activation. No precipitation was noted. Suspension growth relative to the solvent controls was 12% at 50 µg/ml and 0 at 150 µg/ml without S9 activation, and 0 at 1 mg/ml with S9 activation. Based on these results, the maximum concentrations were determined as 100 µg/ml without S9 and 1000 µg/ml with S9.

In mutagenesis assays, AL-6221 produced cytotoxic effects evidenced by inhibition of cell growth with S9 activation (↓71% at 500 µg/ml) or without S9 activation (↓80% at 60 µg/ml). The drug

The bone marrow was harvested 24, 48 and 72 hr after dosing. The frequency of micronucleated cells was expressed as percent micronucleated polychromatic erythrocytes (MNPCE) over polychromatic erythrocytes (PCE). The proportion of PCE to total erythrocytes was also measured.

Results:

No mortality was noted in any group during the study. Clinical signs observed following dosing included lethargy in all AL-6221-treated groups and diarrhea in male and female mice at 100 mg/kg.

The results of mouse micronucleus assay are summarized in the table below. Mice treated with AL-6221 showed no decrease in the PCE/total erythrocytes ratio, and no increase in the frequency of micronucleated PCEs compared to the vehicle control. Therefore, AL-6221 was not clastogenic under the present testing conditions.

Results of micronucleus assay

	Males						
Compound	Dosage (mg/kg)	PCE/Total RBC (24 hr)	MNPCE/PCE % (24 hr)	PCE/Total RBC (48 hr)	MNPCE/PCE % (48hr)	PCE/Total RBC (72 hr)	MNPCE/PCE % (72 hr)
Vehicle	0	0.57±0.04	0.08±0.11	0.48±0.06	0.06±0.06	0.51±0.03	0.10±0.07
AL-6221	25	0.53±0.06	0.04±0.06	0.53±0.02	0.06±0.06	0.50±0.01	0.08±0.13
AL-6221	50	0.53±0.02	0.04±0.06	0.53±0.07	0.02±0.05	0.50±0.01	0.08±0.05
AL-6221	100	0.49±0.08	0.04±0.06	0.48±0.06	0.10±0.07	0.50±0.06	0.06±0.06
CP	60	0.39±0.06	4.76±.44				
	Females						
Compound	Dosage (mg/kg)	PCE/Total RBC (24 hr)	MNPCE/PCE % (24 hr)	PCE/Total RBC (48 hr)	MNPCE/PCE % (48hr)	PCE/Total RBC (72 hr)	MNPCE/PCE % (72 hr)
Vehicle	0	0.57±0.04	0.08±0.05	0.48±0.04	0.10±0.07	0.53±0.08	0.06±0.06
AL-6221	25	0.62±0.06	0.03±0.13	0.53±0.06	0.14±0.11	0.53±0.06	0.08±0.08
AL-6221	50	0.53±0.06	0.06±0.09	0.55±0.06	0.08±0.13	0.50±0.06	0.10±0.14
AL-6221	100	0.53±0.05	0.00±0.00	0.55±0.02	0.10±0.12	0.56±0.03	0.08±0.18
CP	60	0.43±0.03	5.5±1.41				

3.5.1.5 Chromosomal Aberrations In Vivo in Rat Bone Marrow Cells, TR No. 060:30:0300, [redacted] No. 20374-0-452OECD

Study No.: 20374-0-452OECD
Report No.: TR No. 060:30:0300
Study Aim: To evaluate the ability of AL-6221 administered in vivo to induce structural or numerical chromosome abnormalities by examining rat bone marrow cells arrested at mitotic metaphase
Compound: AL-6221 (Lot #: AL-6221-10, purity = 97.7%)
Vehicle: [redacted]
Dose Level: 0, 18.8, 37.5 and 75 mg/10 ml/kg
(+) Control: Cyclophosphamide (CP), 60 mg/kg, oral gavage
Route: Intravenous injection
Dosing Regimen: Single dose
Animal: Male Sprague Dawley rats
Study Location: [redacted]
GLP/QAU: Yes

