CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-756

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

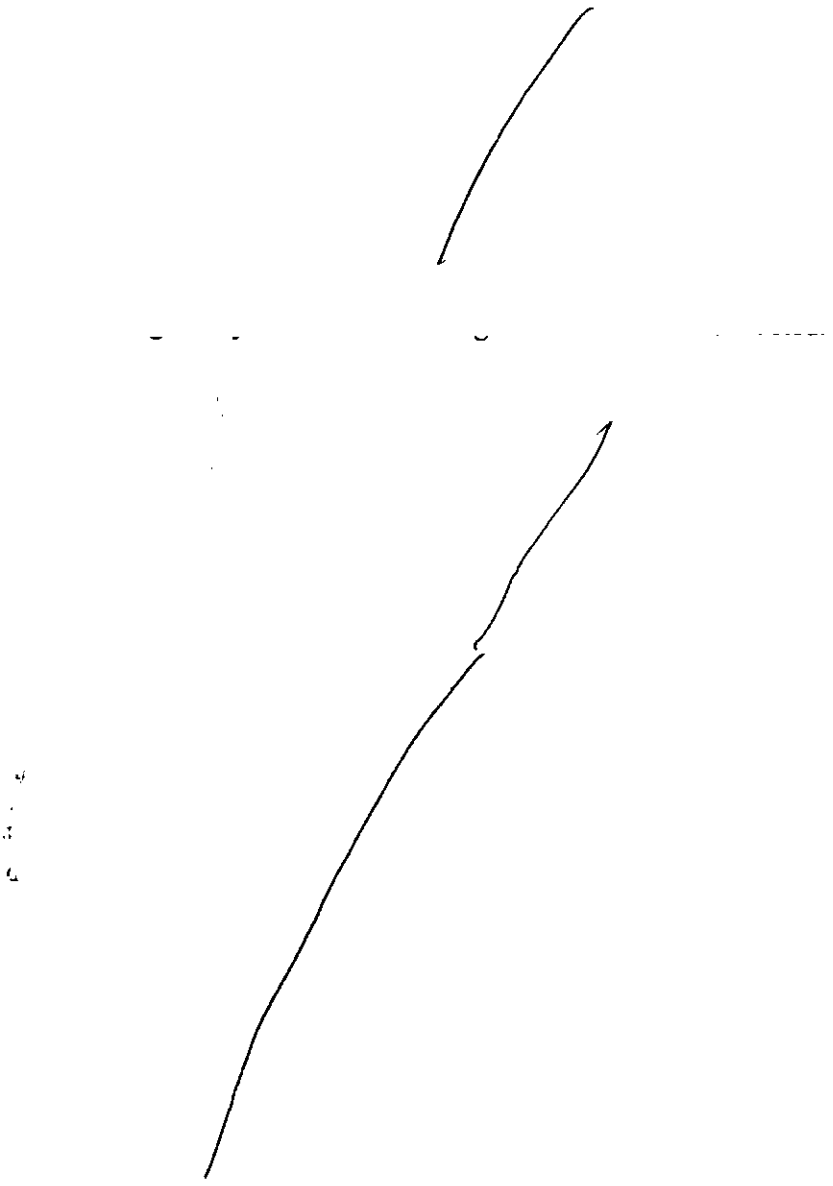
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-756**
SERIAL NUMBER:	**003**
DATE RECEIVED BY CENTER:	**3/17/04**
DRUG NAME:	**Macugen**
INDICATION:	**Exudative age-related macular degeneration**
SPONSOR:	**Eyetech Pharmaceuticals, Inc., Three Times Square, 12th Floor, New York, NY 10036** **Tel: 212-997-9241; Fax: 212-997-9251**
DOCUMENTS REVIEWED:	**Revised labeling provided by the sponsor on October 15, 2004**
REVIEW DIVISION:	**Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550)**
PHARM/TOX REVIEWER:	**Zhou Chen**
PHARM/TOX SUPERVISOR:	**Josie Yang**
DIVISION DIRECTOR:	**Brian Harvey**
PROJECT MANAGER:	**Mike Puglisi**

Date of review submission to Division File System (DFS): November 5, 2004

On October 15, 2004, the sponsor provided a new version of draft labeling. Previously suggested labeling changes as stated in the original NDA pharmacology/toxicology review (Review Number 000/002) were not conveyed. In this NDA review, pharmacology/toxicology-related modifications in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section are recommended based on the sponsor's new draft labeling.

Original version submitted on October 15, 2004:



2 Draft Labeling Page(s) Withheld

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/s/

Zhou Chen
11/5/04 09:33:44 AM
PHARMACOLOGIST

Josie Yang
11/5/04 01:21:47 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-756**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**3/17/04**
DRUG NAME:	**Macugen**
INDICATION:	**Exudative age-related macular degeneration**
SPONSOR:	**Eyetech Pharmaceuticals, Inc., Three Times Square, 12th Floor, New York, NY 10036** **Tel: 212-997-9241; Fax: 212-997-9251**
DOCUMENTS REVIEWED:	**Module 4**
REVIEW DIVISION:	**Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550)**
PHARM/TOX REVIEWER:	**Zhou Chen**
PHARM/TOX SUPERVISOR:	**Josie Yang**
DIVISION DIRECTOR:	**Brian Harvey**
PROJECT MANAGER:	**Mike Puglisi**

Date of review submission to Division File System (DFS): September 8, 2004

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 21-756
Review number: 001
Sequence number/date/type of submission: 000/March 17, 2004/Commercial
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Eyetech Pharmaceuticals, Inc., Three Times Square, 12th Floor, New York, NY 10036
Manufacturer for drug substance: —

—

Reviewer name: Zhou Chen
Division name: Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
HFD #: 550
Review completion date: September 8, 2004

Drug:

Trade name: **Macugen**
Generic name: Pegaptanib sodium
Code name: NX11838, NX1838, and EYE001
CAS registry number: 222716-86-1
Molecular formula/molecular weight: $C_{294}H_{342}F_{13}N_{107}Na_{28}O_{188}P_{28}[C_2H_4O]_n$ (where n is approximately 900), MW: approximately 50 kilodaltons.

Relevant INDs/NDAs/DMFs: IND 56,503

Drug class: VEGF antagonist, Aptamer, a pegylated synthetic RNA oligonucleotide composed of 28 nucleotide bases

Indication: Exudative (wet) age-related macular degeneration

Route of administration: Intravitreous injection

Proposed use: 0.3 mg/eye, once every 6 weeks

This review contains responses to the comments on the original NDA review for NDA 21-756 (Macugen) from Dr. Abigail Jacobs (Associate Director for Pharmacology/Toxicology, ODE IV and V) on 8-20-2004.

Overall: I recognize that this was a rather complicated review and there is more than one way to view the issues. The multiple of the human exposure should be revised. It should probably be noted that the animals in the embryofetal studies could have tolerated higher doses. The pharmacologic activity suggests that potential developments tox is biologically plausible at sufficiently high exposure, and animals may be less sensitive than humans.

p.87- embryofetal development. Studies don't seem to have achieved maternal toxicity.

The labeling: teratogenic effects: I think the multiple of the human dose is inappropriate and should be based on nominal doses converted to body surface comparison, assuming 100% bioavailability. The resulting multiple will be much lower. I think that based on its pharmacologic activity and systemic bioavailability, macuagen probably should not be used in pregnant women. Thus, although the developmental studies in animals did not demonstrate a risk, these studies could have been conducted at higher doses (no maternal toxicity was seen) and the pharmacologic activity suggests a potential risk. To warrant a category B shouldn't the studies go up to a maternally toxic dose and not have a pharmacologic concern? The binding constant for VEGF is rather low.

Response: During the PreNDA meeting on 1/25/2001, the sponsor —

r ← The sponsor was told that reproductive toxicity studies were not necessary for the AMD indication —

—

. In an email dated December 20, 2001 regarding the segment II toxicity studies for EYE001 (IND 56,503), the sponsor asked if the mouse was an acceptable test species, and if the segment II study in a second species could be waived. The sponsor was informed that mouse was an acceptable species for segment II study for this IND, and the segment II study in second species could be waived if the results from mouse study showed negative results —

/

In this NDA submission, the sponsor included a segment II study in mice. The reviewer agrees that higher doses should have been used in the embryofetal study. An MTD was not reached in this study. This will be mentioned in the labeling recommendation (see amended review). The pharmacologic activity suggests a potential risk and does not appear to benefit embryo/fetal development. However, the high dose used in mice (40 mg/kg or 120 mg/m^2) was 650 time human dose (0.3 mg/eye, 0.005 mg/kg or 0.185 mg/m^2) using surface area. The safety margin is relatively great. At the dose of 40 mg/kg/day, mean pegaptanib plasma concentrations at 5 min post injection on gestation day 15 were about 2000 µg/ml and the mean AUC_{tau} was approximately 8000 µg.hr/ml. In humans, at the maximum dose administered of 3 mg/study eye given every 4 weeks, mean C_{max} values were about 90 ng/ml (or 0.09 µg/ml) and AUC_{tau} values were approximately 25 µg.hr/ml. Thus, pegaptanib maximum plasma concentrations and AUC values after daily IV dosing in pregnant CD-1 mice were greater than 20000-fold and 300-fold those seen in humans receiving intravitreous injections of 3 mg/eye, respectively. Considering the proposed clinical dose is only 0.3 mg/eye and dosing interval is 6 weeks, a higher safety margin is assured.

Macugen was $VEGF_{165}$ ($VEGF_{164}$ in rodents) specific. Macugen binds to $VEGF_{165}$ with high affinity and specificity, thereby inhibiting $VEGF_{165}$ binding to its VEGF receptors. In humans, there are several VEGF isoforms. It is reported (Susumu Ishida, et. al., *J. Exp. Med.*, 198, 483–489, 2003) that when pegaptanib sodium, a $VEGF_{164}$-specific neutralizing aptamer, was administered, it suppressed the leukocyte adhesion and pathological neovascularization, whereas it had little or no effect on physiological neovascularization. In parallel experiments, [redacted] $VEGF_{164}$-deficient ($VEGF_{120/188}$) mice exhibited no difference in

physiological neovascularization when compared with wild-type controls. VEGF isoforms other than $VEGF_{164}$, in combination, may be sufficient to promote normal physiological neovascularization.

Considering the great safety margin in the mouse study, multiple publications showing that genetically altered $VEGF_{164}$ deficient mice had normal vasculature, and indication of the drug, the reviewer believes that it is acceptable to maintain the "Teratogenic Effects" in "Pregnancy Category" as a B.

I didn't follow the basis of waiving the carcinogenicity studies? p. 86 and elsewhere. The systemic exposure is substantial, effects would be expected to be cumulative, even though exposure is only once every 6 weeks; SHE cell negatives don't usually support waiving of carcinogenicity, although they do allow clinical trials to continue. Genetox results don't usually correlate with carc results for nucleotide/nucleoside analogs and often genetox results are due to secondary mechanisms and perturbation of the nucleotide/nucleoside pools. Nucleoside analogs have given positive carcinogenicity results and results in chronic studies are not predictive of carcinogenicity. Since the waiver was granted, nothing can be done at this time.

Response: [redacted] has been proposed as a useful model to assess the carcinogenicity potential of diverse chemicals. In a presentation given at PharmTox Retreat in fall 2002 by Anita Bigger and Jui Shah, it was indicated that SHE cell assay had the lowest false positive and false negative rate. SHE cell assay detected both genotoxic and non-genotoxic carcinogens. The FDA analysis of the validation data provided by GSK demonstrated that SHE cell assay exhibited a high concordance with the rodent bioassay and therefore, should be a useful short-term *in vitro* tool for predicting rodent carcinogenicity.

p.31 What is meant by "appeared to be" nonmutagenic in the Ames test? Later you say that it wasn't mutagenic in that assay.

Response: The reviewer has changed "appeared to be" to "was" (see amended review).

p. 7 says the drug will be given every 6 weeks but p. 24 says the drug will be given every 4 weeks?

Response: It should be every 6 weeks. In some clinical trials, the sponsor used 4-week intervals.

p. 25 PK measurements and interpretation are not straightforward because of complicated metabolism and breakdown (oligonucleotides smaller than the parent drug could have activity and incorporation of breakdown products and metabolism into endogenous material); nominal doses for humans are 120 ug/kg (assuming >95% systemic bioavailability) and nominal doses for mice in the iv repro study are 3.5 mg/kg. The relevant multiple of the human exposure could be much lower than the values given in the review. Furthermore humans have been found to be more sensitive to angiogenesis inhibition than mice, rats, or rabbits. The potential effects on development (inhibition of angiogenesis) might not correlate with Cmax or AUC but on time above a threshold and relative effects also depend on intracellular conversion to the triphosphate.

Response: The nominal dose for humans in this clinical trial (3 mg/eye) was 60 μg/kg and nominal dose for mice in the iv reproductive study was 40 mg/kg. The proposed clinical dose is 6 μg/kg (0.3 mg/eye) or 12 μg/kg for 2 eyes.

p.17 2.63 is blank ; it looks as if notes to reviewers were not deleted.
p. 32 2.6.10 and 2.65 have blank spaces; it looks as if notes to reviewers were not deleted.
p. 100 2.6.6.10 . has blank spaces; it looks as if notes to reviewers were not deleted.

Response: I will delete the notes (see amended review).

p. 62 The toxicity of FIAU in rats did not exactly mimic what was seen in humans; the effects described in the review are the human effects not the effects in rats In the rat studies the severity or spectrum of the human effects was not demonstrated.
Studies of 2-fluorouridine and 2-fluorocytidine:
a. As I recall rats were rather insensitive to FIAU relative to humans; the studies for this NDA were conducted by iv, so the liver did not receive as large a dose as in oral studies.
b. Studies were not conducted in parallel with FIAU to see if the effects of FIAU would have been detected in the animals under the current conditions. As I also vaguely recall, results in woodchucks depend on the time of year.
c. Perhaps the limitations of the studies should be mentioned (especially FIAU not being studied at the same time
p. 96. The key effect regarding serious consequences is not incorporation into DNA or RNA but whether the chain is terminated or the incorporated nucleotide resembles thymidine, such that the chain continues growing. If the chain is not terminated, there could be adverse consequences at a time later than that measured in the woodchuck tox studies

2'-Fluoropyridimes have structural similarities and differences to FIAU. All three have a fluorine substitution at the 2'-position of the sugar. FIAU contains an arabinose sugar with 2'-OH substituted with a fluorine. The 2'-Fluoropyridimes contain a ribose sugar with the 2'-OH substituted with fluorine. FIAU contains a uracil base with an iodine at the 5-position, while the 2'-Fluoropyridimes contain either uracil or cytosine with no substitution at the 5 position. Whether these structural differences between FIAU and 2'-Fluoropyrimidines can prevent 2'-Fluoropyrimidines from producing the long-term toxic effects caused by FIAU is not known.

Nonclinical studies demonstrated that monomer nucleotide 2'-Fluorouridine was incorporated into cellular DNA and RNA of all tissues examined following subchronic (3 months) iv administration. Based on the structure of 2'-Fluorouridine (OH in 3' position), the chain will not be terminated as in AZT but continue to grow. Regarding mitochondrial toxicity, no differences in mtDNA content of heart, spleen, liver, testes, and kidney were observed between treatment groups and the control group in rats and woodchucks after 3-month treatment. It is proposed that the toxicity of FIAU in woodchuck was similar to that in humans. Although there was no direct evidence if 2'-Fluorouridine inhibited mitochondrial DNA polymerase γ (as the case of FIAU) , and these two 3-month studies were conducted without inclusion of FIAU, clinical studies with Macugen for 2 years showed no apparent systemic issues and no delayed toxicity. The toxicity similar to FIAU does not seem to be a concern for the treatment with Macugen.

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/s/

Zhou Chen
9/9/04 10:28:54 AM
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Josie Yang
9/9/04 10:35:42 AM
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-756**
SERIAL NUMBER:	**000/002**
DATE RECEIVED BY CENTER:	**3/17/04**
DRUG NAME:	**Macugen**
INDICATION:	**Exudative age-related macular degeneration**
SPONSOR:	**Eyetech Pharmaceuticals, Inc., Three Times Square, 12th Floor, New York, NY 10036** **Tel: 212-997-9241; Fax: 212-997-9251**
DOCUMENTS REVIEWED:	**Module 4**
REVIEW DIVISION:	**Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550)**
PHARM/TOX REVIEWER:	**Zhou Chen**
PHARM/TOX SUPERVISOR:	**Josie Yang**
DIVISION DIRECTOR:	**Brian Harvey**
PROJECT MANAGER:	**Mike Puglisi**

Date of review submission to Division File System (DFS): September 9, 2004

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

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

B. Recommendation for nonclinical studies

No recommendation is necessary.

C. Recommendations on labeling

Minor modifications of labeling in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section (see Labeling Review) are recommended.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Macugen, a pegylated modified oligonucleotide, is a selective vascular endothelial growth factor (VEGF) antagonist. VEGF plays a very important role in the progression of the exudative form of age-related macular degeneration. Macugen binds to the major pathological VEGF isoform, extracellular $VEGF_{165}$, with high affinity and specificity, thereby inhibiting $VEGF_{165}$ binding to its VEGF receptors.

PK studies were conducted in rabbits, monkeys and dogs in which NX1838 was administered intravitreously. Vitreous drug levels remained high for a long period of time, and decreased very slowly with a $T_{1/2}$ value approximate 100 hr for each species. The systemic bioavailability in monkeys was high (>95%) following intravitreous injection. Following intravitreal administration to rabbits with ^{14}C-pegaptanib, high radioactivity was found in vitreous fluid and retina. Following both intravitreal and intravenous administrations of ^{14}C-pegaptanib to rabbits, highest concentrations of radioactivity were obtained in the kidney, spleen, bone marrow, lymph node and liver. Studies in mice, rabbits, rats and dogs showed that the volume of distribution was low and close to the plasma volume, suggesting that the drug was distributed primarily into plasma volume and was not extensively distributed to peripheral tissues after intravenous administration. Pegaptanib sodium was degraded by nucleases into nucleotides in all species. In a rabbit study, 2'-fluorouridine was found in both plasma and urine. PEG conjugation was proved to be important in increasing plasma drug residence time by reducing plasma drug clearance rate, possibly through decreasing renal filtration and by increasing the resistance to nucleases. Following intravenous or intravitreal administration in rabbits, the predominant route of excretion was via urine.

Several repeated dose ocular toxicity studies were conducted with duration up to 3 months in monkeys, 6 months in rabbits, and 9 months in dogs. No drug-induced systemic and ocular toxicity were observed. However, injection-procedure-induced ocular lesions were seen in all studies in both control and

treated animals. These alterations included clinical signs (conjunctival injection, bruising, swelling, conjunctival/scleral hemorrhage, squinting, ocular discharge, tearing, and corneal/lens opacity), ophthalmology examination findings (tapetal scars - in dogs only because tapetum is not present in the rhesus monkey and the rabbit, subcapsular cataracts, focal retinal hemorrhages and partial retinal detachments), and cellular infiltration, fibrosis or lens changes in histopathological examinations. All these changes were reversible. The sponsor indicated that the most frequently reported adverse events in clinical studies were ocular, transient and for the most part related to the injection procedure. These events occurred in ≥ 10% of patients who received 0.3 mg Macugen. Based on nonclinical study results, the NOAEL levels were determined as 0.5 mg/eye for monkeys, 2.0 mg/eye for rabbits, and 3.0 mg/eye for dogs.

In a 3-month systemic toxicity study in SD rats, chronic progressive nephropathy and lymphoid depletion in the spleen were observed in all groups but with increased incidence and severity in MD and HD groups. The clinical significance of these findings was not known since the doses used in the study (6 and 60 mg/m^2) were much higher than that proposed for humans (0.4 mg/m^2).

The potential of 2'-fluorouridine and 2'-fluorocytidine, two degradation products of NX1383, to induce toxicity similar to that of FIAU was investigated by intravenous injection to male Fisher 344 rats at 5, 50 or 500 mg/kg/day, and to woodchucks at 0.75 or 7.5 mg/kg/day for 90 days. Neither compound showed evidence of toxicity similar to that induced by the antiviral drug, FIAU.