did not increase the mutant frequency with or without S9 activation in the initial trial. In the confirmatory assay, one non-activated culture treated with 60 µg/ml and all ten S9-activated cultures showed mutant frequencies which were at least 60 mutants per 10^6 clonable cells over that of the solvent control. There was no dose-response trend with or without S9 activation. One S9-activated culture treated with 350 µg/ml showed mutant frequency 117 mutants per 106 clonable cells over that of the solvent control. In conclusion, under the conditions of this study, AL-6221 was negative in the absence of S9 activation and equivocal in the presence of S9 activation.

Mutant frequency in the confirmatory assay

Without S9				With S9			
DMSO	Mutant frequency	Induced mutant frequency	% total growth	DMSO	Mutant frequency	Induced mutant frequency	% total growth
10 µl/ml	42			10 µl/ml	79		
10 µl/ml	36			10 µl/ml	59		
AL6221 (µg/ml)				AL6221 (µg/ml)			
30	50	11	92	350	186	117	87
30	45	6	95	350	158	89	76
40	62	23	64	375	154	85	86
40	52	13	76	375	165	96	85
50	57	18	60	400	150	82	88
50	55	16	62	400	147	79	82
55	41	2	69	450	165	96	59
55	36	-2	61	450	162	93	51
60	86	47	24	500	151	83	38
60	99	60	17	500	133	64	20
MMS				DMBA			
10 µg/ml	328	289	46	2.5 µg/ml	246	177	77
20 µg/ml	673	634	14	4 µg/ml	455	386	48

3.5.1.4 Micronucleus Cytogenetic Assay in Mice, TR No. 055:30:0300, [redacted] G96CB63.122 (Vol. 2.42)

Study No.: G96CB63.122
Report No.: TR No. 055:30:0300
Study Aim: To evaluate the clastogenic potential of AL-6221 to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female mice
Compound: AL-6221 (Lot #: AL-6221-05, purity = 99.31%)
Vehicle: [redacted]
Dose Level: 0, 25, 50 and 100 mg/10 ml/kg
(+) Control: Cyclophosphamide (CP)
Route: Intravenous injection
Dosing Regimen: Single dose
Animal: ICR mice, 6-8 weeks old, ♂: 31.7-37.2 g, ♀: 24.5-30.6 g
Study Location: [redacted]
GLP/QAU: Yes
Study Initiation Date: 11/14/1996
Study Design:

Group	Compound	Dosage (mg/kg)	N (24 hr harvest)	N (48 hr harvest)	N (72 hr harvest)
1	Vehicle	0	5♂5♀	5♂5♀	5♂5♀
2	AL-6221	25	5♂5♀	5♂5♀	5♂5♀
3	AL-6221	50	5♂5♀	5♂5♀	5♂5♀
4	AL-6221	100	5♂5♀	5♂5♀	5♂5♀
5	CP (Positive control)	60	5♂5♀		

* Only 5 mice were used for preparation of slides.

Study Initiation Date: 3/3/1999
Study Design:
Treatment protocol

Group	Compound	Dosage (mg/kg)	N (18 hr harvest)*	N (42 hr harvest)*
1	Vehicle	0 (iv)	6	6
2	AL-6221	18.8 (iv)	6	
3	AL-6221	37.5 (iv)	6	
4	AL-6221	75 (iv)	6	6
5	CP	60 (po)	6	

* Only 5 rats/group were used for preparation of slides.

The bone marrow was harvested 18 or 42 hr after dosing. Due to high mortality at 75 mg/kg dose level, the 42-hr sampling timepoint was repeated at 0 and 50 mg/kg.

Results:

Mortalities were noted in all treated groups (3/9 at 18.8 mg/kg, 1/7 at 37.5 mg/kg, 1/12 at 50 mg/kg, and 11/19 at 75 mg/kg. Clinical signs including urine and fecal stains, soft feces or diarrhea, and chromodacryorrhea were noted in animals at the doses of 37.5 mg/kg or higher.