NX1838 was negative in *in vitro* and *in vivo* genotoxicity studies. Genetic toxicology studies were also conducted with monomer nucleotides. In chromosomal aberrations studies with human whole blood lymphocytes in the absence of S9 activation, results were negative for all 2'-fluoropyrimidines and 2'-O-methylpurines. In the Ames test, results for all nucleotides were negative in the *Salmonella* strains. In *E. coli*, results for the 2'-O-methylpurines were negative, while 2'-fluoropyrimidines yielded a marginal but reproducible positive response.

In an embryofetal development study in pregnant CD-1 mice, EYE001 at iv doses up to 40 mg/kg/day did not cause any maternal toxicity. However, at 40 mg/kg/day, fetal weights were significantly reduced for both male and female fetuses. A reduction in the average number of ossified forepaw phalanges was also noted. However, these changes were within the historical control ranges. In addition, no body weight differences were seen in naturally delivered pups. Therefore, these findings might not be toxicologically significant. No other Cesarean-sectioning or litter parameters were affected. No fetal gross external, soft tissue or skeletal malformations were caused by EYE001. It should be pointed out that an MTD in the dams was not reached in this study. The dams could have tolerated higher doses.

B. Pharmacologic activity

Pegaptanib, a synthetic oligonucleotide angiogenesis inhibitor, appeared to have very high affinity and specificity to human $VEGF_{165}$, a major pathological VEGF isoform. The aptamer showed a potent inhibition of VEGF binding to human VEGF receptors, KDR and Flt-1, expressed on porcine endothelial cells, and to HUVEC VEGF receptors. In HUVECs, VEGF-induced calcium flux and cell proliferation were inhibited by NX1838. In *in vivo* studies conducted in guinea pigs, Sprague-Dawley rats and mice, NX1838 was able to fully inhibit VEGF-induced vascular leakage, greatly reduce VEGF-induced angiogenesis, and inhibit the growth of human A673 tumor. In safety pharmacology studies, pegaptanib showed no neurologic, cardiovascular, and pulmonary effects at plasma pegaptanib concentrations up to 1080 ng/ml,

approximately 10-fold higher than mean plasma concentrations observed in human clinical studies after a monocular 3 mg/eye dose.

C. Nonclinical safety issues relevant to clinical use

There are no drug-related safety issues relevant to clinical use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

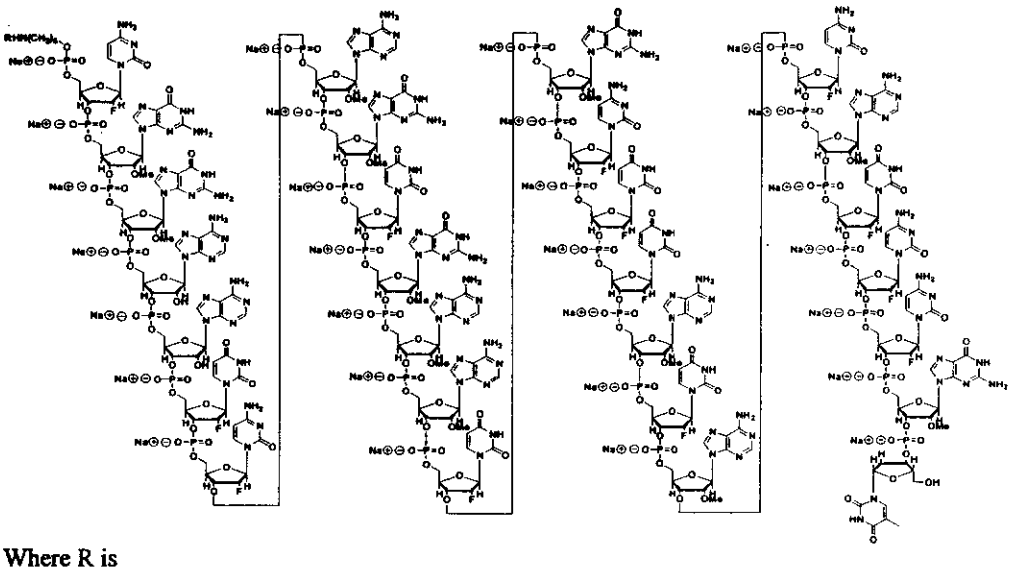
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 21-756
Review number: 002
Sequence number/date/type of submission: 000/March 17, 2004/Commercial
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Eyetech Pharmaceuticals, Inc., Three Times Square, 12th Floor, New York, NY 10036
Manufacturer for drug substance: [redacted]

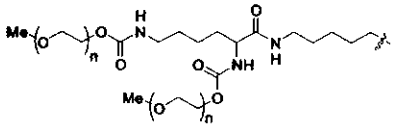
Reviewer name: Zhou Chen
Division name: Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
HFD #: 550
Review completion date: July 15, 2004

Drug:

Trade name: **Macugen**
Generic name: Pegaptanib sodium
Code name: NX11838, NX1838, and EYE001
Chemical name: RNA, ((2'-deoxy-2'-fluoro)C-Gm-GmA-A-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-Am-Gm-(2'-deoxy-2'-fluoro)U-Gm-Am-Am-(2'-deoxy-2'-fluoro)U-Gm-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)U-Am-(2'-deoxy-2'-fluoro)U-Am-(2'-deoxy-2'-fluoro)C-Am-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)C-Gm-(3'→3')-dT), 5'-ester with α,α'-[4,12-dioxo-6-[[[5 (phosphoonoxy)pentyl]amino]carbonyl]-3,13-dioxa-5,11-diaza-1,15 pentadecanediyl]bis[ω-methoxypoly(oxy-1,2-ethanediyl)], sodium salt
CAS registry number: 222716-86-1
Molecular formula/molecular weight: $C_{294}H_{342}F_{13}N_{107}Na_{28}O_{188}P_{28}[C_2H_4O]_n$ (where n is approximately 900), MW: approximately 50 kilodaltons.
Structure:



Where R is



and n is approximately 450.

Relevant INDs/NDAs/DMFs: IND 56,503, DMFs —

Drug class: VEGF antagonist, Aptamer, a pegylated synthetic RNA oligonucleotide composed of 28 nucleotide bases

Indication: Exudative (wet) age-related macular degeneration

Clinical formulation of Macugen

Component	Standard quantity per 100% (w/v)
Pegaptanib sodium	0.3
Sodium chloride, USP	—
Sodium phosphate monobasic, monohydrate, USP	—
Sodium phosphate dibasic, heptahydrate, USP	—
Sodium hydroxide, NF	pH
Hydrochloride acid, NF	pH
Water for injection, USP	

Route of administration: Intravitreous injection

Proposed use: 0.3 mg/eye, once every 6 weeks

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Pharmacology:

Primary pharmacodynamics

109-97001-R: 2'-Fluoropyrimidine RNA-based aptamers bind with picomolar affinities to $VEGF_{165}$ through the exon-7-encoded domain and inhibit its binding to both FLT-1 and KDR
RD-RPT-0001: IC_{50} of pegaptanib *in vitro*
RD-RPT-0002: The interaction of the anti-VEGF aptamers with VEGF isoforms (VEGF121, VEGF165, VEGF188) and family members (VEGF-B, VEGF-C, PDGF-BB, PlGF)
RD-RPT-0003: The mechanism of action of Macugen activity: inhibition of VEGF binding to VEGF-R1, VEGF-R2, and Neuropilin-1
109-97001-I: *In vitro* inhibition of VEGF receptor binding by VEGF aptamer NX1838
109-97002-I: Inhibition of VEGF-induced endothelial cell proliferation *in vitro* by VEGF aptamer NX1838
109-97003-I: Inhibition of VEGF-induced calcium mobilization *in vitro* by VEGF aptamer NX1838

Drug activity related to proposed indication

109-97001-P: *In vivo* inhibition of VEGF-induced dermal microvascular leakage in guinea pig by NX1838
109-97002-P: *In vivo* inhibition of VEGF-induced corneal angiogenesis in Sprague-Dawley rats by NX1838
109-97004-P: NX1838 inhibition of retinal angiogenesis induced by hyperoxia in neonatal C57BL/6 mice

Secondary pharmacodynamics

109-97003-P: *In vivo* anti-tumor efficacy of NX1838 in a human A673 rhabdomyosarcoma nude mouse xenograft model

Safety pharmacology

SP103-012: The assessment of the effects of pegaptanib on the central nervous system–gross behavior (Irwin Test) in rats
RR3167: Safety pharmacology-blood pressure, heart rate, and cardiac rhythm effects of pegaptanib in beagle dogs
SPR03-021: Assessment of the effects of pegaptanib on respiratory function in conscious Sprague Dawley rats

PK:

Absorption:

109-97001-B: Pharmacokinetics of intravitreously administered single dose NX1838 in New Zealand white rabbits
109-97002-B: Pharmacokinetics of intravitreously administered single dose NX1838 in Dutch-belted rabbits
0460LE15-001: A 6-month toxicity study in rabbits of EYE001 by intravitreous administration
0472DE15-001: A nine-month local tolerance and toxicity study of EYE001 given by intravitreous injection to beagle dogs
109-98005-B: Plasma pharmacokinetics of an anti-VEGF aptamer, NX1838, following subcutaneous administration in rhesus monkeys

109-98004-B: Plasma pharmacokinetics of an anti-VEGF aptamer, NX1838, following intravenous administration in rhesus monkeys
109-98006-B: Pharmacokinetics of an anti-VEGF aptamer, NX1838, following intravitreal administration in rhesus monkeys
109-980142-T: An acute intravitreous safety study (0699-35) with NX1838 in rhesus monkeys

Distribution:

109-97003-B: Dose dependent pharmacokinetics of intravenous administered single dose NX1838 in CD-1 mice
109-98001-B: Pharmacokinetics of intravenous administered single dose of NX1838 in Dutch-belted rabbits
1005-004: Intravenous developmental toxicity study of EYE001 in mice
109-97004-B: Pharmacokinetics of intravenous administered single dose of NX1838, NX22270 (20K PEG), NX22271 (no PEG) in Sprague-Dawley rats
3164: Toxicokinetic analysis for the acute intravenous toxicokinetic study of pegaptanib in dogs (PGRD Study # 3164)
45943: Pharmacokinetics, tissue distribution, excretion and metabolism of radioactivity in male Dutch Belted rabbits following a single intravitreal or intravenous injection of ^{14}C-pegaptanib sodium

Metabolism

400014: Metabolic stability of ^{14}C-pegaptanib sodium (EYE001) using endonuclease, 3'-exonuclease, 5'-exonuclease, ribonuclease and human, rabbit, dog and monkey plasma

Toxicology:

Single dose studies

109-97002-T: Twelve-day pilot intravitreous toxicity study associated with intravitreous NX1838 administration
109-97003-T: Single dose intravenous toxicity study of NX11838 in rats with a 30-day observation period
109-98006-B: Pharmacokinetics of an anti-VEGF aptamer, NX1838, following intravitreal administration in rhesus monkeys
109-98011-T: An acute toxicity study of NX1838 given by intravenous administration to rhesus monkeys
109-980142-T: An acute intravitreous safety study (0699-35) with NX1838 in rhesus monkeys

Repeated dose studies

109-98003-T: An 11-week intravitreous toxicity study of VEGF aptamer NX1838 [40K PEG] in Dutch-belted rabbits
109-98004-T: A 13-week intravenous toxicity study of NX11838 in rats
109-98010-T: A 3-month intravitreous toxicity study (0640-35) with NX1838 in rhesus monkeys
144-002: Three-week local tolerance and toxicity study of EYE001 administered by intravitreous administration to beagle dogs
0460LE15-001: A six-month toxicity study in rabbits of EYE001 by intravitreous administration

0472DE15-001: A nine-month local tolerance and toxicity study of EYE001 given by intravitreous injection to beagle dogs
109-97008-T: A 90-day toxicity study of 2'-fluorouridine and 2'-fluorocytidine –HCl in male F-344 rats with a 90-day reversibility
109-97009-T: A 90-day toxicity study of 2'-fluorouridine and 2'-fluorocytidine–HCl in woodchucks (Marmota Monox)

Genetic toxicology

109-98001-T: Mutagenicity test on NX11838 in the L5178Y $TK^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay
109-98002-T: Mutagenicity test with NX11838 in the *Salmonella–Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay
7167-117: *In vitro* transformation of Syrian hamster embryo (SHE) cells by 7-day exposure to pegaptanib sodium
0676-1521: *In vivo* for chemical induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells
109-97004-T: Mutagenicity test on 2'-fluoro-cytidine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation
109-97005-T: Mutagenicity test on 2'-fluoro-uridine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation
109-97006-T: Mutagenicity test with 2'-fluoro-cytidine hydrochloride in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay
109-97007-T: Mutagenicity test with 2'-fluoro-uridine in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay
109-98006-T: Mutagenicity test on 2'-O-methylguanosine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation
109-98007-T: Mutagenicity test on 2'-O-methyladenosine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation
109-98008-T: Mutagenicity test with 2'-O-methylguanosine in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay
109-98009-T: Mutagenicity test with 2'-O-methyladenosine in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay
7167-118: *In vitro* transformation of Syrian hamster embryo (SHE) cells by 7-day exposure to 2'-fluoro-2'-deoxy-uridine
7167-119: *In vitro* transformation of Syrian hamster embryo (SHE) cells by 7-day exposure to 2'-fluoro-cytidine

Reproductive and developmental toxicology

1005-003P: Intravitreous dosage-range developmental toxicity study of EYE001 in rabbits
1005-004: Intravenous developmental toxicity study of EYE001 in mice

Special toxicology

109-98013-T: Incorporation of 2'-FU into DNA of rats following long-term administration of NX1838

109-98014-T: DNA and RNA incorporation of 2'-Fluorouridine into rats and woodchucks following long-term administration
109-97001-T: Immunogenicity of NX1838 in an *in vitro* lymphocyte stimulation assay
109-98005-T: Immunogenicity of NX1838 in BALB/c mice, Sprague-Dawley rats, Dutch-belted rabbits

Studies not reviewed within this submission:

Pharmacokinetics

100008: Qualification of a method for the determination fluoro-2-deoxyuridine in tris [hydroxymethyl]aminomethane buffer; human, dog, monkey and rabbit plasma (heparin); plasma (EDTA), and rabbit urine by liquid chromatography-mass spectrometry (LC-MS —
BA0012-P-VP-VA: — HPLC protocol and validation for the determination of NX1838 in Sprague Dawley plasma
BA0013-VP-VA: — HPLC protocol and validation for the determination of NX1838 in rabbit plasma and vitreous
BA98001-P1-VP-VR-VRA-VRB: — HPLC protocol and validation for the determination of NX1838 in human plasma
BA98001-XVP1-XVR1A-XVR1B: — HPLC cross-validation protocol and validation for the determination of NX1838 in rhesus monkey plasma
BA98001-XVP2-XVR2A-XVR2B: — HPLC cross-validation protocol and validation for the determination of NX1838 in rhesus monkey vitreous
BA98002-P2-VP-VP2-VR2: — dual hybridization protocol and validation for the determination of NX1838 in human EDTA plasma
BA98006-P1: — enzyme-linked immunoassay for the detection of human or rhesus monkey antibodies against NX1838
ICD183-1: Eyetech dual hybridization protocol and validation for the determination of EYE001 in canine EDTA plasma
ICD183-2: Eyetech dual hybridization protocol and validation for the determination of EYE001 in rabbit plasma
ICD183-3: Eyetech dual hybridization protocol and validation for the determination of EYE001 in mouse EDTA plasma
ICD183-4: Eyetech dual hybridization protocol and validation for the determination of EYE001 in mouse amniotic fluid
ICD183-6: Eyetech dual hybridization protocol and validation for the determination of EYE001 in rabbit urine

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The human VEGF gene is composed of 8 exons and differential alternative splicing results in the synthesis of multiple VEGF isoforms of 121, 145, 165, 189, and 206 amino acids ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$, respectively). All VEGF isoforms share a common N-terminal domain of

115 residues that comprises of exons 1 to 5 covering the binding sites for the VEGF receptors (VEGFR-1 and -2).

Pegaptanib, a synthetic oligonucleotide angiogenesis inhibitor, has very high affinity and specificity to human $VEGF_{165}$, a major pathological VEGF isoform. The aptamer showed potent ability to inhibit binding of VEGF to the human VEGF receptors, kinase domain receptor (KDR; also known as VEGFR-2 or Flk-1) and fms-like tyrosine kinase (Flt-1; also known as VEGFR-1), expressed on porcine endothelial cells, and to HUVEC (human umbilical vein endothelial cell) VEGF receptors. In the HUVECs, VEGF-induced calcium flux and cell proliferation were inhibited by NX1838. In *in vivo* studies conducted in guinea pigs, Sprague-Dawley rats and mice, NX1838 was observed to be able to fully inhibit VEGF-induced vascular leakage, greatly reduce VEGF-induced angiogenesis, and inhibit the growth of human A673 tumor. In safety pharmacology studies, pegaptanib showed no neurologic, cardiovascular, and pulmonary effects.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

109-97001-R: 2'-Fluoropyrimidine RNA-based aptamers bind with picomolar affinities to $VEGF_{165}$ through the Exon-7-encoded domain and inhibit its binding to both FLT-1 and KDR

The purpose of this study was to isolate and characterize high affinity aptamers that antagonize VEGF activities. Aptamers are oligonucleotides isolated from enormous randomized libraries of RNA, DNA, or modified nucleic acids that bind with high affinity and specificity to various molecular targets. In this study, aptamers (2'-F-pyrimidine RNA oligonucleotide ligands) to human $VEGF_{165}$ were isolated through the methods of systemic evolution of ligands by exponential enrichment (SELEX) from 2'-F-pyrimidine RNA libraries. Representative aptamers (including NX1838) from three distinct sequence families were truncated to the minimal sequence capable of high affinity binding to VEGF (23-29 nucleotides). To improve the aptamer's stability against nucleases, replacement of 2'-O-methyl for 2'-OH was made at all ribopurine positions where the substitution was tolerated. The Kd values ranged from 49 to 130 pM (49 pM for NX1838), and Ca^{++} was required for high affinity binding to VEGF. These aptamers bound to human $VEGF_{165}$ and its mouse homologue $VEGF_{164}$ with high affinity, but the affinity to reduced $VEGF_{165}$, $VEGF_{121}$, and placental growth factor (PlGF) was very low. NX1838 could also bind to $VEGF_{165}$/PlGF heterodimer with a reduced affinity (Kd = 430 pM). All three aptamers can form a cross-link to $VEGF_{165}$ at residue Cys_{137} within the exon 7-encoded domain. In the VEGF receptor binding study, the aptamers inhibited the binding of $VEGF_{165}$ to 2 VEGF receptors (Flt-1 and KDR) expressed on porcine endothelial cells. NX1838 also showed a significant inhibitory effect on intradermal VEGF-induced vascular permeability *in vivo*.

RD-RPT-0002: The interaction of the anti-VEGF aptamers with VEGF isoforms (VEGF121, VEGF165, VEGF188) and family members (VEGF-B, VEGF-C, PDGF-BB, PlGF)

The purpose of this study was to assess the interaction of NX1839 with different VEGF isoforms and its associated family members. Purified VEGF isoforms were allowed to bind with the ^{32}P-labeled NX1838 in an effort to determine its binding specificity and affinity as well as its potential effectiveness at limiting VEGF/receptor activities. — assay and — assay, a method designed to detect protein interactions, were performed.

NX1838 showed high-affinity and specific binding for $VEGF_{165}$ with an apparent binding affinity (KD) of about 75 pM to 137 pM. Regarding associated family members and additional growth factors, the anti-VEGF aptamer exhibited no specific binding for any of the family members tested ($VEGF_{121}$, $VEGF-B_{167}$, VEGF-C, PDGF-BB and PlGF). This lack of binding suggested that the anti-VEGF aptamer binding to the VEGF molecule depend on the presence of exon 7. $VEGF_{164}$ and $VEGF_{188}$ did bind to ^{32}P-labeled anti-VEGF aptamer. The anti-VEGF aptamer binds to the major heparin-binding VEGF isoform ($VEGF_{165}$) with strong affinity and $VEGF_{188}$ with a lower but significant affinity. The sponsor indicated that the interaction with $VEGF_{188}$ might be due to lack of purity for the $VEGF_{188}$ protein used in the — analysis.