There were no statistically significant increases in either structural or numeric chromosome aberrations at any dose level, including those associated with mortalities and clinical signs. Travoprost was concluded to be negative for induction of chromosome damage under the conditions of this study.

3.6 SPECIAL TOXICITY

3.6.1.1 *In Vitro* Transformation of Syrian Hamster Embryo Cells, TR No. 123:30:0500, [redacted] No. 19287-1-485F and 19287-1-485R (Vol. 2.43)

Study №.: 19287-1-485F and 19287-1-485R
Report №.: TR No. 123:30:0500
Study Aim: To determine the potential of AL-6221 to induce increases in the frequency of morphologically transformed colonies in Syrian hamster embryo cells
Compound: AL-6221 (Lot #: 1376, purity = 96.06%)
Dose Level: 24 hr exposure: 0, 7.5, 12.5, 17.5, 22.5, 27.5, 32.5 and 37.5 µg/ml; 7-day exposure: 0, 5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/ml (The doses were selected based on the results of a dose range finding study.)
(+) Control: Benzo(a)pyrene 1.25-5.0 µg/ml
Vehicle Control: [redacted]
Indicator Cell: Syrian golden hamster embryo (SHE) cells
Study Location: [redacted]
GLP/QAU: Yes
Study Initiation Date: 3/12/1998
Study Design: SHE cells were exposed to AL-6221 for 24 hr or 7 days (45 dishes/dose). The total colony number and the number of colonies with transformed morphology were recorded. Based on this data, plating efficiency and transformation frequency were calculated.
Evaluation of Results:
Positive: A statistically significant increase in morphological transformation frequency in at least 2 doses compared to concurrent controls, or a statistically

significant treatment-related increase in one dose with an indication of a statistically significant positive dose trend
Negative: There is no dose with a statistically significant treatment-related increase and the uppermost dose of test material demonstrates a sufficient level of toxicity (50% reduction in plating efficiency or colony density).

Results:

In dose range finding assay, cells were treated with AL-6221 at 10-450 μg/ml for 24 hr. Cytotoxicity was measured by the relative reduction in plating efficiency (RPE). The results showed that RPE was reduced to 85% at 10 μg/ml, 38% at 20 μg/ml and totally inhibited at the concentrations ≥ 40 μg/ml. Based on the cytotoxicity data, the maximum doses selected were 37.5 μg/ml for 24 hr exposure and 20 μg/ml for 7-day exposure.

In 24 hr transformation assay, AL-6221 produced cytotoxic effects evidenced by inhibition of RPE by 52% at 32.5 μg/ml. The drug did not increase the morphological transformation frequency at any concentrations. In 7-day transformation assay, AL-6221 at the highest concentration, 20 μg/ml, reduced the colony number by 38% and colony size by 74%. At the same concentration, a statistically significant increase in transformation frequency was noted (0.412% vs. control's 0.174%). Since statistically significant increase in the transformation was seen in only 1 concentration and no positive trend was noted, AL-6221 was considered as negative in this assay. In conclusion, AL-6221 was negative for potential to induce morphological transformation following either 24-hr or 7-day continuous exposure.

3.6.1.2 Delayed Contact Sensitization Study in Guinea Pigs with AL-6221, TR No. 057: 30: 0300, [redacted] No. 96-8231-21 (Vol. 2.43)

Study No.: 96-8231-21
Report No.: TR No. 057:30:0300
Study Aim: To determine the delayed contact hypersensitivity response in guinea pigs to AL-6221
Compound: AL-6221 (Lot #: AL-6221-04, purity = 96.46%)
Vehicle: [redacted]
(+) Control: Hexylcinnamaldehyde (5% in acetone)
Animal: Hartley guinea pigs, 10/sex for AL-6221 group, 5/sex/group for positive, vehicle and naive control
Study Location: [redacted]
Compliance with GLP/QAU: Yes
Study Initiation Date: 9/20/1996
Study Design: The study was divided into 5 phases.