RD-RPT-0003: The mechanism of action of Macugen activity: inhibition of VEGF binding to VEGF-R1, VEGF-R2, and neuropilin-1

The purpose of this study was to determine the efficacy of pegaptanib (IC_{50}) in inhibiting the binding of $VEGF_{165}$ to three high-affinity cell surface receptors: KDR, Flt-1, and neuropilin-1 (Npn-1) *in vitro*. — of the different VEGF receptors were used in this — , assay to examine their specific interactions with VEGF. A set of five-fold dilutions of the pegaptanib ranging from 1 μM (or 2 μM) to 0.512 pM (or 1.024 pM) were each mixed with about 0.01 μCi of ^{125}I-$VEGF_{165}$ in binding buffer. All tubes were incubated at 37°C (for KDR and Flt-1) or at room temperature (for neuropilin-1) for 15 to 20 min. The radioactivity of each well was determined by scintillation counting.

The data showed that pegaptanib effectively inhibited $VEGF_{165}$ binding to its cellular receptors, VEGFR-1 (IC_{50}=0.47 nM), VEGFR-2 (IC_{50}=1.10 nM), and Npn-1 (IC_{50}=0.23 nM).

109-97001-I: *In vitro* inhibition of VEGF receptor binding by VEGF aptamer NX1838

The inhibitory effects of NX1838 on VEGF binding to human umbilical vein endothelial cell (HUVEC) VEGF receptors (including KDR and Flt-1 tyrosine-kinase receptors) were assessed in this *in vitro* study. HUVECs were cultured in 96-well plates. NX1838 (10^{-5} to 10^{3} nM), monoclonal antibody, irrelevant antibodies, and/or scrambled sequence aptamer controls were then titrated across plates as appropriate, followed by ^{125}I-VEGF addition (10 ng/ml) to culture plates. Results showed that NX1838 concentration-dependently inhibited the binding of VEGF to the endothelium with the IC_{50} ranging from 0.03 to 1.4 nM. In conclusion, NX1838 was an effective inhibitor of VEGF binding to HUVECs *in vitro*.

109-97003-I: Inhibition of VEGF-induced calcium mobilization *in vitro* by VEGF aptamer NX1838

VEGF binds to endothelial cells via KDR and Flt-1 tyrosine kinase receptors. Many events following the binding are associated with calcium mobilization. The purpose of this study was to determine whether NX1838 could inhibit VEGF-induced calcium mobilization *in vitro*. Ca^{2+} mobilization in human umbilical vein endothelial cells (HUVECs) was determined via a — HUVEC suspensions were labeled with Indo-1 and intracellular Ca^{2+} mobilization (both magnitude and duration) was assessed prior to and following addition of various treatments. The results demonstrated that the addition of VEGF (10 ng/ml) to HUVECs induced significant calcium mobilization, which could be attenuated in a concentration-dependent manner by VEGF monoclonal antibody (IC_{50} = 2.6 nM). The mobilization could also be inhibited by NX1838 ($10^{-0.5}$ to $10^{1.5}$ nM) in a dose-dependent fashion similar to VEGF monoclonal antibody (IC_{50} = 0.74-3.18 nM). In conclusion, NX1838 was an effective inhibitor of VEGF-induced calcium flux in endothelial cells.

RD-RPT-0001: IC_{50} of pegaptanib *in vitro*

This study was designed to assess the IC_{50} of pegaptanib in an *in vitro* cell based assay. $VEGF_{165}$-induced tissue factor expression was used to examine the inhibitory profile (IC_{50}) of pegaptanib. Low passage human umbilical vein endothelial cells (HUVECs) (< passage 3) were incubated with $VEGF_{165}$ (12.5 ng/ml) in the presence or absence of 50% normal human plasma (NHP) for one hr. Following treatment, RNA was isolated and tissue factor expression was evaluated by the [redacted] analysis. To determine an IC_{50} of pegaptanib, cells were treated as above but pegaptanib was added at varying concentrations (10^{-3} to 10^{3} nM). Results revealed an IC_{50} for pegaptanib inhibition of $VEGF_{165}$-induced tissue factor expression of 0.53-1.38 nM. The IC_{50} for inhibition of $VEGF_{165}$-induced tissue factor expression in the absence of 50% NHP (i.e., medium alone) was 0.39-0.64 nM. In conclusion, pegaptanib inhibited VEGF induce-tissue factor expression in HUVECs in a dose-dependent manner.

Drug activity related to proposed indication:

109-97002-I: Inhibition of VEGF-induced endothelial cell proliferation *in vitro* by VEGF aptamer NX1838

The purpose of this study was to determine whether VEGF-induced HUVEC proliferation could be inhibited by treatment with NX1838 or VEGF monoclonal antibody (MAb). [redacted] ^{3}H-thymidine-incorporation assays were used to assess VEGF-induced proliferation of HUVECs *in vitro*. Cells were exposed to VEGF (10 ng/ml), either alone, or in the presence of various concentrations of NX1838 (10^{-4} to 10^{3} nM) or VEGF MAb, for a 48 hr incubation in the presence of ^{3}H-thymidine. Cellular incorporation of ^{3}H-thymidine into DNA was determined by [redacted] counting. The results showed that VEGF stimulated HUVEC proliferation, the proliferation was attenuated by VEGF monoclonal antibody with an IC_{50} of 0.07-0.11 nM, and NX1838 also inhibited VEGF-induced HUVEC proliferation with an IC_{50} of 0.4-2.9 nM. In conclusion, NX1838 was an effective inhibitor of VEGF-induced endothelial cell proliferation.

109-97001-P: *In vivo* inhibition of VEGF-induced dermal microvascular leakage in guinea pig by NX1838

The purpose of this study was to evaluate effects of NX1838 on inhibiting VEGF-induced vascular leakage of macromolecules using a dermal vascular permeability assay (Miles assay) in the guinea pig. VEGF (20 nM) was mixed with different concentrations of NX1838 (1000, 300, 100, and 30 nM) and injected intradermally. Evans Blue dye was administered intravenously later, and vascular leakage of albumin was quantitated using video image analysis at the VEGF injection sites. Results showed that VEGF alone resulted in a marked increase in vascular permeability near the injection site. Co-injection of NX1838, at the concentrations greater than 100 nM, with VEGF fully inhibited vascular leakage. In conclusion, NX1838 effectively inhibited VEGF-induced dermal vascular permeability in the guinea pig.

109-97002-P: *In vivo* inhibition of VEGF-induced corneal angiogenesis in Sprague-Dawley rats by NX1838

The purpose of this study was to assess effects of NX1838 on inhibiting VEGF-induced corneal angiogenesis using a rat corneal pocket model. VEGF (3.5 pmol) was surgically implanted bilaterally in the male rat corneal stroma. Animals were then treated with PBS or NX1838 (1, 3 and 10 mg/kg, iv) twice daily for 5 days. Results showed that intravenous administration of NX1838 attenuated vessel growth

concentration-dependently (10 mg/kg: ↓ 58-65%; 3 mg/kg: ↓ 57%; 1 mg/kg: ↓ 39%). In conclusion, systemic administration of NX1838 significantly inhibited new vessel growth, and NX1838 was an effective antagonist of VEGF.

109-97004-P: NX1838 inhibition of retinal angiogenesis induced by hyperoxia in neonatal C57BL/6 mice

The purpose of this study was to determine effects of NX1838 on vascular proliferation inhibition in a mouse model of retinopathy of prematurity. After 5 days exposure to 75% oxygen, 12-day old mice were returned to room air, and treated with or without NX1838 for 5 days. In the first study, NX1838 treated mice (10 mg/kg, ip, qd x 5 days) and untreated animals produced similar proliferative retinopathy changes. In the second study, abnormal retinal vessels were developed in the untreated mice and mice receiving 1 mg/kg of NX1838, but at doses of 3 mg/kg and 10 mg/kg, retinopathy was significantly reduced. The sponsor could not explain the differences between Study 1 and Study 2. Additional experiments may help to draw the conclusion.

2.6.2.3 Secondary pharmacodynamics

109-97003-P: *In vivo* anti-tumor efficacy of NX1838 in a human A673 rhabdomyosarcoma nude mouse xenograft model

The purpose of this study was to determine the *in vivo* anti-tumor efficacy of NX1838 in the A673 human rhabdomyosarcoma nude mouse xenograft model. Tumors were established by subcutaneously injecting A673 cells (1×10^7) on the flank of mice. For non-established tumor growth studies, animals were sorted on Day 0 according to weight and implanted with cells. Approximately 14-16 hr later, dosing of compounds was initiated. For established tumor growth studies, tumors were grown until approximately 200 mm^3 in size, and dosing of compound was initiated.

Treatment of mice with NX1838 (twice daily ip for 3 weeks, 10 mg/kg and 40 mg/kg) inhibited the growth of non-established A673 tumors by 75% and 80%, respectively, which were similar to the inhibition achieved by anti-VEGF monoclonal antibody (100 μg, twice weekly x 3 weeks, 83% inhibition). In another anti-tumor experiment, a dose-response effect was observed with doses from 0.03 mg/kg (49% inhibition) to 10 mg/kg (84% inhibition). For the already established A673 tumors, the growth was inhibited by 59% with the treatment of NX1838 at the dose of 10 mg/kg. In conclusion, NX1838 demonstrated inhibition effects on the growth of both non-established and established human A673 tumors in nude mice.

2.6.2.4 Safety pharmacology

Neurological effects:

SP103-012: The assessment of the effects of pegaptanib on the central nervous system – gross behavior (Irwin test) in rats

The purpose of this study was to assess effects of pegaptanib on the gross behavioral and physiological state of rats following intravenous bolus doses of 7, 20, and 65 μg/kg in a primary observation test (Irwin test). Plasma levels were determined from satellite groups. Thirty male Sprague Dawley rats

(6/group) were treated with control (phosphate buffered saline), reference substance (2 mg/kg chlorpromazine), or pegaptanib. The dose volume for all treatments was 1 ml/kg. The parameters outlined in the Irwin test were systematically evaluated for each rat at 15, 60, 240, and 480 min postdose. Blood samples were collected predose and approximately 15, 60, 240, 360, and 480 min postdose from TK animals. [Reviewer's comments: No TK data were provided.]

There were no behavioral or physiological changes recorded during the 15, 60, 240, or 480 min postdose observation periods in rats receiving pegaptanib at 7, 20, or 65 µg/kg when compared to vehicle-treated animals. Rats treated with chlorpromazine exhibited behavioral and physiological effects, which were consistent with its known pharmacological activity.

Cardiovascular effects:

AA3167: Safety pharmacology - blood pressure, heart rate, and cardiac rhythm effects of pegaptanib in beagle dogs

The purpose of this study was to evaluate effects of pegaptanib, given intravenously, on blood pressure, heart rate, and cardiac rhythm parameters in dogs. Four male beagle dogs with radiotelemetry transmitters were treated with single intravenous bolus doses of 0, 4.5, 13.5, and 45 µg/kg (in a 3- or 4-day intervals) pegaptanib diluted in phosphate buffered saline (0.25 ml/kg). Immediately following bolus dosing, animals were given 60-min infusions (2, 6, and 20 µg/kg/hr) to maintain blood levels of pegaptanib during cardiovascular monitoring. Blood pressure, heart rate, and ECG parameters were recorded for about 30 min prior to dosing, during bolus dosing, and during the 60-min maintenance infusion. Blood samples were collected predose, 15 and 30 min, and 1 and 2 hr after bolus dosing and plasma pegaptanib concentrations were measured in drug-treated animals.

There were no drug-related effects on heart rate, blood pressure, or ECG parameters in dogs at plasma pegaptanib concentrations up to 1080 ng/ml, approximately 10-fold higher than mean plasma concentrations observed in human clinical studies after a monocular 3 mg/eye dose.

Pulmonary effects:

SPR03-021: Assessment of the effects of pegaptanib on respiratory function in conscious Sprague Dawley rats

The purpose of this study was to assess effects of pegaptanib following intravenous bolus doses of 7, 20, and 65 µg/kg on respiration rate and tidal volume in rats. Male Sprague Dawley rats (8/group) were treated with a single intravenous dose of 7, 20, or 65 µg/ml/kg pegaptanib, vehicle 1 ml/kg (sterile phosphate buffered saline), or 20 mg/2 ml/kg morphine. Respiratory rate and tidal volume were evaluated at 15, 30, 60, 240, and 480 min postdose.

No significant differences were recorded in the respiration rate or tidal volume of rats treated with pegaptanib at intravenous doses of 7, 20, and 65 µg/kg when compared to the vehicle-treated group at 15, 30, 60, 240, and 480 min postdose. Treatment with morphine resulted in significant decreases in respiration rate and tidal volume. These effects are consistent with its known pharmacological activity.

2.6.2.5 Pharmacodynamic drug interactions

No drug interaction studies were conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No tabulated summary was provided by the sponsor.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

In PK studies conducted in rabbits, monkeys and dogs in which NX1838 was administered intravitreously, the vitreous levels of NX1838 remained high for a long period, and decreased very slowly. The systemic bioavailability in animals was also high.

In a rabbit study, following intravitreal administration, high radioactivity concentrations were found in vitreous fluid and retina. Following both intravitreal and intravenous administrations, highest concentrations of radioactivity were obtained in the kidney, spleen, bone marrow, lymph node (mesenteric) and liver. Very low levels of radioactivity were found in the eye following systemic administration. Little radioactivity was associated with red blood cells. Pegaptanib did not seem to bind to melanin. In studies with different species, the volume of distribution at steady state was low and close to the plasma volume, suggesting minimal tissue uptake. In a study in pregnant mice, pegaptanib crossed the placenta and was found in the amniotic fluid.

PEG conjugation was proved to be important in increasing plasma drug residence time by reducing plasma drug clearance rate, possibly through decreased renal filtration, and by increasing resistance to nuclease. It was projected that by 1008 hr following intravitreal or intravenous injection in rabbits, approximately 28 to 51% pegaptanib dose were met[redacted]2'-fluoro-2'-deoxyuridine.

In a study in rabbits, the predominant route of excretion was via urine following iv or po dosing. Feces were only a minor route of excretion, suggesting little biliary elimination of radioactivity.

2.6.4.2 Methods of Analysis

See descriptions under individual study reviews.

2.6.4.3 Absorption

109-97001-B: Pharmacokinetics of intravitreously administered single dose NX1838 in New Zealand white rabbits

Study Nº: 109-97001-B
Compound: NX1838 (Lots: NX1838.08 and NX1838.04)
Route: Intravitreous
Dose Level: 0.5 mg/eye (40 µl/eye, both eyes)
Dosing Regimen: Single dose

Animal: Male New Zealand white rabbits
GLP: No

The purpose of this study was to investigate intravitreous levels and plasma pharmacokinetics of NX1838 in rabbits over a 28-day period. Blood samples were collected at 24, 72, 168, 336, and 672 hr following dosing. Vitreous samples were collected at 1, 6, 24, 72, 168, 336, and 672 hr following dosing. HPLC and — assays were used in this study.

Results:

PK parameters for this study are summarized in the following table. Twenty-eight days after dosing, drug levels in the vitreous and plasma dropped to 1.7 µg/ml and 0.005 µg/ml, respectively. The data in this study supported that a significant portion of NX1838 was cleared slowly from the eye into the plasma.

PK parameters of NX1838 after single intravitreous administration (0.5 mg/eye, both eyes) in rabbits

	Vitreous	Plasma
Tmax (hr)	6	24
Cmax (µg/ml)	357.7	0.092
T1/2 (hr)	83	84
$AUC_{0-\infty}$ (µg-hr/ml)	47129	15.7

109-97002-B: Pharmacokinetics of intravitreously administered single dose NX1838 in Dutch-belted rabbits

Study N^{o}: 109-97002-B
Compound: NX1838 (Lot: 1838.04)
Route: Intravitreous
Dose Level: 0.5 mg/eye (40 µl/eye, both eyes)
Dosing Regimen: Single dose
Animal: Male Dutch-belted rabbits
GLP: No

The purpose of this study was to investigate the intravitreous levels and plasma pharmacokinetics of NX1838 in Dutch-belted rabbits. Blood samples were collected 1, 6, 24, 72, 168, 336, 504 and 672 hr following dosing. Vitreous samples were collected 1, 72, 168, 336 and 672 hr after dosing. HPLC and — assays were used in this study.

Results:

PK parameters for this study are summarized in the following table. Twenty-eight days after dosing, the drug levels in the vitreous and plasma dropped to 4 µg/ml and < 0.030 µg/ml, respectively. Plasma levels were very low with values less than 0.6 µg/ml in all samples.

PK parameters of NX1838 after single intravitreous administration (0.5 mg/eye, both eyes) in rabbits

	NX1838	
	Vitreous	Plasma
Tmax (hr)	1	24
Cmax (µg/ml)	501.9	0.364
T1/2 (hr)	111	51.3
$AUC_{0-\infty}$ (µg-hr/ml)	42824	22.5

0460LE15-001: A 6-month toxicity study in rabbits of EYE001 by intravitreous administration

Study N°: 0460LE15-001
Compound: EYE001 (Lot #s: HP1214-030-2, 3, and 4)
Route: Intravitreous
Dose Level: 0 (buffered PSS), 0.2, 0.67, or 2.0 mg/eye (67 µl/eye, both eyes)
Dosing Regimen: Once every 2 weeks x 13 (6 months)
Animal: New Zealand white rabbits, 5-6 months old, 3.1-4.2 kg, 7/sex/group in LD and MD groups and 9/sex/group for control and HD groups
Test facility: —

GLP: Yes
Study initiation: 6/28/2001

The purpose of this study was to determine the local tolerance, potential toxicity and toxicokinetics of EYE001 when administered to NZW rabbits by bilateral intravitreous injection once every 2 weeks for 6 months. Pharmacokinetics was assessed as shown in the table below. The right eye (4 animals/sex/group for main study animals and 1 animal/sex/group for recovery animals) was collected for PK evaluation. A — assay was used in this study.

Pharmacokinetics assessment

Group	Dose (mg/eye)	Number of Rabbits/sex/dose	Blood sampling time	Termination
1	0	9	3 animals/time: 1, 3, 6, 9, 24, 48, 96, and 168 hr post dose for the 1st, 6th and 12th doses	7 animals/sex following dose 13 (Week 25) and 2 animals in Week 31
2	0.2	7		Following dose 13 (Week 25)
3	0.67	7		Following dose 13 (Week 25)
4	2.0	9		7 animals/sex following dose 13 (Week 25) and 2 animals in Week 31

Results:

Vitreous drug concentrations were not measured because the sponsor later chose another lab for the measurement. When the samples arrived at the lab, they were considered too old for use. Plasma PK parameters summarized in the table below demonstrated that the Cmax and AUC levels increased with increasing intravitreal doses of the drug. A slight accumulation was noted as measure by Cmax. However, comparable AUC values were noted following a single or multiple-dose intravitreous injections. No differences between male and female animals were noted.

Plasma pegaptanib PK data

No. Dose	Dose (mg/eye)	Total Dose (mg)	T1/2 (hr)	Tmax (hr)	Cmax (ng/ml)	$AUC_{0-\infty}$ (µg-hr/ml)
1	0.2	0.4	141	9	89	13.1
1	0.67	1.34	102	24	166	35.7
1	2	4	153	24	556	113.3
6	0.2	0.4	111	9	71	10.2
6	0.67	1.34	122	24	214	27.9
6	2	4	115	9	776	87.3
12	0.2	0.4	77	9	82	9.6
12	0.67	1.34	111	6	229	30.5
12	2	4	136	9	780	80.1

0472DE15-001: A nine-month local tolerance and toxicity study of EYE001 given by intravitreous injection to beagle dogs

Study N^{o}: 0472DE15-001
Compound: EYE001 (Lot #s: 241001A, 111002A, 131002A and N06004F)
Route: Intravitreous
Dose Level: 0 (buffered PSS), 0.3, 1.0, and 3.0 mg/eye (100 µl/eye, both eyes)
Dosing Regimen: Once every 2 weeks x 20 (9 months)
Animal: Beagle dogs, 15-16 months old, 8.7-11.8 kg for males and 7.3-10.1 kg for females, 5/sex/group in LD and MD groups and 7/sex/group for control and HD groups
Test facility: —

—

GLP: Yes
Study initiation: 2/21/2001

The purpose of this study was to determine the local tolerance, potential toxicity and toxicokinetics of EYE001 when administered to beagle dogs by bilateral vitreous injection once every 2 weeks for 9 months. Pharmacokinetics was assessed as shown in the table below. The right eye (3 animals/sex/group for main study animals) was collected for PK evaluation. A dual hybridization assay was used in this study.