Phase 1. Irritation screening: One male and one female were used in the primary irritation screen. Each animal was injected intradermally at 8 sites with concentrations of 0, 0.01%, 0.05% and 0.1% AL-6221 and graded for skin response after 3 days. For topical irritation screen animals were treated for 24 hr at 8 sites each with occlusive patches containing 0.4 ml of the same concentrations and the sites were evaluated for skin response 24 and 48 hr later.

Phase 2. Induction: Intradermal injections: Main study animals were injected intradermally on Day 0 at 6 sites (3 on each side). Two sites received 0.1 ml of either AL-6221, vehicle or positive control;

two sites were treated with AL-6221, vehicle or positive control in a 50% (v/v) FCA emulsion (Freund's Complete Adjuvant); and two sites received injections of a 50% (v/v) FCA/water emulsion.

Phase 3. Induction: Topical application: The purpose of this phase was to continue the induction exposure initiated through intradermal injection by topical exposure route. On Day 7, the animals were exposed topically to 0.8 ml of respective test or control solutions applied using an occlusive patch. The patches were removed 48 hr later.

Phase 4. Primary challenge: Topical application: The purpose of this phase was to investigate the elicitation of response. On Day 21, all animals were topically challenged at a naive skin site with 0.4 ml of the respective test or control solution using occlusive patches. The patches were removed 24 hr later, and application sites were scored 24 and 48 hr after application.

Phase 5. Rechallenge: The purpose of this phase was to confirm the findings exhibited at the primary challenge phase. On Day 35, the animals of different groups were topically rechallenged with 0.4 ml of the respective test and control solutions at 3 naive sites using occlusive patches for 24 hr. Application sites were scored for erythema at 24 and 48 hr after application.

The test sites were graded using the following scale:
0 = no perceptible reaction or any occurrence of slight, ill-defined erythema
1 = slight but confluent, or moderate patchy erythema
2 = moderate erythema
3 = severe erythema with or without edema

Results:

During the irritation scerning phase, no treatment-related reactions were noted 3 days after intradermal injection or 24 and 48 hr after topical application of AL-6221. Based on these results, a concentration of 0.1% AL-6221 was chosen for the induction phase.

Following the primary challenge, the incidence of grade 2 responses in the AL-6221, vehicle control and positive control groups was 1 of 20 (5%), 0 of 10 and 1 of 10 (10%), respectively.

Following the rechallenge, the incidence of grade 2 responses in the AL-6221, naive control and positive control groups was 1 of 20 (5%), 0 of 10 and 4 of 10 (40%), respectively.

In conclusion, incidence of 5% responses at the primary challenge and rechallenge suggested a little or no potential for delayed hypersensitivity for AL-6221. The drug was not considered a sensitizer.

3.7 PACKAGING BIOLOGICAL EVALUATION STUDIES

The following study reports (Vols. 43-44) were submitted but not reviewed.

[redacted]

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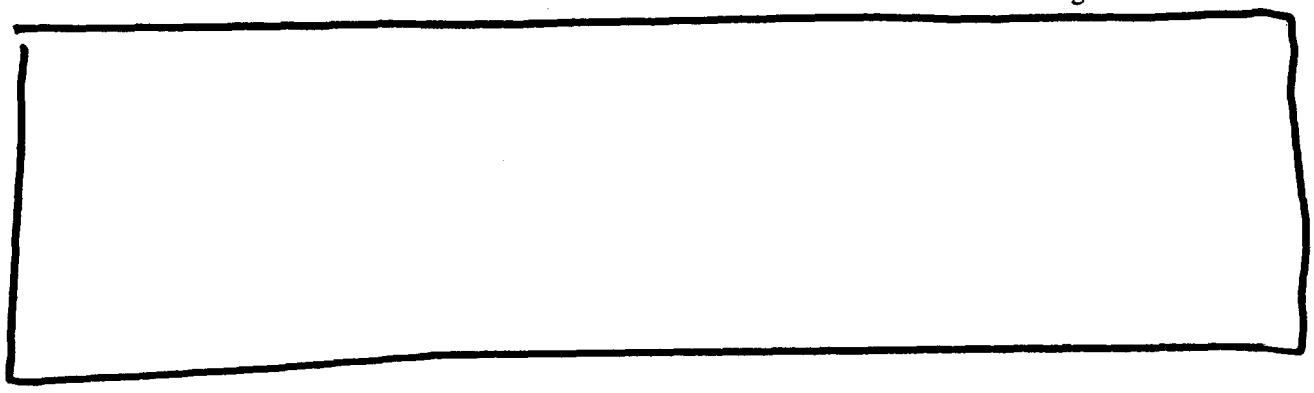

4 Labeling Review

Original version:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Travoprost was not mutagenic in bacteria, in one mouse lymphoma assay, in the mouse micronucleus tests nor in the rat chromosome aberration assay. In another mouse lymphoma assay, higher concentrations of travoprost were slightly mutagenic only in the presence of activation enzymes.

In-life and early post-mortem evaluations of carcinogenicity studies in rats and mice suggest no evidence of a carcinogenic potential.

Travoprost did not affect mating or fertility indices in male or female rats and mice at subcutaneous doses up to 10 ug/kg/day (250 times the recommended human dose). The mean number of corpora lutea was slightly reduced at that dose, but was not affected at 3 ug/kg/day (75 times the maximum recommended human dose).

Pregnancy: Teratogenic Effects

Pregnancy Category: C

In reproduction studies conducted in pregnant rats and mice, travoprost reduced fetal viability when administered during gestation at doses as low as 1.0 ug/kg/day (25 times the maximum recommended human dose) with the lowest no effect level at 0.3 ug/kg/day (7.5 times the maximum recommended human dose). The incidence of skeletal malformations was increased in fetuses of rat dams receiving travoprost by subcutaneous injection at 10 ug/kg/day (250 times the maximum recommended human dose), but not at 3 ug/kg/day (75 times the maximum recommended human dose). No fetal abnormalities were observed in mice at 1.0 ug/kg/day (25 times the maximum recommended human dose). No adequate and well-controlled studies have been performed in pregnant women. TRAVATAN™ may interfere with the maintenance of pregnancy and should not be used by women during pregnancy or by women attempting to become pregnant.

Nursing Mothers

A study in lactating rats demonstrated that radiolabeled travoprost and/or its metabolites were excreted in milk. It is not known whether this drug or its metabolites are excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when TRAVATAN™ is administered to a nursing woman.

Revised version:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Travoprost was not mutagenic in Ames test, mouse micronucleus test (at the doses up to 100 mg/kg, or 300 mg/m^2, po) and rat chromosome aberration assay (at the doses up to 75 mg/kg, or 450 mg/m^2, iv). However, a slight increase in the mutant frequency was observed in one of two mouse lymphoma assays in the presence of rat S-9 activation enzymes.

Travoprost did not affect mating or fertility indices in male or female rats at subcutaneous doses up to 10 µg/kg/day [250 times the maximum recommended human ocular dose of 0.04 µg/kg/day on a µg/kg basis for a 50 kg adult (MRHOD)]. At 10 µg/kg/day, the mean number of corpora lutea was reduced, and the post-implantation losses were increased. These effects were not observed at 3 µg/kg/day (75 times the MRHOD).

Pregnancy: Teratogenic Effects

Pregnancy Category C

Travoprost was not teratogenic in mice at subcutaneous doses up to 1.0 µg/kg/day (25 times the MRHOD). Travoprost was not teratogenic in rats at iv doses up to 3 µg/kg/day (75 times the MRHOD); however, at an iv dose of 10 µg/kg/day (250 times the MRHOD), increases in the incidence of skeletal malformations as well as external and visceral malformations, such as fused sternebrae, domed head and hydrocephaly, were observed. Travoprost produced an increase in post-implantation losses and a decrease in fetal viability in rats at iv doses > 3 µg/kg/day (75 times the MRHOD) and in mice at subcutaneous doses > 0.3 µg/kg/day (7.5 times the MRHOD).