Pharmacokinetics assessment

Group	Dose (mg/eye)	Number of dogs/sex/dose	Blood sampling time	Termination
1	0	7	3 animals/sex at 1, 3, 6, 9, 24, 48, 96, and 168 hr post dose for the 1st, 9th and 18th doses	5 animals/sex within 3 days after dose 20 (Week 39) and 2 animals in Week 45
2	0.3	5		Within 3 days following dose 20 (Week 39)
3	1.0	5		Within 3 days following dose 20 (Week 39)
4	3.0	7		5 animals/sex within 3 days after dose 20 (Week 39) and 2 animals in Week 45

Results:

Vitreous drug concentrations were not measured due to a long storage period between sampling and measurement. P[redacted] the table below demonstrated that the Cmax and AUC levels increased with increasing intravitreal doses of the drug. No apparent accumulation was noted. No differences between male and female animals were noted.

Plasma pegaptanib PK data (mean ±SD)

No. Dose	Dose (mg/eye)	Total Dose (mg)	T1/2 (hr)	Tmax (hr)	Cmax (ng/ml)	$AUC_{0-\infty}$ (μg-hr/ml)
1	0.3	0.6	35± 20	4± 2	109± 68	2.2± 0.8
1	1	2	52± 18	3± 1	496± 163	8.2± 1.4
1	3	6	59± 18	5± 2	1548± 822	26.4± 5.1
9	0.3	0.6	21± 17	4± 2	154± 71	1.9± 0.4
9	1	2	45± 22	4± 1	573± 230	8.7± 1.9
9	3	6	55± 14	6± 5	1329± 640	26.2± 4.0
18	0.3	0.6	18± 8	6± 7	107± 42	2.1± 0.3
18	1	2	37± 23	5± 2	453± 169	8.5± 1.5
18	3	6	45± 16	4± 2	1746± 473	28.8± 5.0

109-98005-B: Plasma pharmacokinetics of an anti-VEGF aptamer, NX1838, following subcutaneous administration in rhesus monkeys
109-98004-B: Plasma pharmacokinetics of an anti-VEGF aptamer, NX1838, following intravenous administration in rhesus monkeys

Report Nº: 109-98005-B and 109-98004-B
Compound: NX1838 (Lot: 11838) dissolved in PBS.
Dosing Regimen: Single dose (intravenous and subcutaneous injections in the same animals separated by 16 days), 1 mg/0.5 ml/kg
Animal: Six female rhesus monkeys (4-5 years old, 3.5-5.2 kg)
Study Site: —
GLP: Yes

The purpose of this study was to determine plasma PK of NX1838 in rhesus monkeys following a single iv and sc injections. Blood samples were collected prior to dosing and at 5 min (iv only) and 0.25, 0.5, 1, 2, 4, 8, 12, 24, 32, 48 and 72 hr after dosing. The samples were analyzed by an — — assay.

Results:

PK data are summarized in the table below.

PK parameters of NX1838 following a single 1 mg/kg iv or sc injection in monkeys (mean ± SD)

	Cmax (μg/ml)	$AUC_{0-\infty}$ (μg-hr/ml)	T1/2 (hr)	Tmax (hr)	Bioavailability (%)
Intravenous injection	25.5 ± 2.4	165 ± 22.9	9.3 ± 1.5		
Subcutaneous injection	4.9 ± 1.5	128. 5± 33.2	12.0 ± 0.8	9.3 ± 2.1	78 ± 14

109-98006-B: Pharmacokinetics of an anti-VEGF aptamer, NX1838, following intravitreal administration in rhesus monkeys

Report Nº: 109-98006-B
Compound: NX1838 (Lot: D0905F)
Dosing Regimen: Single dose (intravitreal injection), 0.5 mg/0.067 ml/eye
Animal: Six female rhesus monkeys (4-5 years old, 3.4-5.2 kg)
Study Site: —
GLP: Yes

The purpose of this study was to determine plasma and vitreal PK of NX1838 in rhesus monkeys following a single intravitreal injection. Three animals (Group 1) were terminated on Day 7 and three animals (group 2) were terminated on Day 28. Blood samples were collected prior to dosing and at 1, 2, 4, 8, 12, 24, 32, 48 and 72 hr after dosing, and on Day 7. Additional blood samples were collected from Group 2 animals on Days 14, 21 and 28. Vitreous and aqueous humor samples were collected on Days 7 and 28 from each eye. The samples were analyzed by an HPLC assay and a double hybridization assay.

Results:

Results are summarized in the table below. The dual hybridization assay consistently showed about 80% of the HPLC values. The sponsor believed that data from the dual hybridization assay were more accurate. NX1838 appeared completely absorbed into plasma with bioavailability of 95%. Vitreous drug concentrations on both Days 7 and 28 were greater than the equilibrium dissociation constant of NX1838 for $VEGF_{165}$ (200 pM).

PK parameters of NX1838 following a single 0.5 mg/eye intravitreal injection in monkeys (mean ± SD)

	Plasma					Vitreous (µg/ml)	
	Cmax (µg/ml)	$AUC_{0-\infty}$ (µg-hr/ml)	T1/2 (hr)	Tmax (hr)	Bioavailability (%)	Day 7	Day 28
HPLC	0.415±0.088	39.6± 13.8	65.0± 30.6	18.7± 8.3	96±23	49.21± 12.65	1.5± 0.64
Hybridization	0.318± 0.047	38.2± 3.6	104.8± 14.4	12.7± 5.9	95±11		

109-980142-T: An acute intravitreous safety study (0699-35) with NX1838 in rhesus monkeys

Report Nº: 109-980142-T
Compound: NX1838 (Lot #: RN98000122)
Dosing Regimen: Single dose (intravitreal injection), 1.5 and 2.0 mg/0.065 ml/eye, both eyes
Animal: Four rhesus monkeys, 2/dose
Study Site: —
GLP: No

The purpose of this study was to determine plasma PK of NX1838 in rhesus monkeys following a single intravitreal injection. Blood samples were collected at 2, 4, 8, 12, and 24 hr and 2, 4, 7, 14, 21 and 26 days after dosing. Samples were analyzed by an HPLC assay.

Results:

Results are summarized in the table below.

PK parameters of NX1838 following a single 1.5 and 2.0 mg/eye intravitreal injection in monkeys (mean ± SD)

	Plasma				
	Cmax (µg/ml)	$AUC_{0-\infty}$ (µg-hr/ml)	T1/2 (hr)	Tmax (hr)	Bioavailability (%)
1.5 mg/eye	1.05± 0.33	135± 7.1	86± 40.3	12-24	98± 3
2.0 mg/eye	1.99± 0.51	239± 17	105± 6.4	12-24	135± 35

2.6.4.4 Distribution

109-97003-B: Dose dependent pharmacokinetics of intravenous administered single dose NX1838 in CD-1 mice

Study N^{o}: 109-97003-B
Compound: NX1838
Route: Intravenous
Dose Level: 0.1, 1 and 10 mg/kg
Dosing Regimen: Single dose
Animal: Female CD-1 mice
GLP: No

The purpose of this study was to investigate plasma pharmacokinetics and dose-dependent effects of NX1838 on PK parameters following a single intravenous injection in mice. Blood samples were collected 15, 45, 120, 240, 480 and 1440 min following dosing, and were analyzed with a double hybridization assay.

Results:

PK parameters are shown in the table below. Plasma concentrations for all dose groups decreased over the 24 hr time period approximately 200-fold from initial values. The volume of distribution at steady state was close to the plasma volume and ranged from — ml/kg, suggesting minimal tissue uptake. Little dose-dependent effects on volume of distribution, t1/2 and plasma clearance were noted.

PK parameters of NX1838 following a single iv injection

Parameter	0.1 mg/kg	1 mg/kg	10 mg/kg
Cmax (μg/ml)	1.0	12.4	147.5
$AUC_{0-\infty}$ (μg-min/ml)	325	4968	41473
T1/2 (hr)	4	3.5	4.5
Tmax (min)	45	15	15
Cl (ml/min-kg)	0.308	0.201	0.241
V (ml/kg)	83.5	58.8	62.6

109-98001-B: Pharmacokinetics of intravenous administered single dose of NX1838 in Dutch-belted rabbits

Study N^{o}: 109-98001-B
Compound: NX1838
Route: Intravenous
Dose Level: 1 mg/kg
Dosing Regimen: Single dose
Animal: Male Dutch-belted rabbits
GLP: No

The purpose of this study was to investigate intravitreous levels and plasma pharmacokinetics of NX1838 following a single iv injection of NX1838 in rabbits. Blood samples were collected prior to, 5, 10, 20, 30 min, and 1, 2, 4, 8, 24, 48 and 72 hr following dosing from 3 animals/time point. Vitreous samples were collected at 24 and 72 hr after dosing. The HPLC technique was used in this study to determine plasma and vitreous drug levels.

Results:

In all eye samples, concentrations of NX1838 were below the limit of quantitation (— μg/ml). Plasma PK parameters are summarized in the table below. The plasma concentration of NX1838 decreased

approximately 200-fold from a mean value of 17.3 to 0.081 µg/ml for samples collected over a time period of 5 min to 24 hr. Samples taken after 24 hours were all below the limit of quantitation. The volume of distribution at steady state of NX1838 was quite low, 57.7 ml/kg, approximated the plasma volume.

Plasma PK parameters for rabbit following a single intravenous injection (1 mg/kg)

Parameter	Cmax	AUC	T1/2	Tmax	Cl	V
Value (Mean ± SE)	(µg/ml)	(µg-min/ml)	(min)	(hr)	(ml/min-kg)	(ml/kg)
	17.3± 0.5	4158	210	0.08	0.241	57.7

1005-004: Intravenous developmental toxicity study of EYE001 in mice

Study N°: 1005-004
Study site: —
Compound: NX1838 (Lot #: C22001L003)
Route: Intravenous
Dose Level: 40 mg/5 ml/kg
Dosing Regimen: Once daily from gestation day (GD) 6 to day 15
Animal: 36 mated - CD-1(ICR)BR female mice, 69-79 days old, 23-33 g
GLP: Yes

This was the TK part of a reproductive study. Blood samples were collected on gestation days 6 and 15 at approximately 5 and 20 min and 1, 3, 9 and 24 hr postdose from 3 mice/time point. Additionally, fetuses and amniotic fluid were collected from TK mice sacrificed on gestation day 15. The amniotic fluid was collected, pooled by litter and transferred to labeled tubes. A dual hybridization procedure was used in this study.

Results:

Results are summarized in the table below. IV injections of pegaptanib at 40 mg/kg/day from gestation day 6 to gestation day 15 resulted in a slight accumulation of pegaptanib in the plasma. Mean pegaptanib plasma terminal half-life was 4 hr. Pegaptanib crossed the placenta and was found in amniotic fluid with the maximal amniotic fluid concentration of 0.58µg/ml at 1 hr after dose on gestation day 15. Pegaptanib amniotic fluid concentrations measured at 1 hr post dosing on day 15 were 0.04% of the corresponding mean pegaptanib plasma concentration of 1400 µg/ml at 1 hr.

TK parameters in CD-1 mouse plasma and amniotic fluid

	Plasma		Amniotic fluid
Gestation day	**6**	**15**	**15**
T1/2 (hr)	3.6	3.8	3.5
Tmax (hr)	0.083	0.083	1
Cmax (µg/ml)	1880	2177	0.58
AUC_{tau} (µg-hr/ml)	6936	8190	3.8

At the dose of 40 mg/kg/day administered from gestation days 6 to 15 to pregnant CD-1 mice, mean pegaptanib plasma concentrations at 5 min post injection on gestation day 15 were about 2000 µg/ml and the mean AUC_{tau} was approximately 8000 µg.hr/ml. In humans, at the maximum dose administered of 3 mg/study eye given every 4 weeks, mean C_{max} values were about 90 ng/ml (or 0.09 µg/ml) and AUC_{tau} values were approximately 25 µg.hr/ml. Thus, pegaptanib maximum plasma concentrations and AUC values

after daily IV dosing in pregnant CD-1 mice were greater than 20000-fold and 300-fold those seen in humans receiving intravitreous injections of 3 mg/eye, respectively.

109-97004-B: Pharmacokinetics of intravenous administered single dose of NX1838, NX22270 (20K PEG), NX22271 (no PEG) in Sprague-Dawley rats

Study Nº: 109-97004-B
Compound: NX1838 (with 40K PEG), NX22270 (with 20K PEG) and NX22271 (no PEG)
Route: Intravenous
Dose Level: 1 mg/kg
Dosing Regimen: Single dose
Animal: Sprague-Dawley rats
GLP: No

The purpose of this study was to determine the influence of PEG conjugation on plasma PK parameters of NX1838 in rats. The drugs used in this study were identical oligonucleotides with different PEG conjugates to explore effects of molecular weight on plasma clearance. Blood samples were collected at 2, 5, 10, 20, 30, 60, 120, 180, 240, 480 and 1440 min after dosing. A double hybridization assay was used in this study.

Results:

PK parameters are [redacted] in the following table. Significant differences in plasma clearance were seen as a function of PEG molecular weight. These differences appeared to be mainly due to two factors, the elimination half life and the volume of distribution. Low levels of volume distribution and clearance for PEG conjugates suggested that these compounds were mainly confined to the plasma, and these compounds might have a low renal filtration. PEG was shown to increase the molecule's resistance to nuclease, which also contributed to increased plasma residence time of the drug.

PK parameters of NX1838, NX22270 and NX22271 after a single iv injection (1mg/kg)

Parameter	NX1838 (PEG40K)	NX22270 (PEG20K)	NX22271 (No PEG)
Cmax (µg/ml)	14.9	14.2	2.3
$AUC_{0-\infty}$ (µg-min/ml)	7712	1248	21.2
T1/2 (hr)	6	3.2	0.3
Tmax (min)	10	10	5
Cl (ml/min-kg)	0.13	0.8	47.2
Vd (ml/kg)	58	89	306

3164: Toxicokinetic analysis for the acute intravenous toxicokinetic study of pegaptanib in dogs (PGRD Study # 3164)

Study Nº: 3164
Study site: — , Pfizer Global Research & Development, La Jolla, CA
Compound: Pegaptanib (Lot #s: 241201A and 131201A) diluted in PBS
Route: Intravenous
Dose Level: 5 and 50 µg/0.25 ml/kg
Dosing Regimen: Single dose
Animal: Male beagle dogs (2/dose group), 1 to 4 years old, 8 to 12 kg
GLP: Yes

The purpose of this study was to determine the PK profile of pegaptanib following a single iv dose to male beagle dogs. Blood samples were collected immediately postdose, at 15 and 30 min, and 1, 2, 4, 7, 12, 24 and 32 hr after dosing. A [redacted] assay was used in this study.

Results:

PK parameters are summarized in the following table. There was a dose proportionality between the low and high doses. A 10-fold difference was seen in Cmax and AUC levels between these two doses.

Plasma PK parameters for dogs following a single intravenous injection (5 and 50 µg/kg)

Dose (µg/kg)	Cmax (ng/ml)	AUC (ng-hr/ml)	T1/2 (hr)	Tmax (hr)	Cl (ml/min-kg)	V (ml/kg)
5	113.0	247.0	1.99	0.033	20.8	54.3
50	1084.0	2320.0	1.80	0.133	21.7	49.0

45943: Pharmacokinetics, tissue distribution, excretion and metabolism of radioactivity in male Dutch belted rabbits following a single intravitreal or intravenous injection of ^{14}C-pegaptanib sodium

Study N°: 45943
Study site: [redacted]
Compound: ^{14}C-pegaptanib sodium, Lot #: CFQ12942, 369 mCi/mmol, purity = [redacted]
Route: Intravenous (group 3) or intravitreal (Group 2) injection
Dose Level: 1.33 mg/eye (both eyes) for Group 2 rabbits and 1.33 mg/kg iv for Group 3 rabbits
Animal: Male Dutch belted rabbits, 5 months old, 1.9-2.2 kg
GLP: Yes
Study initiation: 5/28/2003
Study design

Group	Dose	N	Route	Radioactivity
1	Untreated	1	N/A	
2	1.33 mg/50 µl/eye, both eyes	7	Intravitreal	50 µCi/eye
3	1.33 mg/0.5 ml/kg	7	Intravenous	10 µCi/animal

The purpose of this study was to determine the following in male Dutch belted rabbits following a single intravitreal (Group 2) or intravenous injection (Group 3) of ^{14}C-pegaptanib sodium:

Plasma and red blood cell PK parameters
Tissue distribution
Excretion of radioactivity
Melanin binding of radioactivity
Determination of 2'-fluorou[redacted]cytidine in plasma and urine

Blood, urine, and feces samples were collected from Groups 2 and 3 animals at selected timepoints (see table below) after dosing. The tissue distribution of radioactivity was determined by a quantitative whole-body autoradiography (QWBA) in one animal per timepoint from both Groups 2 and 3. Melanin binding of radioactivity was assessed from qualitative and quantitative data of the eye (uveal tract) and the skin from autoradioluminograms of Group 2 and 3 animals. Groups 2 and 3 plasma and urine samples were analyzed for the presence of 2'-fluorouridine (2'-fluoro-2'-deoxyuridine) using a liquid chromatography [redacted] mass spectrometry. Group 2 and 3 plasma and urine samples were also analyzed

for the presence of pegaptanib (oligonucleotide content) by a dual hybridization assay. The blood and eye structures collected from the Group 1 animal were used to determine background levels of radioactivity in the respective matrix. The radioactivity was analyzed by liquid scintillation spectroscopy.

Animal No.	Collection Timepoints (hr post-dose) for Group 2 animals														
	0.5	1	2	6	12	24	48	72	96	120	144	168	312	504	1008
201						B/T									
202									B/T						
203												BT			
204													BT		
205	B	B	B	B	B	B/U	B/U	U	B/U	U	U	B/U	B/U	B/U	B/U/T
206	B	B	B	B	B	B/U	B/U	U	B/U	U	U	B/U	B/U	B/U	B/U
207	B	B	B	B	B	B/U	B/U	U	B/U	U	U	B/U	B/U	B/U	B/U
Animal No.	Collection Timepoints (hr post-dose) for Group 3 animals														
	0.5	1	2	6		24	48	72	96	120	144	168	504		
301			B/T												
302				B/T											
303						B/T									
304									B/T						
305	B	B	B	B		B/E	B/E	E	B/E	E	E	B/E	B/U/ T		
306	B	B	B	B		B/E	B/E	E	B/E	E	E	B/E	B/U		
307	B	B	B	B		B/E	B/E	E	B/E	E	E	B/E	B/U		

B = Blood collection, U = Urine collection, E = Excreta collection (urine, feces, cage wash), T = Animal euthanized for tissue analysis by [redacted]

Results:

Plasma and RBC PK parameters

Results are summarized in the table below. Following both intravitreal and intravenous administrations, red blood cells to plasma [redacted] of radioactivity ranged between 0.10 to 0.33, indicating that little radioactivity was partitioned into red blood cells.

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PK parameters in plasma and red blood cells

	Tmax (hr)	Cmax	T1/2	$AUC_{0-\infty}$
Group 2	(hr)	(µg eq/ml)	(hr)	(µg eq h/ml)
Animal #	**Plasma**			
205				
206				
207				
Mean	**212**	**0.142**	**NC (not calculated)**	**NC**
S.D.	**173**	**0.0451**	**NC**	**NC**
	Red blood cells			
205				
206				
207				
Mean	**276**	**0.024**	**NC**	**NC**
S.D.	**248**	**0.002**	**NC**	**NC**
Group 3	**Plasma**			
Animal #	(hr)	(µg eq/ml)	(hr)	(µg eq h/ml)
305				
306				
307				
Mean	**0**	**11.3**	**132**	**84.6**
S.D.	**0**	**0.859**	**18.5**	**7.53**
	Red blood cells			
305			170	15.7
306			162	14.4
307			186	17.4
Mean	**0**	**2.21**	**173**	**15.8**
S.D.	**0**	**0.515**	**11.8**	**1.51**

Tissue distribution

Following intravitreal administration, radioactivity was mainly distributed in vitreous fluid (administration site), retina and aqueous fluid at 24 hr post-dose. Very low levels of radioactivity were found in the lens. A lower but still important increase of radioactivity over the study duration was observed in the cornea.