In the offspring of female rats that received travoprost subcutaneously from Day 7 of pregnancy to lactation Day 21 at the doses ≥ 0.12 µg/kg/day (3 times the MRHOD), the incidence of postnatal mortality was increased, and neonatal body weight gain was decreased. Neonatal development was also affected evidenced by delayed eye opening, pinna detachment and preputial separation, and by decreased motor activity.

No adequate and well-controlled studies have been performed in pregnant women. TRAVATAN™ may interfere with the maintenance of pregnancy and should not be used by women during pregnancy or by women attempting to become pregnant.

Nursing Mothers

A study in lactating rats demonstrated that radiolabeled travoprost and/or its metabolites were excreted in milk. It is not known whether this drug or its metabolites are excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when TRAVATAN™ is administered to a nursing woman.

5 SUMMARY

5.1 PHARMACOLOGY

- Travoprost is a highly selective FP prostanoid receptor agonist.
- Travoprost solutions reduce IOP in ocular hypertensive lasered cynomolgus monkeys after topical ocular instillation of single or multiple doses.
- Travoprost produces a dose-related reduction of IOP in lasered eyes of monkeys, with an apparent maximal response produced by 0.3 to 1.0 μg doses.
- Once daily dosing with travoprost lowers IOP to a similar extent as BID dosing. The duration of response to once daily dosing with travoprost is expected to provide adequate IOP control for clinical anti-glaucoma therapy.
- After topical ocular instillation of multiple doses, travoprost increases optic nerve head blood flow with no significant changes in systemic blood pressure or arterial blood gases or pH.
- Conjunctival hyperemia may be produced by topical ocular administration of travoprost; however, if hyperemia is observed, it is expected to be relatively mild.
- No meaningful responses were noted in the systemic pharmacology studies at doses less than 250-fold excess above the therapeutic dose (2 µg/day), suggesting that systemic untoward effects with the proposed clinical doses are unlikely to occur.

5.2 ADME

Following topical ocular administration of ^{3}H-AL-6221 in rabbits, the drug was absorbed into the eye with high concentrations noted in the anterior tissues (iris-ciliary body and aqueous humor; Tmax: 1-2 hr). Low concentrations were found in the posterior tissues. In most ocular tissues, the drug was rapidly eliminated with half-life of 0.4-2.6 hr. In both rabbit and monkey studies, systemic exposure to the drug was very low after topical ocular administrations.

Following subcutaneous administration to rats, travoprost was well absorbed with Cmax and AUC increasing in a dose-dependent manner. In distribution assays with ^{3}H-AL-6221, the highest concentrations of radioactivity were observed in kidneys, liver, lungs and plasma. Very low concentrations were found in brain, fat and muscle. In studies conducted in pregnant rats or lactating rats with ^{3}H-AL-6221, low levels of radioactivity were found in amniotic fluid and fetal tissues (2-4% of maternal plasma level), and in the milk of lactating rats.

The extent of binding of the drug to rat, monkey and human plasma proteins was similar at approximately 80%. Over a 1000-fold concentration range of [redacted] ng/ml, the percent of bound drug for these species was independent of drug concentration.

Systemically, travoprost free acid was rapidly and extensively oxidized to inactive metabolites. Biotransformations included β-oxidation of the α(carboxylic acid) chain to give the 1,2-dinor and 1,2,3,4-tetranor analogs, oxidation of the 15-hydroxyl moiety, as well as reduction of the 13,14 double bond. The metabolites identified included 1;2,3,4-tetranor-13,14-dihydro-15-

oxo-AL-5848, 1,2-dinor-13,14-dihydro-15-oxo-AL-5848, 1,2,3,4-tetranor-15-oxo-AL-5848 and 1,2-dinor-15-oxo-AL-5848.