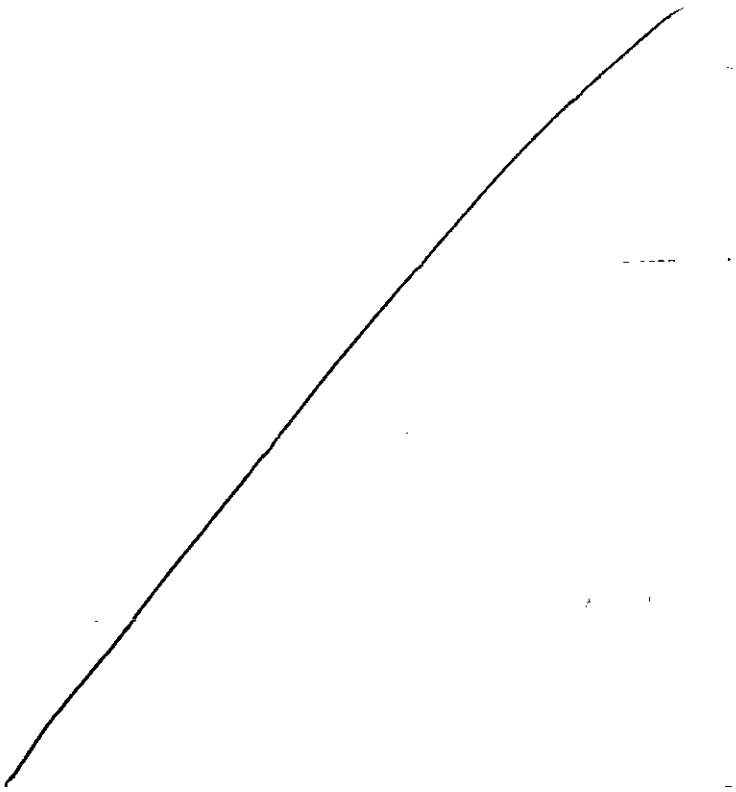
Ocular distribution of pegaptanib after single intravitreal injection in rabbits (µg-eq/g)

	Animal 201	Animal 202	Animal 203	Animal 204	Animal 205
Sample	**24 hr**	**96 hr**	**168 hr**	**312 hr**	**1008 hr**
Aqueous Fluid					
Cornea					
Dissection Rinse					
Dissection Swab					
Iris					
Lens					
Optic Nerve					
Retina					
Sclera (+ choroid)					
Vitreous Fluid					

Following intravitreal injection, QWBA results showed high levels of radioactivity in the eye up to the last timepoint of 1008 hr. Following both intravitreal and intravenous administrations, highest concentrations of radioactivity were obtained in the kidney followed by the spleen, bone marrow (vertebra), lymph node (mesenteric) and liver. The lowest concentrations were found in the brain, spinal cord, skeletal muscle (dorsal) and bone (vertebra). Following the intravenous administration, low levels were also found in the eye.

Tissue distribution after iv administration

	Animal 301	Animal 302	Animal 303	Animal 304	Animal 305
Sample	2 hr	6 hr	24 hr	96 hr	504 hr
Adipose Tissue (Brown Fat)					
Adipose Tissue (White Fat)					
Adrenal Gland					
Bone (Vertebra)					
Bile from Gall Bladder					
Bone Marrow (Vertebra)					
Brain					
Epididymis					
Eye					
Kidney					
Large Intestine Contents					
Large Intestine Wall					
Liver					
Lung					
Lymph Node (Mesenteric)					
Skeletal Muscle (Dorsal)					
Myocardium					
Pancreas					
Pituitary Gland					
Prostate Gland					
Rectum Wall					
Rectum Contents					
Salivary Gland (Parotid)					
Small Intestine Wall					
Small Intestine Contents					
Skin					
Spinal Cord					
Spleen					
Stomach Wall					
Stomach Contents					
Seminal Vesicle					
Testis					
Thymus					
Thyroid/Parathyroid Gland					
Urinary Bladder Wall					
Urinary Bladder Contents					



Following intravitreal administration, it was not possible to assess binding of radioactivity to melanin in the uveal tract due to high radioactivity levels in the eye up to the last timepoint. Low levels of radioactivity in the skin of Group 2 animals as well as in the eye and skin of Group 3 animals suggested that little or no binding of radioactivity to melanin occurred.

Excretion of radioactivity

Following intravitreal administration, urinary excretion represented a slow but continuous pathway for elimination of radioactivity. By 168 hr, a mean of 36% of the administered radioactive dose was recovered in the urine. At 1008 hr post-dose, low levels of radioactivity were still present in the kidney and urinary bladder. Feces were only a minor route of excretion, suggesting little biliary elimination of radioactivity.

Mean excretion of radioactivity by rabbits following a single intravitreal injection of ^{14}C-pegaptanib sodium

Time Interval (hr)	Percent of Dose Urine	Cumulative Percent of Dose Total Urine
0 - 24	8.911 ± 5.956	8.911 ± 5.956
24 - 48	6.064 ± 1.989	14.975 ± 7.926
48 - 72	4.445 ± 0.884	19.421 ± 7.904
72 - 96	4.814 ± 1.281	24.234 ± 6.645
96 - 120	4.063 ± 0.650	28.297 ± 5.997
120 - 144	3.469 ± 1.440	31.766 ± 4.562
144 - 168	4.397 ± 0.433	36.163 ± 4.449
288 - 312	2.577 ± 0.900	>38.741 ± 4.640
480 - 504	1.169 ± 0.269	>39.910 ± 4.395
984 - 1008	0.107 ± 0.013	>40.017 ± 4.386

Following intravenous administration, the excretion of radioactivity was characterized by a rapid elimination phase during the first 24 hr followed by a more prolonged elimination over the remaining interval (24-168 hr). The predominant route of excretion was via urine with approximately 86% of the administered dose excreted by 168 hr. Over a 90% of the dose was recovered in urine, feces and cage washes by 168 hr. Excretion of radioactivity in feces accounted for approximately 3% (up to 168 hr post-dose).

Mean excretion of radioactivity by rabbits following a single iv injection of ^{14}C-pegaptanib sodium (%, Mean ±SD)

Time Interval	Percent of Dose				Cumulative Percent of Dose			
(hr)	Urine	Feces	Cage wash	total	Urine	Feces	Cage wash	total
0 - 24	73.816 ±2.804	1.825 ±0.170	1.088 ±0.344	76.729 ±2.793	73.816 ±2.804	1.825 ±0.170	1.088 ±0.344	76.729 ±2.793
24 - 48	4.753 ±0.260	0.244 ±0.094	0.226 ±0.069	5.224 ±0.355	78.569 ±2.891	2.069 ±0.187	1.314 ±0.307	81.953 ±2.827
48 - 72	2.561 ±0.292	0.245 ±0.006	0.131 ±0.073	2.937 ±0.249	81.131 ±3.056	2.314 ±0.191	1.445 ±0.315	84.889 ±2.899
72 - 96	1.756 ±0.409	0.249 ±0.171	0.052 ±0.011	2.057 ±0.259	82.886 ±3.438	2.563 ±0.329	1.497 ±0.320	86.947 ±3.092
96 - 120	1.167 ±0.038	0.146 ±0.063	0.051 ±0.015	1.365 ±0.040	84.054 ±3.473	2.710 ±0.389	1.548 ±0.316	88.312 ±3.054
120 - 144	0.904 ±0.043	0.095 ±0.016	0.036 ±0.014	1.035 ±0.037	84.958 ±3.495	2.805 ±0.397	1.584 ±0.312	89.346 ±3.048
144 - 168	0.586 ±0.051	0.083 ±0.006	0.029 ±0.015	0.698 ±0.046	85.544 ±3.493	2.888 ±0.403	1.613 ±0.310	90.044 ±3.030
480-504	0.067 ±0.007			0.067 ±0.007	>85.611 ±3.487	>2.888 ±0.403	>1.613 ±0.310	>90.112 ±3.023

Determination of 2'-fluoro-2'-deoxyuridine

Following intravitreal administration, highest concentrations of 2'-fluoro-2'-deoxyuridine were obtained in both plasma and urine at 96 and 168 hr post-dose from two animals and at 24 hr from the other animal. For the same timepoints, approximately 4 to 13 times more 2'-fluoro-2'-deoxyuridine were obtained in urine compared to plasma. The sponsor estimated that approximately 47, 51 and 31% of administered pegaptanib dose were metabolized to 2'-fluoro-2'-deoxyuridine by 1008 hr for these three animals, respectively.

Following intravenous administration, highest concentrations of 2'-fluoro-2-deoxyuridine were obtained in plasma at 2 and 6 hr post-dose and in urine at 24 hr post-dose. For the same timepoints, approximately 7 to 25 times more 2'-fluoro-2'-deoxyuridine were measured in urine compared to plasma. The sponsor estimated that approximately 49, 28 and 42% of administered pegaptanib dose were metabolized to 2'-fluoro-2'-deoxyuridine by 1008 hr for the three animals included in this test, respectively.

2.6.4.5 Metabolism

400014: Metabolic stability of ^{14}C-pegaptanib sodium (EYE001) using endonuclease, 3'-exonuclease, 5'-exonuclease, ribonuclease and human, rabbit, dog and monkey plasma

Study N°: 400014
Study site: —
Compound: ^{14}C-pegaptanib sodium, Lot #: CFQ12942, 7.1 µCi/mg, purity = —
Pegaptanib sodium, Lot #: 2880-AG-2P-158, purity > —
Positive control: 27-oligomer with 3'-terminus cap with reverse thymidine, Lot #: GAUEA-0001
Test systems: Human plasma, Dutch belted rabbit plasma, rhesus monkey plasma, beagle dog plasma, endonuclease, 3'-exonuclease, 5'-exonuclease and ribonuclease (A nuclease solution was prepared in 0.1 M Tris-HCl buffer, pH 8.0 that contained 0.2 units/ml of endonuclease, ribonuclease A and phosphodiesterase II and 0.02 units/ml of phosphodiesterase I.)
GLP: No

The purpose of this study was to assess the metabolic stability of ^{14}C-pegaptanib sodium using endonuclease, 3'-exonuclease, 5'-exonuclease and ribonuclease and plasma from humans, rabbits, dogs and monkeys. Various concentrations of ^{14}C-pegaptanib sodium (50, 100 and 250 ng/ml) were incubated with a mixture of endonuclease, 3'-exonuclease, 5'-exonuclease and ribonuclease or plasma from humans, rabbits, dogs or monkeys for 0, 2.5 or 5 hr. Samples were then analyzed for the remaining parent compound by an HPLC and the formation of 2'-fluoro-2'-deoxyuridine by an LC-MS — Control incubations using an unprotected 27-oligomer with the same sequence as the oligonucleotide portion of pegaptanib sodium were also performed.

Results:

Results are summarized in the table below. Significant resistance to nucleases was observed for ^{14}C-pegaptanib sodium when compared to the degradation of an unmodified 27-oligomer positive control oligonucleotide. However, the modifications did not render ^{14}C-pegaptanib sodium completely resistant to degradation by nucleases. A consistent disappearance of the parent compound, with a concomitant increase of 2'-fluoro-2'-deoxyuridine in all incubations suggested that the oligonucleotide portion was degraded by nucleases into nucleotides in plasma from all species. The extent of degradation of parent compound and formation of 2'-fluoro-2'-deoxyuridine in plasma from different species was likely to be associated with the amount of nucleases released from blood cells during the preparation of plasma.

[redacted] peak area of ^{14}C-pegaptanib sodium following incubation with nuclease solution or human, dog, monkey, or rabbit plasma for various time points

Concentration	50 ng/ml			100 ng/ml			250 ng/ml		
Incubation Time	0 hr	2.5 hr	5 hr	0 hr	2.5 hr	5 hr	0 hr	2.5 hr	5 hr
Human plasma	>100	>100	>100	>100	>100	98.1	>100	98.8	>100
Rabbit plasma	96.8	61.7	41.9/94.8	90.1/>100	54.7	57.1	96.6/>100	85.5	65.5
Dog plasma	>100	23.2/98.0	21.6/99.2	>100	22.9	12.9	>100	25.1	14.0
Monkey plasma	>100	>100	94.7	>100	>100	94.2	>100	94.0	96.0
Nuclease solution	>100	23.3	17.6	>100	23.0	15.3	90.3	6.3	6.1

2.6.4.6 Excretion

Excretion evaluation was included in other studies reviewed above. Following intravitreal administration in rabbits, urinary excretion represented a slow but continuous pathway for elimination of radioactivity. Feces were only a minor route of excretion, suggesting little biliary elimination of radioactivity. No extra specific excretion studies were provided.

2.6.4.7 Pharmacokinetic drug interactions

No studies were provided.

2.6.4.9 Discussion and Conclusions

In animals receiving NX1838 intravitreously, vitreous levels of NX1838 remained high for a long period, and decreased very slowly.

Following intravitreal administration, high radioactivity concentrations were found in rabbit vitreous fluid and retina. Following both intravitreal and intravenous administrations, highest concentrations of radioactivity were obtained in the kidney, spleen, bone marrow, lymph node (mesenteric) and liver of rabbits. Very low levels of radioactivity were found in the eye following systemic administration. Pegaptanib did not seem to bind to melanin. The volume of distribution was low and close to the plasma volume, suggesting minimal tissue uptake. In pregnant mice, pegaptanib crossed the placenta and was found in the amniotic fluid.

In vitro studies with plasma from humans, rabbits, dogs, and monkeys suggested that pegaptanib sodium be degraded by nucleases into nucleotides in all species. The extent of degradation of parent compound and formation of 2'-fluoro-2'-deoxyuridine in different plasma and species was likely associated with the amount of nucleases released from blood cells during the preparation of plasma. Pegaptanib sodium exhibited significant resistance to nucleases. PEG conjugation was proved to be important in increasing plasma drug residence time by reducing plasma drug clearance rate, possibly through decreased renal filtration, and by increasing the resistance of drug to nucleases.

Following intravenous or intravitreal administration, the predominant route of excretion was via urine. Feces were only a minor route of excretion, suggesting little biliary elimination of radioactivity.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

No tabulated summary was provided by the sponsor.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

In single dose systemic toxicity studies using intravenous injection, NX1838 at doses up to 450 mg/kg in SD rats and 5 mg/kg in monkeys was well tolerated. In single dose intravitreous injections in NZW rabbits and monkeys, NX1838 at doses of 0.5 mg/eye in rabbits and 1.5 mg/eye in monkeys was well tolerated. In monkeys, at 2 mg/eye, mild ocular inflammation, characterized by minimal to mild conjunctival reddening and pupil constriction was seen at 24 hr to Day 8 after dosing. Similar responses were seen in repeated dose studies that were attributed to minor trauma to the eye or mild infection secondary to the injection procedure.

Several repeated dose ocular toxicity studies were conducted with duration up to 3 months in monkeys, 6 months in rabbits and 9 months in dogs. No drug-induced systemic and ocular toxicity was observed. However, injection-procedure-induced ocular changes were seen in all studies in both control and treated animals. The lesions included clinical signs (conjunctival injection, bruising, swelling, conjunctival/scleral hemorrhage, squinting, ocular discharge, tearing, corneal/lens opacity, and/or prominent third eyelid), ophthalmology examination findings (tapetal scars - in dogs only, subcapsular cataracts, focal retinal hemorrhages and partial retinal detachments), and cellular infiltration, fibrosis or lens changes in histopathological examinations. All these changes were reversible. Based on study results, NOAELs were determined as 0.5 mg/eye for monkeys, 2.0 mg/eye for rabbits, and 3.0 mg/eye for dogs.

In a systemic toxicity study, SD rats were treated with daily iv doses of NX1838 at 0.1, 1 and 10 mg/kg for 13 weeks. Chronic progressive nephropathy and lymphoid depletion in the spleen were observed in all groups but with higher incidences and severity in MD and HD groups. The clinical significance of these findings was not known since doses used in this study (6 and 60 mg/m^2) were much higher than that proposed for humans (0.4 mg/m^2). Phagocytized pegaptanib evidenced by vacuolated macrophages were found in many tissues including bone marrow, kidney, liver, pancreas, salivary gland, testis, ovary, and so forth in all HD animals and MD males. These changes were not related to tissue damages. The dose of 1 mg/kg was determined to be the NOAEL for both male and female animals.

The potential of 2'-fluorouridine and 2'-fluorocytidine, two major degradation products of NX1838, to induce toxicity similar to that of FIAU was investigated by intravenous injection to male Fisher 344 rats at 5, [redacted] mg/kg/day, and to woodchucks at 0.[redacted] or 7.5 mg/kg/day for 90 days. Neither compound showed evidence of toxicity similar to that induced by the antiviral drug, FIAU.

Genetic toxicology:

NX1838 was nonmutagenic in the Ames test and in the L5178Y/$TK^{+/-}$ mouse lymphoma mutagenesis assay. The drug was also negative in *in vivo* micronucleus assay and *in vitro* SHE cell assay.

Genetic toxicology studies were also conducted with monomer nucleotides. In chromosomal aberrations studies with human whole blood lymphocytes in the absence of S9 activation, results were negative for all 2'-fluoropyrimidines and 2'-O-methylpurines. In the Ames test, results for all nucleotides were negative in the *Salmonella* strains. In *E. coli*, results for the 2'-O-methylpurines were negative, while 2'-fluoropyrimidines yielded a marginal but reproducible positive response.

Carcinogenicity:

No carcinogenicity studies were conducted on NX1838. A waiver for carcinogenicity studies was granted by the review division on August 8, 2003.

Reproductive toxicology:

In a non-GLP dose-range finding study in rabbits, EYE001 at doses up to 2 mg/eye (intravitreous injection on gestation days 6, 13 and 19) caused no general and developmental toxicity.

In an embryofetal development study in pregnant CD-1 mice, EYE001 at iv doses up to 40 mg/kg/day did not cause any maternal toxicity. However, at 40 mg/kg/day, fetal weights were

significantly reduced for both male and female fetuses. A reduction in the average number of ossified forepaw phalanges was also noted. These changes were within the historical control ranges. In addition, no body weight differences were seen in naturally delivered pups. Therefore, these findings might not be toxicologically significant. No other reproductive or litter parameters were affected. No fetal gross external, soft tissue or skeletal malformations were caused by EYE001. It should be pointed out that an MTD in the dams was not reached in this study. The dams could have tolerated higher doses.

Natural delivery and litter observations were unaffected and no clinical or necropsy observations in the F1 generation pups were attributable to the treatment. Pups in MD and HD groups showed an increase in heart rate and a decrease in RR, PR, and QT intervals in ECG examination performed on DL 13, 14, 15, 16 or 17, but not on DL10. The toxicological significance was not known.

Special toxicology:

In the immunogenicity study, no antibody against NX1838 was detected in mice, rats and rabbits after intravenous, intravitreous or subcutaneous administrations. Unlike DNA-based oligonucleotides, NX1838 and RNA-based oligonucleotides induced little or no immune stimulation in human or mouse lymphocytes *in vitro*. The monomer 2'-fluorouridine was incorporated into cellular DNA and RNA of all tissues examined following subchronic iv administration of NX1838 and 2'-FU to SD rats, Fisher 344 rats and woodchucks.

2.6.6.2 Single-dose toxicity

109-97002-T: Twelve-day pilot intravitreous toxicity study associated with intravitreous NX1838 administration

Report Nº: 109-97002-T
Compound: NX1838 (Lot #: 1838.04) suspended in PBS with Ca^{++} and Mg^{++}
Dose: 0.5 mg/eye (40 µl, one eye; the other eye was assigned to PBS control.)
Dosing regimen: Single dose
Route: Intravitreous
Animal: Male New Zealand white rabbit (2.02-2.37 kg, n = 6)
Study site: [redacted]
Study initiation: 7/10/1997
GLP: No

The purpose of this study was to determine the ocular toxicity of NX1838 following intravitreous administration of the drug in the rabbit. Toxicity was assessed as shown below.

Toxicity assessment for Study 109-97002-T

Parameter	Procedure
Ophthalmologic examinations	Prior to, immediately after injection, and on Days 5 and 12 post-injection
Histopathologic examinations	On Day 12 following the final ophthalmologic examination, ocular tissues

Results:

Ophthalmologic examinations: Cataracts were observed in the NX1838-treated eyes in 2 rabbits, and were considered iatrogenic because of their focal nature and the absolute lack of any cataract formation in the other 4 NX1838-treated eyes. No changes were noted in the choroidal or retinal structures.

Histopathologic examinations: No treatment-related changes were noted.

Conclusion: Single intravitreous injection of NX1838 at the dose of 0.5 mg/eye was well tolerated. No ocular toxicity related to NX1838 was observed.

109-97003-T: Single dose intravenous toxicity study of NX11838 in rats with a 30-day observation period

Report N^{o}: 109-97003-T
Compound: NX1838 (Lot #: 11838.26) dissolved in PBS
Dose: 0 (PBS), 50, 150 and 450 mg/kg
Route: Intravenous
Dosing regimen: Single dose at a dose volume of 7.5 ml/kg
Animal: Sprague-Dawley rats, 7 weeks old, 194-222 g for males and 143-165 g for females, 5/sex/group
Study site:
Study initiation: 11/10/1997
GLP: Yes

The purpose of this study was to evaluate the acute toxicity of NX1838 following a single intravenous injection of NX1838 in rats. The day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment for Study 109-97003-T

Parameter	Procedure
Clinical observations	Twice daily
Body weights	Weekly
Food consumption	Weekly
Gross pathology	A complete gross postmortem examination was conducted on all animals on Day 31.

Results:

Clinical observations: No treatment-related clinical signs were noted.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No treatment-related effects on food consumption were observed.

Gross pathology: No treatment-related, toxicologically significant changes were observed.

In conclusion, no treatment-related effects on survival, clinical observations, body weights, food consumption, and gross pathology were noted following a single intravenous injection of NX1838 in rats. The no observable effect level (NOEL) was 450 mg/kg or higher.

109-98006-B: Pharmacokinetics of an anti-VEGF aptamer, NX1838, following intravitreal administration in rhesus monkeys

Report Nº: 109-98006-B
Compound: NX1838 (Lot: D0905F)
Dosing regimen: Single dose (intravitreal injection), 0.5 mg/0.067 ml/eye
Animal: Six female rhesus monkeys (4-5 years old, 3.4-5.2 kg)
Study site: —
Study initiation: 6/23/1998
GLP: Yes

The purpose of this study was to determine plasma and vitreal PK of NX1838 in rhesus monkeys following a single intravitreal injection. PK/TK analysis was reviewed in detail under section 2.6.4.3 Absorption. The toxicology part is reviewed here. The day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Clinical observations: Twice daily
PK/TK: See section 2.6.4.3 Absorption.
Ophthalmic examinations: All animals: pre-study and Days 1 and 6; Group 2 animals: Day 27
Necropsy: Eyes only, three animals (Group 1) on Day 7 and three animals (Group 2) on Day 28

Results:

Clinical signs and mortality: No mortality occurred in this study. No drug-related clinical signs were observed.

Ophthalmologic examinations: No drug-related abnormalities were noted. On Day 27, hyper-reflectivity in the fundic area was observed in one animal. This condition, attributed to reflection from the light of the ophthalmoscope, was occasionally seen in untreated animals, and was not considered as biologically relevant.

Necropsy examinations: No visible lesions of the eye were noted at either Day 7 or Day 27 sacrifices.

Conclusion: Single intravitreous injection of NX1838 at the dose of 0.5 mg/eye to rhesus monkeys was well tolerated. No ocular toxicity related to NX1838 was observed.

109-98011-T: An acute toxicity study of NX1838 given by intravenous administration to rhesus monkeys

Report Nº: 109-98011-T
Compound: NX1838 (Lot: D0908F) in PBS
Dosing regimen: Single dose (intravenous injection), 5 mg/kg
Animal: Six female rhesus monkeys (2.8-4.7 years old, 3.0-4.1 kg)
Study site: —
Study initiation: 6/4/1998
GLP: No

Study design

Group	N	Treatment	Dose (mg/kg)	Dosing volume (ml/kg)
1	1• ; 1• •	Vehicle	0	4
2	2• ; 1• •	NX1838	5	3.82

The purpose of this study was to evaluate the acute toxicity of NX1838 following a single intravenous injection of NX1838 in rhesus monkeys. The day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Clinical observations: Twice daily
Food consumption: Daily
Body weights: Weekly
Clinical pathology: Pre-study, 24 hr after dosing, and on Day 7
Complement split (C4d and Bb): Pre-study and 1 and 24 hr after dosing
TK: 1, 8 and 24 hr after dosing
Ophthalmic examinations: All animals: pre-study and Days 1 and 6; Group 2 animals: Day 27
Necropsy: Group 2 animals on Day 8

Results:

Clinical signs and mortality: No deaths occurred in this study. No drug-related clinical signs were observed.

Food consumption: Not reported.

Body weights: No treatment-related abnormalities were noted.

Clinical pathology: No drug-related, toxicologically significant changes in hematology and clinical chemistry were seen. Regarding coagulation parameters, increased activated partial thromboplastin time was noted in treated animals (see table below). Similar finding was not seen in other toxicity studies. Hence, it was not considered as toxicologically significant.

Activated partial thromboplastin time (APTT) in monkeys treated with NX1838

Animal #	Sex	Pre-study	Day 1	Day 2	Day 7
Control					
R6793M	M	21.8	23.1	25.0	23.1
R6755F	F	19.2	22.1	20.3	19.5
Treated					
R5725M	M	20.5	27.7	24.1	25.6
R5878M	M	20.5	26.9	25.2	22.4
R6789F	F	19.7	26.9	23.7	25.0

Complement split (C4d and Bb): No drug-related differences in Bb and C4d complement factor levels were noted.

Necropsy examinations: No visible lesions were noted in any animals.

Conclusion: Single intravenous injection of NX1838 at the dose of 5 mg/kg to rhesus monkeys was well tolerated. No toxicity related to NX1838 was observed.

109-980142-T: An acute intravitreous safety study (0699-35) with NX1838 in rhesus monkeys

Report N^{o}: 109-980142-T
Compound: NX1838 (Lot #s: RN98000122 and RN97000690)
Vehicle: PBS
Dosing Regimen: Single dose (intravitreal injection), 0.25, 0.5, 1.0, 1.5 and 2.0 mg/0.065 ml/eye, both eyes
Animal: Rhesus monkeys
Study Site: —
GLP: No
Study design

Group	N/sex	Dose (mg/eye)	Lot #
1	1*	Vehicle	RN97000690
2	1**	0.25	
3	1	0.5	
4	1#	1.0	
1	1#	0.25	RN98000122
2	1	0.5	
3	1**	1.0	
4	1	1.5	
5	2 females*	2.0	

*, **, and #: same animals with treatment intervals of 29 days

The purpose of this study was to determine the potential toxicity of two clinical lots of NX1838 in rhesus monkeys following a single intravitreal injection at doses of 0.25 to 2.0 mg/eye. The day of dosing was designated as Day 1. A direct ophthalmic examination was performed on all animals at 24 hr following dosing. An additional examination was conducted in animals receiving 1.5 mg/eye 6 days after dosing. For animals treated at 2.0 mg/eye, the examination was performed on Days 2, 4, 8, and 29.

Results:

No systemic toxicity was seen in animals treated at doses up to 2.0 mg/eye. Regarding ocular examinations, slight ocular discharge and slight conjunctival reddening were noted in all groups including control animals at 24 hr time point. These changes were not considered as drug-related since mild ocular discharge and conjunctival reddening were common findings related to the intravitreal injection procedure. In animals at 2 mg/eye, mild ocular inflammation, characterized by minimal to mild conjunctival reddening (in two animals) and pupil constriction (in one animal), was seen at 24 hr to Day 8. No abnormalities were seen on Day 29. The sponsor indicated that similar responses were seen in other studies that were attributed to minor trauma to the eye or mild infection secondary to the injection procedure.

Conclusion: Single intravitreal injection of NX1838 from both lots at doses up to 1.5 mg/eye in rhesus monkeys was well tolerated. An injection at 2.0 mg/eye caused a mild inflammatory response that could not be definitely attributed to NX1838.

2.6.6.3 Repeated-dose toxicity

Study title: 109-98003-T: An 11-week intravitreous toxicity study of VEGF aptamer NX1838 [40K PEG] in Dutch-belted rabbits

Key study findings: Repeated intravitreous injection of NX1838 produced no systemic toxicity. Slight attenuation of the retinal vessels, and mild cyclitis associated with macrophage infiltration in the vitreous cavity and optic papilla were attributed to NX1838. The NOAEL was 1 mg/eye.

Study no.: 109-98003-T
Volume #, and page #: ECTD
Conducting laboratory and location:

Date of study initiation: 11/26/1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NX1838, Lot: 26, purity >
Study Design:

Groups	Dose (mg/eye)	Dose volume	N/sex
1. Control (PBS)	0	50 µl/eye	5
2. NX1838	0.1 (for Weeks 1, 3, 5 and 7)/2.0 (for Weeks 9 and 11)	50 µl/eye	5
3. NX1838	0.3	50 µl/eye	5
4. NX1838	1.0	50 µl/eye	5

The purpose of this study was to evaluate the toxicity of NX1838 following intravitreous administration of the drug every 2 weeks for 11 weeks in the rabbit.

Methods

Doses: 0.1, 0.3, 1.0 or 2.0 mg/eye, qow for 11 weeks (6 injections/each animal)
Species/strain: Dutch-belted rabbits
Number/sex/group or time point (main study): 5
Route, formulation, volume, and infusion rate: Intravitreous, PBS, 50 µl/eye
Satellite groups used for toxicokinetics or recovery: N/A
Age: 5-6 months old
Weight (nonrodents only): 1.7-2.3 kg for males and 1.8-2.2 kg for females
Unique study design or methodology (if any): No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Ophthalmoscopy: Prior to each dose, 48 hr after the first dose, and 1 week following the last dose

Hematology: Prior to each treatment, and immediately prior to necropsy

Clinical chemistry: Prior to each treatment, and immediately prior to necropsy

Antibody titer: All animals, prior to necropsy

Gross pathology: All animals, Week 12

Organ weights (specify organs weighed if not in histopath table): The following organs from each animal were weighed: adrenal, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid and parathyroids, and uterus.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Histopathologic examinations were conducted on the right eyes (Groups 1 and 2 only) and left eyes, as well as heart, liver, lung, kidney, spleen and gross lesions from all animals.

Toxicokinetics: See section 2.6.4.3 Absorption.

Results:

Mortality: One Group 3 female was found dead in Week 5. The cause of the death was due to anesthetic administration and was not drug-related.

Clinical observations: Transient ocular swelling was noted after dosing in Weeks 7, 9 and 11, which was injection procedure-related, but not drug-related.

Body weights: No treatment-related differences in body weights were noted.

Ophthalmology: Slight attenuation of the retinal vessels was observed in all treated groups in the late treatment period (after Week 9), which was considered NX1838 treatment-related. Mild cyclitis was a most commonly observed finding (beginning in Week 2), and was also attributed to NX1838. The clumps of undissolved NX1838 were observed during biomicroscopic examinations in Weeks 9 and 11. No treatment-related differences in ERG and intraocular pressure were noted. Signs of irritation (slight aqueous flare, mild photophobia, and/or incomplete mydriasis) on most eyes were observed 48 hr after the first injection, which disappeared in 10 days, and were considered injection procedure related. The following table shows the incidence of cyclitis and retinal vascular attenuation on Day 77.

Incidence of cyclitis and retinal vascular attenuation on Day 77

Ocular lesion	Total number of eyes per group			
Group	1	2	3	4
Number of eyes	20	20	18	20
Cyclitis (slight)	2	13	11	16
Cyclitis (moderate)	0	6	1	3
Attenuation of retinal vessels (slight)	0	5	5	7
Attenuation of retinal vessels (moderate)	0	2	0	0

Clinical pathology: No treatment-related, toxicologically significant differences in hematology and clinical chemistry were noted.

Organ weights: No treatment-related differences were noted.

Gross and histopathological examinations: No treatment-related differences were noted in gross examinations. Drug-related differences observed in histopathologic examinations were listed in the following table. The macrophage infiltration into the vitreous cavity was consistent with clinically observed cyclitis. Slight retinal degeneration characterized by disorganization of the outer layers of the retina was noted in 1 of the 10 animals in Group 2 (2 mg/eye).

Incidence of histologic examination in male and female rabbits

	Male				Female			
Treatment group	1	2	3	4	1	2	3	4
Left eye								
Infiltration of macrophage in vitreous cavity	0/4	5/5	5/5	5/5	0/5	5/5	2/5	5/5
Infiltration of macrophage in optic papilla	0/4	4/5	1/5	3/5	0/5	1/5	1/5	4/5
Basophilic material in vitreous cavity	0/4	5/5	4/5	5/5	0/5	4/5	2/5	4/5
Right eye								
Infiltration of macrophage in vitreous cavity	0/1	5/5	0/5	0/5	0/2	5/5	0/5	0/5
Infiltration of macrophage in optic papilla	0/1	4/5	0/5	0/5	0/2	5/5	0/5	0/5

In conclusion: Intravitreous injection of NX1838 produced no systemic toxicity. Slight attenuation of the retinal vessels, and mild cyclitis associated with macrophage infiltration in the vitreous cavity and optic papilla were attributed to NX1838. The NOAEL was 1 mg/eye.

Study title: 109-98004-T: A 13-week intravenous toxicity study of NX11838 in rats

Key study findings: Histopathologic examinations indicated spleen and kidney changes. The necrosis of the kidneys could explain the weight increases and total plasma protein decreases. The dose of 1 mg/kg was determined to be the NOAEL for both male and female animals.

Study no.: 109-98004-T
Volume #, and page #: ECTD
Conducting laboratory and location: —
Date of study initiation: 10/17/1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NX1838, Lot: 11838.2f — purity: —

The purpose of this study was to evaluate the subchronic toxicity of NX1838 in a 13-week intravenous injection study in rats.

Methods

Doses: 0 (PBS), 0.1, 1 and 10 mg/ml/kg, qd x 13 weeks at a dose volume of 1 ml/kg
Species/strain: Sprague-Dawley - CD®BR VAF/Plus® rats
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: intravenous, PBS
Age: 7 weeks old
Weight: 219-239 g for males and 154-176 g for females

Observation times and results

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: All rats received an indirect ophthalmoscopic examination during the pretest period and prior to their scheduled sacrifice.

Hematology: Prior to terminal sacrifice
Clinical chemistry: Prior to terminal sacrifice
Gross pathology: All animals
Organ weights (specify organs weighed if not in histopath table): The absolute and relative weights of the following organs were measured: brain, adrenal, heart, kidney, liver, pituitary, spleen, testis, thymus, thyroid/parathyroid, and ovary.
Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
TK: Days 1 and 91 at 30 min and 24 hr postdose.

Results:

Clinical observations: A control male died on Day 35 and a female at 1.0 mg/kg died on Day 46. The cause of death for the treated animal was unknown but the sponsor indicated that it was the result of NX11838 administration. No treatment-related clinical signs were noted.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No treatment-related effects on food consumption were observed.

Ophthalmoscopic examinations: No treatment-related ocular effects were observed.

Clinical pathology: No treatment-related, toxicologically significant changes in hematology were noted. In clinical chemistry, changes possibly related to the treatment with NX1838 were listed in the table below. Changes in total protein and albumin in males receiving 10 mg/kg/day of NX1838 may be related to the chronic progressive nephropathy.

Clinical chemistry changes following multiple intravenous administration of NX1838

Dose (mg/kg/day)	0	0.1	1	10	0	0.1	1	10
	Males				Females			
Total protein (g/dl)	6.8	6.7	6.9	6.3	6.9	7.1	6.9	7.8
Albumin (g/dl)	3.4	3.4	3.4	**3.1**	3.5	3.5	3.4	3.5
Cholesterol	57	58	56	**89**	69	68	73	**88**

Macroscopic examinations: No NX1838-related gross changes were noted.

Organ weights: Statistically significant differences were limited to the kidney, spleen, liver, testis and pituitary (see the table below). The weight increases were due to the presence of vacuolated macrophages within these tissues except for the kidney, in which the increased severity of chronic nephropathy could be the cause of increased kidney weight in the 10 mg/kg/day males.

Terminal organ weights following multiple intravenous administration of NX1838 (g)

Dose (mg/kg/day)	0	0.1	1	10	0	0.1	1	10
	Males				Females			
Kidney	4.26	4.51	4.47	4.87	2.30	2.57	2.33	2.55
Liver	17.01	18.86	17.92	21.42	8.59	9.35	8.98	10.77
Spleen	0.86	0.96	0.96	1.14	0.61	0.70	0.66	0.75
Testis	3.74	3.92	3.80	4.08				
Pituitary	16	16	16	17	18	19	20	23

Histopathology: Renal and spleen changes are summarized in the table below. In addition, vacuolated macrophages were found in many tissues including bone marrow, kidney, liver, pancreas, salivary gland, testis, ovary, and so forth in all HD animals and MD males. The incidence and severity increased with dose. No vacuolated macrophages were found in the 0.1 mg/kg/day animals. These changes were not related to tissue damages. The vacuolated macrophages may represent phagocytized PEG and/or NX1383. The table below shows treatment-related changes in spleen and kidney.

Histopathologic changes in the spleen and kidney following multiple iv administration of NX1838

Dose (mg/kg/day)	0	0.1	1	10	0	0.1	1	10
	Males				Females			
Kidney								
Chronic progressive nephropathy	3/10	6/10	6/10	10/10	3/9	2/10	2/9	4/10
--trace	3	6	6	4	3	2	2	3
--mild				6				1
Spleen								
Lymphoid depletion	0/10	0/10	2/9	8/10	1/10	1/10	0/10	7/9
--trace			1	4				3
--mild			1	4		1		4
--moderate					1			

In summary: SD rats were treated with daily iv doses of NX1838 at 0.1, 1 and 10 mg/kg for 13 weeks. The drug was generally well tolerated. However, chronic progressive nephropathy and lymphoid depletion in the spleen were observed in all groups but with higher incidences and severity in MD and HD groups. The clinical significance of these findings was not known since doses used in this study (6 and 60 mg/m^2) were much higher than that proposed for humans (0.4 mg/m^2). Phagocytized PEG and/or NX1383 evidenced by vacuolated macrophages were found in many tissues including the bone marrow, kidney, liver, pancreas, salivary gland, testis, ovary, and so forth in all HD animals and MD males. These changes were not related to tissue damages. The dose of 1 mg/kg was determined to be the NOAEL for both male and female animals.

109-98010-T: A 3-month intravitreous toxicity study (0640-35) with NX1838 in rhesus monkeys

Key study findings: Single intravitreous injection of NX1838 (0.5-2.0 mg/eye) produced inflammation-related changes. In repeated treatment section, compound-related changes (blurring fundus and brown haze, and histological changes) were observed only in the 0.5 mg/eye group and only after dose 1 with Lot 11838.26 — . The endotoxin level in this lot — unit/mg NX1838) was about 2-fold higher than that of the drug preparation (— unit/mg NX1838) used for Doses 2-4. In this study, 0.5 mg/eye was considered the no observed effect level (NOEL).

Study no.: 109-98010-T
Volume #, and page #: ECTD
Conducting laboratory and location: —
Date of study initiation: 5/17/1998
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NX1838, Lot: 11838.26 ; — , purity: — , D09805F, D09806F, D09807F, D09809F, D09810F, and D09812F

The purpose of this study was to evaluate the potential toxicity of NX1838 when administered as 6 semi-monthly intravitreal injections at doses of 0.25 and 0.5 mg/eye, or 4 semi-monthly intravitreal

injections at a dose of 0.1 mg/eye followed by 2 semi-monthly injections at 1 mg/eye. In addition, effects of different lots of NX1838 administered as a single intravitreal injection at doses of 2.0 and 1.0 mg/eye were evaluated. The day of the 1st dose was designated as Day 1.