In rats, rabbits and dogs following intravenous administration of ^{3}H-AL-6221, plasma concentrations of radioactivity decreased in a biphasic manner with half-lives for radioactivity elimination of 35, 48 and 26 hr for rats, rabbits and dogs, respectively. In rats, 95% of a subcutaneous radiolabeled dose was eliminated within 24 hours. The major route of elimination was via the bile (61%).

5.3 TOXICOLOGY

5.3.1 TOPICAL OCULAR STUDIES

Repeat dose topical ocular toxicity studies with AL-6221 were conducted in rabbits and monkeys. In rabbit studies with the duration ranging from 4 weeks to 6 months and drug concentrations of 0.001% to 0.01% (bid or tid for both eyes), no significant toxicity was observed. In a monkey study, animals were treated with AL-6221 in the clinical formulations (concentrations ranging from 0.0015% to 0.012%) for 1 year (bid, one eye). No significant systemic toxicity was noted. However, an increase in palpebral fissure, noted clinically as "big eye", and iris pigmentation was noted in treated groups. These ocular findings were consistent with those reported for latanoprost in monkeys.

5.3.2 SINGLE DOSE ACUTE TOXICITY STUDY

Species/strain	N/sex/group	Dose level	Regimen	Duration	Findings
Rat/Sprague Dawley	5	10 mg/kg	Single dose, iv	14-day follow up	The drug was well tolerated. No toxicity was observed in clinical observations, body weight measurement and necropsy examination.

5.3.3 REPEAT DOSE TOXICITY STUDIES

Species/strain	N/sex/group	Dose level	Regimen	Duration	Findings
Mice/CD-1(ICR)BR	10	0, 0.1, 0.3 and 1.0 mg/kg	qd, iv	28 days	1♂ died on day 24. The reason was unknown. In the other animals, no toxicity was observed in any of the evaluations performed.
Rat/ Crl:CD®BR VAF/Plus	10	0, 0.1, 0.3 and 1.0 mg/kg	qd, iv	28 days	2♂ at 0.1 mg/kg, 3♂ and 1♀ at 0.3 mg/kg, and 2♂ at 1 mg/kg were found dead during the study. The reason was unknown. In the other animals, no toxicity was observed in any of the evaluations performed.
Rat/ Crl:CD®BR VAF/Plus	15	0, 0.1, 0.3 and 1.0 mg/kg	qd, iv	13 weeks	7♂, 2♀ at 0.1 mg/kg, 5♂, 4♀ at 0.3 mg/kg, and 3♂, 1♀ at 1 mg/kg were found dead during the study. The reason was unknown. In all treated groups: slight ↓ in RBC parameter, microscopical bone changes (endosteal fibrosis and hyperostosis)
Mice/CD-1(ICR)BR	15	0, 0.1, 0.3 and 1.0 mg/kg	qd, iv for 1 month and ip for 2 months	13 weeks	3♂ at 0.1 mg/kg and 2♂ at 1 mg/kg were found dead during the study. The reason was unknown., 1♀ was moribund on day 34 with abscess in the thymus. In the other animals, no toxicity was observed in any of the evaluations performed.
Rat/ Crl:CD®BR VAF/Plus	25	0, 10, 30 and 100 µg/kg	qd, sc	6 months	1♀ at 10 µg/kg and 5♂ at 100 µg/kg were found dead during the study. The deaths at high dose were considered treatment-related. ♂ at 100 µg/kg: ↓body weight gain (10%). ♀ at 30 and 100 µg/kg: slight ↓ in RBC parameter. Both ♂and ♀ at 30 and 100 µg/kg: microscopical bone changes (endosteal fibrosis and hyperostosis). NOAEL = 10 µg/kg