Methods

Doses: 0 (PBS), 0.1, 0.25, 0.5, 1.0 and 2.0 mg/ml/eye
Species/strain: Rhesus monkeys
Number/sex/group or time point (main study): See study design.
Route, formulation, volume, and infusion rate: Intravitreal, PBS
Satellite groups used for toxicokinetics or recovery: N/A
Age: 3-5 years old
Weight: 2.7-4.5 kg
Unique study design or methodology (if any): See Study Design.

Study Design: The original toxicity study design is presented in the table below:

Group	Dose (mg/eye)	Dosing frequency	Dose volume (µl)	# of sacrificed in Month 12 (M/F)	# of sacrificed in Month 15 (M/F)
1	Control	2X/month	66	3/3	
2	0.5	2X/month	66	3/3	2/2
3	1.0	2X/month	66	3/3	
4	2.0	2X/month	66	3/3	2/2
5	2.0	Monthly	66	3/3	

On May 17, 1998, 19 monkeys were dosed as scheduled with the drug [Lot: 11838.26 — .)]. Twenty-four hr after dosing, mild to severe ophthalmic inflammation was observed in all treated animals. No changes were noted in the 3 animals receiving PBS. As a result, the sponsor changed the whole study protocol. The animals that received 1.0 and 2.0 mg/eye did not receive additional treatment, and were included in Section 1 of the new protocol (see table below).

Section 1 (single injection study): Eleven monkeys were treated with a single intravitreous injection of NX1838 [Lot: 11838.26 —] as shown in the following table.

Dose (mg/eye)	Dose volume (µl)	N (M/F)	Number sacrificed (M/F)	
1.0	66	1M/2F	1/0 (Day 3)	0/2 (Day 32)
2.0	66	4M/4F	2/2 (Day 3)	2/2 (Day 32)

The other animals were included in the re-designed 3-month study (Section 2, see table below) with the original low dose becoming the high dose. The drug with different lot numbers was used (Lots: D09805F, D09806F, D09807F, D09809F, D09810F, and D09812F).

Section 2 (revised 3-month study): Twenty-four animals were treated with NX1838 for 3 months as shown in the following table. Since no adverse effects were noted following 4 injections at 0.5 mg/eye, the Group 2 animals (at 0.1 mg/eye) were dosed at 1.0 mg/eye for the last two doses.

Group	Dose (mg/eye)	Dosing frequency	Dose volume (µl)	N/sex	N sacrificed after 3 months/sex
1	Vehicle control	2/month	66	3	3
2	0.1 (doses 1-4) and 1.0 (doses 5-6)	2/month	66	3	3
3	0.25	2/month	66	3	3
4	0.5	2/month	66	4• 2• *	4• 2• •

* Five of six Group 4 animals were treated with Lot .26 — for the 1st dose.

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Section 1 animals (1 mg/eye and 2 mg/eye groups): Ocular changes were observed following the initial dose. On Days 2 and 16, and at the end of the month, animals were given a complete ophthalmologic evaluation that included tonometry, biomicroscopic and indirect ophthalmoscopic examinations. On Days 3, 5 and 10, direct ophthalmoscopic examinations were performed. ERG was recorded on Day 27.

Section 2 animals: IOP, indirect ophthalmoscopic and slit lamp biomicroscopic examinations were performed prior to every dose, 1 day after each dose, and prior to necropsy. Direct ophthalmoscopic examinations were performed on the animals that were dosed with Section 1 animals at the same time on Days 3, 5 and 10, and 1 day after Dose 2. Direct ophthalmoscopic examinations were also performed 72 hr following Dose 1, 2 and 3, and 1 day after Dose 5 and 6. ERG was recorded prior to Doses 1, 4 and 6, and prior to necropsy.

EKG, BP and HR: Prior to the first dose and terminal sacrifice (for repeated phase only)

Hematology: Pre-study and Weeks 8 and 13 (repeated phase only)

Clinical chemistry: Pre-study and Weeks 8 and 13 (repeated phase only)

Urinalysis: At necropsy

Antibody: Prior to the study, and Weeks 4 and 13 (repeated phase only)

Gross pathology: All animals (single dose animals: Days 3 and 32; repeated dose animals: 2 weeks after the last dose)

Organ weights (specify organs weighed if not in histopath table): The absolute and relative weights of the following organs were measured: brain, adrenal, heart, kidney, epididymides, liver, lungs, pituitary, spleen, testis, prostate, thymus, thyroid/parathyroid, uterus, and ovary.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )
For Section 1 animals, histopathologic examinations were performed on the eyes only.

TK: Blood samples were collected from animals prior to and 24 hr after the first and 6th doses, and 2 weeks after the 1st, 5th, and 6th doses.

Results:

Dose solution analysis: Lot 11838.26 → Lot 1] showed higher endotoxin levels (EU/mg) than other lots (EU/mg).

Clinical observations:

Section 1 animals: Drug-related clinical signs (see table below) were observed, which disappeared on Day 3. The clinical signs were associated with ocular inflammation.

Clinical signs in Section 1 animals

Dose level	Clinical sign	Incidence
1.0 mg/eye	Cloudy cornea right eye	1/3
2.0 mg/eye	Cloudy cornea/corneal opacity (both eye involved)	4/8
2.0 mg/eye	Decreased activity	3/8
2.0 mg/eye	Squinting	1/8

Section 2 animals: Transient and mild clinical signs observed included redness of the conjunctiva, redness or swelling of the area around the eye, and squinting. These signs were not dose-dependent, and appeared only in the treated groups. The sponsor indicated the signs were related to the injection procedure and were seen in other control animals in other studies.

Body weights and food consumption: No treatment-related differences in body weights and food consumption were noted.

Ophthalmology:

Section 1 animals:

Biomicroscopic examinations: Drug-related, dose-dependent inflammatory changes were noted 1 day after dosing. The incidence of these changes is listed in the table below. These inflammatory responses were reversible. No changes were observed at Week 3.

Incidence of the ocular changes observed in biomicroscopic examinations

Incidence (number of animals affected/number of animals in group)		
	2.0 mg/eye	1.0 mg/eye
Iris bombe	Both eyes: 1/8	0/3
Fibrin in anterior chamber/aqueous	Both eyes: 5/8; one eye: 3/8 (mild to severe)	Both eyes: 1/3; one eye: 1/3 (mild to severe)
Conjunctival congestion	Both eyes: 8/8 (minimal to mild)	Both eyes: 3/3 (minimal to mild)
Conjunctival swelling	Both eyes: 1/8; one eye: 1/8 (mild)	0/3
Aqueous flare	Both eyes: 8/8 (minimal to moderate)	Both eyes: 3/3 (minimal to moderate)
Diminished light reflex	Both eyes: 2/8; one eye: 1/8 (mild)	One eye: 1/3 (mild)
Iris involvement	Both eyes: 4/8 (minimal to moderate)	Both eyes: 1/3 (minimal)
Cells in anterior chamber	Both eyes: 4/8; one eye: 1/8 (mild to moderate)	Both eyes: 2/3; one eye: 1/3 (mild to moderate)
Corneal cloudiness	Both eyes: 2/8; one eye: 3/8 (minimal to severe)	One eye: 1/3 (mild to severe)

Indirect ophthalmoscopic findings: The fundus was not visible or blurred in 2/3 animals in the 1 mg/eye group and in 6/8 animals in the 2 mg/eye group. In the Week 3 examinations, no abnormalities were noted.

Direct ophthalmoscopic observations: The findings on Days 3, 5 and 10 in these examinations revealed a gradual improvement in severity. The incidence is summarized in the table below.

Incidence of the findings observed in direct ophthalmoscopic examinations

	Blurred fundus		Brown haze		Fibrin	
Time	1 mg/eye	2 mg/eye	1 mg/eye	2 mg/eye	1 mg/eye	2 mg/eye
Day 3	1/2	4/4	1/2	0/4	0/2	2/3
Day 5	2/2	4/4	1/2	2/4	0/2	0/4
Day 10	0/2	1/4	0/2	0/4	0/2	0/4

Tonometry: The increases in IOP (from 18-22 mmHg to 39-60 mmHg) were observed in 2 animals (one from each dose group) at the first week, which could be related to the ocular inflammation.

ERG: No drug-related findings were observed.

Section 2 animals: The inflammatory responses seen in animals at 0.5 mg/eye were attributed to dosing from high endotoxin material [Lot 11838.26 -].

Biomicroscopic examinations: Conjunctival congestion and discharge were noted 24 hr after each dose in all groups. These transient changes were considered to be related to acute ocular trauma from repeated intravitreous injections. Aqueous flare was noted only after the 1st dose in 4 of 6 animals at 0.5 mg/eye. These animals were treated with Lot 11838.26 -) and might be lot-related.

Indirect ophthalmoscopic findings: The following treatment-related changes were noted 1 day after the first dose: a pinpoint opacity in the right mid-vitreous (1 animal in the 0.1 mg/eye group), an area of pigment clumping adjacent to the left nasal optic disc (1 animal in 0.25 mg/eye group), and blurred/obscured fundus (2 animals in the 0.5 mg/eye group). Most of the changes disappeared at Week 3. The examinations for Doses 2, 3, 4, 5 and 6 were considered within normal limits.

Direct ophthalmoscopic observations: In animals treated with 0.5 mg/eye of NX1838 with the same lot number as used in Section 1 animals, the changes on Day 3 included blurring of the fundus and the presence of mild to moderate brown haze in 4/5 animals. The severity decreased on Day 5. On Day 10, mild

blurring of the fundus in 1/5 animals was the only alteration. No other toxicologically significant findings were observed.

Tonometry: No drug-related findings were observed.

ERG: No drug-related findings were observed.

Clinical pathology: No treatment-related effects were noted in clinical chemistry, hematology, coagulation parameters and urinalysis.

Antibody analysis: No IgG antibodies directed against NX1838 were identified.

Necropsy

Section 1 animals: In the 5 animals sacrificed on Day 3, the predominant lesion in all animals was comprised of slight opacity in the anterior chamber of one or both eyes. In the animals receiving 2 mg/eye of NX1838, increased viscosity of the vitreous humor was noted in 1 animal; conjunctival exudate was observed in another one. Histologically, the opacity was correlated with the presence of fibrin and/or a mixed cell infiltrate in the anterior chamber. In the 6 animals sacrificed on Day 32, no treatment-related findings were noted.

Section 2 animals: No drug-related abnormal changes were observed.

Organ weights: No treatment-related differences were noted.

Histopathology:

Section 1 animals: In 5 animals sacrificed on Day 3, the predominant lesion summarized in the table below was comprised of inflammatory cell infiltration, fibrin deposition and hemorrhage in different regions of the eye. In the 6 animals sacrificed on Day 32, the lesions consisted of minimal hemorrhage in the iris and minimal mixed cell infiltration in the ciliary body and iris.

Incidence of histologic examination in male and female monkeys on Day 3

	Male		Female	Male		Female
	Left			Right		
Dose (mg/eye)	**1.0**	**2.0**	**2.0**	**1.0**	**2.0**	**2.0**
N	**1**	**2**	**2**	**1**	**2**	**2**
Infiltration of mixed cells in anterior chamber				1m		1d
Infiltration of neutrophil in anterior chamber		1m	1d			
Fibrin in anterior chamber			1m		1m	1d
Hemorrhage in anterior chamber			1m			
Hemorrhage in iris		2d	1r	1m	2d	1m1r
Hemorrhage in ciliary body			1r			1m
Infiltration of neutrophil in ciliary body	1d	2d	2d	1m	2d	1m1d
Infiltration of neutrophil in ciliary process		1d			1d	
Fibrin deposition in ciliary processes		2m	1d	1m	1m	
Fibrin deposition in vitreous	1m	2m	1m1d	1d	2d	2m
Infiltration of neutrophil in vitreous	1m	1d	1d	1d	2d	1d
Hemorrhage in retina		2m	1m1r	1m	2m	1r
Retinal degeneration		2m	1m		1m	1m
Retina separation			1d			1d

Vacuolization, nerve			1m			
Infiltration of neutrophil in retina	1m	1m1d	1m1d	1m	1m1d	1m
Peripheral cysts	1m	1m		1m	1d	

m: minimal; d: mild; r: moderate

Section 2 animals: Possibly drug-related ocular findings are summarized in the table below. Minimal extravasation of erythrocytes in the iris was seen in 3 Group 4 animals (0.5 mg/eye). Mononuclear cell infiltration was seen in 2 Group 4 animals. The sponsor indicated that all animals that showed these changes in the iris had received the initial dose from the high endotoxin lot of NX1838 (Lot 11838.26). Similar changes were not seen in animals treated with two doses of 1.0 mg/eye, and in animals of the same group (Group 4) that were not treated with Lot 11838.26. Therefore, these changes in the iris might be related to the high concentrations of endotoxin. No abnormal findings were noted in non-ocular tissues.

Incidence of histologic examination in Section 2 monkeys

	Males				Females			
Group	1	2	3	4	1	2	3	4
N	3	3	3	4	3	3	3	2
Left eye, iris								
Scattered stromal erythrocytes	0	0	0	2m	0	0	0	1m
Mononuclear cell infiltrate	0	0	0	2m				
Right eye, iris								
Scattered stromal erythrocytes	0	0	0	1m				

m: minimal

TK: Twenty-four hr after the first dose, plasma levels of NX1838 were detected in animals at doses $\geq$ 0.25 mg/eye. The drug concentration was proportional to dose. No accumulation was detected. NX1838 was detected in the vitreous humor in all treated animals. The concentration was proportional to the dose. The estimated NX1838 vitreous half-life was 3.9 days.

In conclusion: Single intravitreous injection of NX1838 produced inflammatory changes, which were predominantly localized within the anterior chamber, and were reversible. In repeated treatment section, compound-related changes (blurring fundus and brown haze, and histological changes) were observed only in the 0.5 mg/eye group and only after dose 1 in which NX1838 of Lot 11838.26 (—) was used. The endotoxin level in this lot was — NX1838, which was about 2-fold higher than that of the drug preparation (— NX1838) used for Doses 2-4. The sponsor considered that the inflammatory responses observed after the first dosing of 0.5 mg/eye of the drug were associated with the higher endotoxin level in drug preparation. In this study, 0.5 mg/eye was considered NOAEL.

144-002: Three-week local tolerance and toxicity study of EYE001 administered by intravitreous administration to beagle dogs

Key study findings: Weekly intravitreous injection of NX1838 (2 mg/100 µl/week x 3 weeks) into both eyes of male and female dogs was well tolerated locally and systemically.

Study no.: 144-002
Volume #, and page #: ECTD
Conducting laboratory and location: —

—

Date of study initiation: 11/8/2000
GLP compliance: Yes

QA report: yes (X) no ( )
Drug, lot #, and % purity: NX1838 (EYE001), Lot: 121001E

The purpose of this study was to evaluate the local tolerance and potential systemic toxicity of EYE001 when administered intravitreously once weekly for 3 weeks to beagle dogs. The day of the 1st dose was designated as Day 1.

Methods

Doses: 0 (PSS) and 2.0 mg/0.1 ml/eye, both eyes
Species/strain: Young adult beagle dogs
Number/sex/group or time point (main study): See study design.
Route, formulation, volume, and infusion rate: Intravitreal, PSS
Satellite groups used for toxicokinetics or recovery: N/A
Age: Not indicated
Weight: 8.48-14.69 kg
Unique study design or methodology (if any): See Study Design.

Study Design:

Group	Dose (mg/eye)	Dosing frequency	Dose volume (µl)	# of animals (M/F)	Terminated
1	Control	qw x 3 (Days 1, 8 and 15)	100	0/2*	Day 23
2	2		100	1/1	Day 23

* Originally 1 dog/sex was assigned to this group. Due to an eye abnormality in the male dog on study Day 1, the male was replaced with a female.

Observation times and results

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Not indicated
Ophthalmoscopy: Slit lamp biomicroscopy: pretest and Day 15; photographs: pretest, 24 hr after the first dose, and weekly thereafter; fundus photographs: Days 1 and 15
Gross pathology: All animals, Day 23
Organ weights (specify organs weighed if not in histopath table): Not indicated
Histopathology: Not performed

Results:

Clinical observations: No mortality occurred during the study period. No drug-related clinical signs were noted.

Body weights: No treatment-related differences in body weights were noted.

Ophthalmology:

Gross photographs: No abnormalities in gross photographs of the eyes were noted during the study period.

Biomicroscopic examinations: No drug-related effects were noted in all animals examined on Day 15. Several changes, considered as the mechanic trauma from the intravitreal injection procedure, were noted. These changes included preretinal hemorrhage in the left eye in a control animal, and the appearance of vitreal fibrin in the eye (eyes) of all animals.

Necropsy: No drug-related necropsy findings were observed.

In conclusion: Weekly intravitreous injection of NX1838 (2 mg/100 µl/week x 3 weeks) into both eyes of male and female dogs was well tolerated locally and systemically.

Study title: 0460LE15-001: A six-month toxicity study in rabbits of EYE001 by intravitreous administration

Key study findings: Repeated biweekly intravitreous injection of NX1838 at doses up to 2 mg/eye produced no ocular and systemic toxicity. Injection procedure-related findings were observed in all groups that included clinical signs, ophthalmology examination findings, and minimal cellular infiltration and fibrosis in histopathological examinations. The high dose of 2.0 mg/eye was considered as the NOAEL.

Study no.: 0460LE15-001
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: 6/28/2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: EYE001 (Lot #s: HP1214-030-2, 3, and 4)
Study Design:

Groups	Dose (mg/eye)	Dose volume	N/sex	Terminated in Wk 25	Terminated in Wk 31
1 Vehicle	0 (PBS)	67 µl/eye	9	7	2
2. NX1838	0.2	67 µl/eye	7	7	
3. NX1838	0.67	67 µl/eye	7	7	
4. NX1838	2.0	67 µl/eye	9	7	2

The purpose of this study was to evaluate the toxicity of NX1838 following bilateral intravitreous administration of the drug once every 2 weeks for 6 months in the rabbit (total 13 doses).

Methods

Doses: 0 (buffered PSS), 0.2, 0.67, or 2.0 mg/eye (67 µl/eye, both eyes), Once every 2 weeks x 13 (6 months)
Species/strain: New Zealand white rabbits
Number/sex/group or time point (main study): 7/sex/group in LD and MD groups and 9/sex/group for control and HD groups
Route, formulation, volume, and infusion rate: Intravitreous, PBS, 67 µl/eye
Satellite groups used for toxicokinetics or recovery: No
Age: 5-6 months old
Weight (nonrodents only): 3.1-4.2 kg

Observation times

Mortality: Twice daily

Clinical signs: At least once daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Prior to treatment initiation, prior to and 24 hr post-dose for injections 1 (Week 1), 6 (Week 11) and 13 (Week 25), and in Week 30. Photographs of the fundus and macroscopic photos of the eyes of each animal were taken prior to treatment initiation, prior to and 24 hr post-dose for injections 1 (Week 1), 6 (Week 11) and 13 (Week 25). IOP was measured for all animals prior to study initiation, and during Weeks 4, 12, 24 and 30.

Clinical pathology: Prior to treatment initiation, 24 hr post-dose for injections 7 (Week 13) and 13 (Week 25), and at the end of Week 31

Gross pathology: Within 3 days after the 13th dose (main animals) and in Week 31 (recovery animals)

Organ weights (specify organs weighed if not in histopath table): The following organs from each animal were weighed: adrenal, brain, heart, kidneys, liver, ovaries, spleen, testes, and thyroid and parathyroids.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Histopathologic examinations were performed on all tissues from control and HD animals. The heart and left eye from all animals were examined histologically.

Toxicokinetics: See section 2.6.4.3 Absorption.

Results:

Mortality: One HD male died after the 1st injection only in the left eye. Histological examination findings included primarily hemorrhage and autolysis in various tissues. Nuclear pyknosis was seen in renal medulla, but no other lesions consistent with hypoxia/anoxia were noted. The cause of the death was unknown.