5.3.4 REPRODUCTIVE TOXICOLOGY

Species/strain	N/sex/group	Dose level	Regimen	Duration	Findings
Rat/Sprague Dawley	26	0, 1, 3 and 10 µg/kg/day	sc, qd	♂: 4 wks prior to and through gestation; ♀: 2 wks prior to mating through gestation	No effects on fertility in rats. 10 µg/kg: ↑ early resorptions. NOAEL = 3 µg/kg
Rat/Sprague Dawley	26♀	0, 1, 3 and 10 µg/kg/day	iv, qd	Gestation Days 6-17	10 µg/kg: dams: ↓body weight gain and food consumption, ↓number of viable fetuses/dam, ↑ post-implantation loss. Fetuses: ↓body weights, ↑incidence of malformations and variation NOAEL = 3 µg/kg for dams and fetuses
Mice/CD-1(ICR)BR	30♀	0, 0.1, 0.3 and 1.0 µg/kg	qd, sc	Gestation Days 6-16	1.0 µg/kg: dams: ↑early deliveries, abortions and total litter resorptions, ↓number of viable fetuses/dam, ↑ post-implantation loss. Fetuses: No teratogenic effects NOAEL = 0.3 µg/kg for dams and fetuses
Rat/Sprague Dawley	26♀	0, 0.12, 0.36 and 0.72 µg/kg/day	sc, qd	Gestation Day 7-lactation Day 21	F_0: All doses: ↓food consumption during gestation, ↓gestation length, ↑litters with stillborn pups. 0.72 µg/kg: prolapsed uterus (1 dam), ↑abortions, ↓litters with viable fetuses, ↓number of pups/litter F_1: All doses: ↓ pup survival during Days 1-4 of lactation, ↓body weights, ↓physical development and motor activity F_2: not affected
Rat/Sprague Dawley	26♀	0, 0.01, 0.03 and 0.1 µg/kg/day	sc, qd	Gestation Day 7-lactation Day 21	No significant toxicity effects on maternal parameters and pup development. ↓implantation sites and number of pups/litter at 0.01 and 0.1 µg/kg, which might not be drug-related. NOAEL = 0.1 µg/kg for both dams and pups.

4.3.5. Genetic Toxicology

Al-6221 was negative in Ames test, in in vivo micronucleus cytogenetic assay in mice, and in an in vivo chromosomal aberration assay in rats. Two in vitro mouse lymphoma TK assays were performed. One was negative. The other assay showed equivocal effects with the activation of S9.

6 Evaluation

Travoprost (AL-6221) is a highly selective and potent agonist at the FP prostanoid receptor. Pharmacological studies indicated that travoprost was effective on reducing intraocular pressure in animal models. Travoprost is being developed by the sponsor for the reduction of IOP in patients with open angel glaucoma or ocular hypertension. The recommended dose is one drop (25 µl) per eye, once daily, used as either primary or adjunctive therapy [Total dose could be 2 µg/patient/day or 0.04 µg/kg (1.48 µg/m^2) for a 50 kg adult].

Ocular and systemic toxicity studies were conducted with durations up to 6 months. Topical ocular administration of travoprost in monkeys resulted in an increase in palpebral fissure, noted clinically as "big eye", and iris pigmentation. These ocular findings were consistent with those reported for latanoprost in monkeys. In a 6-month rat study with repeat sc administration, treatment-related mortalities were noted at 100 µg/kg (600 µg/m^2). Microscopic bone changes (endosteal fibrosis and hyperostosis) were noted at the doses > 30 µg/kg (180 µg/m^2). These doses were at least 121-fold the possibly highest human daily dose (1.48 µg/m^2). Therefore, travoprost was generally considered as safe and well tolerated.

This NDA application is approvable from a nonclinical perspective with some modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility section and Pregnancy: Pregnancy Category C section.

7 Conclusion and Recommendation

This NDA application is approvable from a nonclinical perspective with some modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility section and Pregnancy: Pregnancy Category C section.

/S/

10/31/00

Zhou Chen, Ph.D.

Concur by team leader: Yes ☒ No ☐

/S/ 10/31/00

Robert Osterberg, Ph.D.

cc:
NDA 21-257
HFD-550/Division File
/PT Teamleader/Osterberg
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/MO/Lim
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HFD-345

APPEARS THIS WAY
ON ORIGINAL