Clinical observations: Several clinical signs were noted throughout the study. The signs included conjunctival injection, bruising, swelling, squinting and/or prominent third eyelid. Clear fluid filled bubbles on the exterior portion of the sclera above the area of injection were also noted. These clinical signs were noted in all groups with similar incidence and severity, and were considered most likely the result of trauma/irritation of the dosing procedure. No drug-related clinical signs were noted in systemic observations.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No drug-related abnormal findings in food consumption were observed.

Ophthalmology: Ocular lesions are summarized in the table below. The sponsor indicated that these lesions were likely injection-related. Considering that different ocular lesions were seen in different groups, and similar lesions did not appear in every examination, the reviewer agreed that the ocular lesions were most reasonably interpreted as results of technical difficulties in performing intravitreous injections.

Positive findings in ocular examinations

Animal #	Group	Sex	Comments
			24 hr after dose 1
4024	4	M	OS: 1.3 mm superior posterior linear capsular ectasia
4006	1	M	OU: few superior punctate vitreal opacities
4022	3	M	OS: 1 superior anterior vitreal fibrin stand
			Prior to Injection 6
4024	4	M	OS: superior lens ectasia/posterior capsule, posterior cortical cataract
			24 hr after dose 6
4024	4	M	OS: 2 mm axial corneal erosion; 6 mm dorsal posterior lens capsule ectasia, and posterior cortical cataract
4016	2	M	OS: mild conjunctivitis and iritis
			Prior to Injection 13
4024	4	M	OS: 4 mm superior posterior lens ectasia
4009	1	M	OD: 6 mm axial corneal erosion
4054	3	F	OS: few anterior vitreal cells
			24 hr after dose 13
4031	4	M	OS: axial corneal erosion; moderate conjunctival hyperemia; OD: superior conjunctival swelling

IOP: Mean IOP data are summarized in the table below. Male HD animals seemed to have higher IOP levels relative to controls. However, all levels were within the normal range. In addition, similar increase was not seen in females. The sponsor indicated that the difference between control and HD groups was "more likely due to the viscosity of the HD test article and/or the normal variation of this parameter rather than being presentative a toxic effect of the test article." The reviewer agrees.

Mean IOP levels in animals treated with intravitreous NX1838 (mmHg)

Males	Pre-treatment		Week 4		Week 12		Week 24		Week 30	
Group	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD
1	18	14	18	13	12	11	12	11	13	12
2	19	19	22	14	19	21	13	15		
3	20	15	18	13	15	16	14	14		
4	23	18	25	17	21	23	15	16	13	13
Females	Pre-treatment									
Group	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD
1	20	14	21	15	19	15	14	13	15	12
2	22	13	21	14	19	14	14	12		
3	19	15	21	17	16	17	14	14		
4	18	16	20	16	17	18	15	14	12	12

Clinical pathology: No treatment-related, toxicologically significant differences in hematology, coagulation, and clinical chemistry were noted. Male HD animals showed higher hematocrit (HCT) levels (43.1±3.45% in postdose 7 and 44.4±3.07% in postdose 13) relative to the control animals (39.7%±1.49% in postdose 7 and 40.9±1.63% in postdose 13). Similar changes were not seen in females. There were no other changes for RBC parameters. The increase was considered as incidental and non-treatment-related.

Gross necropsy: Positive findings are summarized in the table below. No positive findings occurred in more than two animals. With low incidence and no dose-dependence, these changes were considered not drug-related.

Positive necropsy findings

Main study animals	**Males**				**Females**			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
N	**7**	**7**	**7**	**6**	**7**	**7**	**7**	**7**
A 0.5 cm cyst with a clear, watery content on the **right kidney**			1					
A few red, rounded areas on the caudate lobes of the **lung**				1				
A 0.4 cm red mass near the **spleen**	1							
Dilated **uterine** horns filled with watery fluid							1	
Right uterine horn: Blind areas (no lumen) and a portion of the horn greatly distended and filled with watery fluid								1
Recovery animals								
N	**2**			**2**	**2**			**2**
A cyst on the right **kidney** filled with clear fluid				1				

Organ weights: No treatment-related, toxicologically significant differences were noted.

Histopathological examinations: No treatment-related differences were noted. Positive microscopic findings are summarized in the table below.

For systemic tissues, histological findings consisted primarily of cellular infiltration in a variety of tissues in both control and treated groups. Generally these filtrates were minimal to mild in severity and were variably lymphocytic, subacute (macrophage and heterophils), mixed mononuclear (lymphocytes and macrophages) or pleocellular infiltrates. Steatosis (fatty infiltration) was seen in several sites in control and treated animals. Minimal to mild hepatocellular vacuolation was seen in several female control and treated animals. No NX1838-induced systemic histological changes were noted.

Similar to the systemic examination, minimal cellular infiltration was seen in a subconjunctival location across control and treated animals. A few animals had a small focus of transmural fibrosis resembling a small scar. All of the lesions observed appeared to be secondary to the injection procedure.

Incidence of histologic examination in male and female main study rabbits

	Male				Female			
Treatment group	1	2	3	4	1	2	3	4
Left eye with optic nerve	**7**	**7**	**7**	**6**	**7**	**7**	**7**	**7**
Fibroplasia	0	0	2	2	0	0	0	1
Pleocellular infiltration	0	0	0	1				
Subacute cellular infiltration	0	1	0	0	1	0	0	3
Proteinaceous fluid accumulation					0	0	0	1
Retinal vacuolation					0	0	0	1
Lymphocytic infiltration	0	1	1	0	0	1	1	0
Fibrosis	2	1	0	0	2	1	1	1
Harderian glands	**7**	**0**	**0**	**6**				
Lymphocytic infiltration	0	0	0	1				
Mandibular lymph node					**5**	**0**	**0**	**6**
Sinus heterophilia					0	0	0	1
Salivary glands					**7**	**0**	**0**	**7**
Lymphocytic infiltration					0	0	0	1
Thyroid					**7**	**0**	**0**	**7**
Lymphocytic infiltration					1	0	0	4
Tongue	**7**	**0**	**0**	**6**				
Lymphocytic infiltration	0	0	0	1				
Heart	**7**	**7**	**7**	**6**	**7**	**7**	**7**	**7**
Histiocytic infiltration	0	0	0	1				
Fibroplasia	0	0	1	1				
Lymphocytic infiltration	0	1	0	0	1	0	0	0
Liver					**7**	**0**	**0**	**7**
Hepatocellular vacuolation					4	0	0	5
Pancreas	**6**	**0**	**0**	**5**				
Steatosis	0	0	0	3				

Adrenal glands	7	0	0	6				
Cortical vacuolation	0	0	0	2				
Cecum	7	0	0	6				
Plasmacytic infiltration	0	0	0	1				
Kidney					7	0	0	7
Pigment, renal tubular epithelium					0	0	0	1
Muscle					7	0	0	7
Subacute cellular infiltration					0	0	0	1
Mammary gland					7	0	0	7
Lymphocytic infiltration					0	0	0	1
Uterus					7	0	1	7
Mucosal atrophy					0	0	0	1
Lymphocytic infiltration					0	0	0	1
Cystic dilatation and mucosal atrophy					0	0	1	0

Summary: NZW rabbits were treated with NX1838 (0.2, 0.67, and 2.0 mg/eye) via bilateral intravitreous injection once every 2 weeks for 6 months. One male animal died after the first injection in the left eye. The cause of death was unknown. Generally speaking, the drug was well tolerated. No treatment-related, toxicologically significant abnormal findings were noted in clinical observations, body weights, food consumption, clinical pathology, ophthalmology, necropsy and histopathology examinations. Injection procedure-related findings were observed in all groups that included clinical signs (conjunctival injection, bruising, swelling, squinting and/or prominent third eyelid), ophthalmology examination findings (vitreous cells and fibrin, vitreous opacity, lens damages, corneal erosion, conjunctivitis, and iritis), and minimal cellular infiltration and fibrosis in histopathological examinations. All these changes were reversible. Based on study results, the high dose of 2.0 mg/eye was considered as the NOAEL.

Study title: 0472DE15-001: A nine-month local tolerance and toxicity study of EYE001 given by intravitreous injection to beagle dogs

Key study findings: Repeated biweekly intravitreous injection of NX1838 at doses up to 3 mg/eye produced no ocular and systemic toxicity. Injection procedure-related findings were observed in all groups that included clinical signs, ophthalmology examination findings, and cellular infiltration and lens changes in histopathological examinations. The high dose of 3.0 mg/eye was considered as the NOAEL.

Study no.: 0472DE15-001
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: 2/21/2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: EYE001 (Lot #s: 241001A, 111002A, 131002A, and N06004F)
Study Design:

Groups	Dose (mg/eye)	Dose volume	N/sex	Terminated in Wk 39	Terminated in Wk 45 (recovery)
1 Vehicle	0 (PBS)	100 µl/eye	7	5	2
2. NX1838	0.3	100 µl/eye	5	5	
3. NX1838	1.0	100 µl/eye	5	5	
4. NX1838	3.0	100 µl/eye	7	5	2

The purpose of this study was to evaluate the toxicity of NX1838 following bilateral intravitreous administration of the drug once every 2 weeks for 9 months to beagle dogs (totally 20 doses).

Methods

Doses: 0 (buffered PSS), 0.3, 1.0, or 3.0 mg/eye (100 µl/eye, both eyes), Once every 2 weeks x 9 months

Species/strain: Beagle dogs

Number/sex/group or time point (main study): 5/sex/group in LD and MD groups and 7/sex/group for control and HD groups

Route, formulation, volume, and infusion rate: Intravitreous, PBS, 100 µl/eye

Satellite groups used for toxicokinetics or recovery: 2/sex from the control and HD groups were allowed to have a recovery period of 6 weeks.

Age: 15-16 months old

Weight (nonrodents only): 8.7-11.8 kg for males and 7.3-10.1 kg for females

Unique study design or methodology (if any): No

Observation times

Mortality: Twice daily

Clinical signs: At least once daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Prior to treatment initiation, prior to and 24 hr post-dose for injections 1 (Week 1), 4 (Week 7), 13 (Week 25), 16 (Week 31), 20 (Week 39) and in Week 44. Photographs of the fundus and macroscopic photos of the eyes of each animal were taken prior to treatment initiation, and 24 hr post-dose for injections 1 (Week 1), 4 (Week 7), 13 (Week 25), 16 (Week 31), 20 (Week 39). Macroscopic photos were also taken during Weeks 12 (post-dose 6), 26 (post-dose 13), 38 (post-dose 19) and 44 (recovery animals). ERG and IOP measurement were performed prior to treatment initiation, and during Weeks 4 (post-dose 2), 12 (post-dose 6), 26 (post-dose 13), 38 (post-dose 19) and 44 (recovery animals).

Physical examinations: Weeks 12, 26, and at necropsy, all animals

ECG: Weeks 38 and 44, all animals

Clinical pathology: Prior to treatment initiation, and during Weeks 13 (post-dose 7), 27 (post-dose 14), 37 (post-dose 19), and 44 (recovery animals)

Urinalysis: Weeks 12 (post-dose 6), 26 (post-dose 13), 38 (post-dose 19), and 44 (recovery animals)

Gross pathology: Within 3 days after the 20th dose (main animals) and in Week 45 (recovery animals)

Organ weights (specify organs weighed if not in histopath table): The following organs from each animal were weighed: adrenal, brain, heart, kidneys, liver, ovaries, spleen, testes, and thyroid and parathyroids.

Histopathology: Adequate Battery: yes (X), no ( )—explain

Peer review: yes ( ), no (X)

Histopathologic examinations were performed on all tissues from control and HD animals. The heart and left eye from all animals were examined histologically.

Toxicokinetics: See section 2.6.4.3 Absorption.

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: No drug-related clinical signs were noted in systemic observations. Several ocular signs were noted throughout the study in all groups. These signs included conjunctival injection (mild to severe), conjunctival bruising, conjunctival redness (mild to severe), conjunctival swelling, conjunctival/scleral hemorrhage (predominantly after injection), squinting, ocular discharge, tearing, corneal/lens opacity, and/or prominent third eyelid. These clinical signs were noted in all groups with similar incidence and severity, and were most likely the result of trauma/irritation of the dosing. The most severe inflammatory response was seen in a LD male 24 hr after the first dose. This animal squinted its right eye and the pupil could not be dilated for ocular examinations. Global swelling and high IOP (40 mmHg) were noted and this animal was treated with Buprenex (0.01 mg/ml, sc) for pain management. This animal appeared to be pain free 8 days after the treatment.

Five animals (1 control, 2 LD and 2 HD) showed corneal opacity/ulcers at various times throughout the study. Antibiotic ointment and lubricating ointment were used. All corneal lesions resolved within 2 weeks of treatment. The lesions were considered related to the dosing procedure and ERG procedure.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No drug-related abnormal findings in food consumption were observed.

Physical examinations: No treatment-related abnormal findings were observed.

Ophthalmology: Following Dose 1, changes (see table below) were noted in 11 males (5 controls, 3 LD, 1 MD and 2 HD) and 3 females (1 control, 1 MD and 1 HD).

Positive findings in ocular examinations

Animal #	Group	Sex	Comments
24 hr after dose 1			
11	1	M	OD: focal superior-posterior capsular linear opacity
19	1	M	OS: 2 mm posterior capsular irregularities; OD: focal superior cortical opacity; OU: conjunctival bruise
26	1	M	OD: punctate axial vitreal floater
13	1	M	OS: focal superior-posterior capsular opacity
27	1	M	OD: superior focal posterior capsular opacity
24	2	M	OS: axial punctate posterior capsular opacity
23	2	M	OS: punctate axial vitreal floater
2	2	M	OD: 1+ flare/intense miosis
22	3	M	OD: few vitreal floaters, conjunctival hemorrhage
1	4	M	OD: one vitreal floater
25	4	M	OU: vitreal floaters
52	1	F	OD: one vitreal floater; OS: conjunctival hemorrhage, focal
33	3	F	OD: 1/8 disc diameter mid tapetal lesion, black/surrounding regional edema
48	4	F	OD: focal superior bulbar conjunctival hemorrhage

When examinations were performed prior to Dose 4 administration, several lesions, including tapetal scars, subcapsular cataracts, focal retinal hemorrhages and partial retinal detachments (one low-dose male had a complete retinal detachment) that were not present during the previous ophthalmologic examination were observed. The cause of the majority of these lesions was suspected to be injury due to needle trauma. Therefore, for all subsequent injections, the depth at which the needle was inserted into the globe was reduced to approximately 1/2 to 2/3 of the needle length.

Positive findings in ocular examinations (number of eyes affected)

Prior to Dose 4	Control		Low dose		Mid dose		High dose	
	••	••	••	••	••	••	••	••
Tapetal scar, OD	4	3	4	4	4	4	6	5
Tapetal scar, OS	5	6	3	3	2	3	5	6
Retinal detachment, OD		1	1				2	
Retinal detachment, OS			2	2	1	2	3	
Central tapetal scar with hemorrhage, OD	2	1			1			1
Central tapetal scar with hemorrhage, OS	1				2		1	
Vitreal floaters, OU		1						
Focal posterior vitreal strands, OS						1		
Superior focal posterior capsular/subcapsular cataract, OD	3							
Superior focal posterior capsular/subcapsular cataract, OS	1		1					
Superior posterior lenticonus, OD	1							

In general, similar changes were noted in the following examinations. When examinations were performed prior to Dose 13 administration, similar lesions, including tapetal scars, conjunctival hyperemia, conjunctival ecchymosis, tapetal hemorrhage, and vitreal floaters were observed. However, of the 13 eyes, which were noted to have focal retinal detachments at the Dose 4 examinations, 11 of the focal detachments had resolved completely and 2 remained as residual focal detachments. The low-dose male with the complete retinal detachment remained unchanged. No new significant retinal lesions were noted following Dose 4 after the needle insertion depth was decreased during dose delivery.

On the day following Dose 20, new lesions for all groups, including the vehicle-control group, were observed in 20 animals (10/sex). The findings included mainly conjunctival hyperemia and conjunctival ecchymosis. These findings were considered related to the injection technique by the consulting veterinary ophthalmologist.

Positive findings in ocular examinations (number of eyes affected)

Prior to Dose 20	Control		Low dose		Mid dose		High dose	
	••	••	••	••	••	••	••	••
Tapetal scar, OD						1		
Tapetal scar, OS		1				1	1	
Vitreal strands, OD				1				
Vitreal strands, OS						1		
Focal punctuate posterior capsular opacity, OD							1	
Focal superior anterior vitreal clot, OS	1							
Focal superior anterior vitreal clot, OD							1	
Focal conjunctival hemorrhage/dorsal 2 tapetal scars, OS	1							
Conjunctival ecchymosis, OS		1	1			1	1	2
Conjunctival ecchymosis, OD			1			1	1	1
Mild conjunctival hyperemia, OS					3	1	1	2
Mild conjunctival hyperemia, OD					3			2
One small anterior vitreal bubble, OD					1			
Mild conjunctival hyperemia 2 tapetal scars, OS								
Vitreal pigment cells, OD		1						
Vitreal pigment cells, OS						1		1

Lesions were noted in the 2 animal/sex/group in both Groups 1 and 4 near the end of the recovery period. Most lesions were tapetal scars (resolution of previously noted lesions). There were 3 posterior cortical opacities noted (1 control male, 1 high-dose male and 1 high-dose female). The consulting veterinary ophthalmologist attributed these lesions to injection technique imperfections.

Positive findings in ocular examinations (number of eyes affected)

Recovery	Control		Low dose		Mid dose		High dose	
	••	••	••	••	••	••	••	••
Tapetal scar, OD	2	2					1	2
Tapetal scar, OS	2	2					2	2
Superior posterior capsular/posterior cortical opacity, OD	1							
Superior posterior capsular/posterior cortical opacity, OS							1	1
Vitreal clot, OD							1	
Central corneal opacity		1						

The sponsor indicated that the lesions were considered likely injection-related. Considering that the ocular lesions were seen in all groups with similar incidence and severity, the reviewer agrees that the ocular lesions were most reasonably interpreted to represent the results of technical difficulties in performing intravitreous injections. These lesions were not drug-related.

ERG: No treatment-related, toxicologically significant abnormal findings in ERG examinations were observed.

IOP: Mean IOP values among the groups fluctuated throughout the study but remained within the normal range of the canine species according to the published literature (11-29 mmHg).

EKG: No drug-related effects were observed in the EKG measurement.

Clinical pathology: No treatment-related, toxicologically significant differences in hematology, coagulation, and clinical chemistry were noted.

Urinalysis: No treatment-related abnormal findings in urinalysis were noted.

Gross necropsy: Positive findings are summarized in the table below. None of these findings were considered drug-related.

Positive necropsy findings

Main study animals	**Males**				**Females**			
Group	1	2	3	4	1	2	3	4
N	5	5	5	5	5	5	5	5
Urinary bladder: cyst-like outer surface, 5 mm, firm				1				
Spleen: grey-tan-orange plaques on capsular surface					1			
Ileocolic junction: red and raised mucosal surface					1			
Recovery animals								
N	2			2	2			2
Uterus: enlarged thickened areas, thick mucosa and right horn filled with puss				1				

Organ weights: No treatment-related, toxicologically significant differences were noted.

Histopathological examinations: Positive microscopic findings are summarized in the table below.

Microscopic ocular findings included lymphocytic infiltration in the episcleral tissue associated with the injection tract. The infiltration was not associated with any other lesions and was not considered test article-related by the veterinary pathologist. Also noted was a lens capsule disruption (high-dose male) and lenticular fiber swelling (control male and mid-dose female). The sponsor indicated that these changes were not associated with inflammatory or degenerative lesions, but were artifactual in nature.

For systemic tissues, positive findings consisted primarily of lymphocytic infiltration, generally minimal to mild in severity. All systemic histopathological findings were considered spontaneous in origin and not considered test article-related.

Incidence of histologic examination in male and female main study dogs

	Male				Female			
Treatment group	1	2	3	4	1	2	3	4
Left eye with optic nerve	5	5	5	5	5	5	5	5
Lens capsule disruption	0	0	0	1				
Lymphocytic infiltration	0	1	2	2	0	0	1	1
Lenticular fiber swelling	1	0	0	0	0	0	1	0
Stomach					5	0	0	5
Lymphocytic infiltration					0	0	0	1
Third eyelid gland	0	0	0	1	1	0	0	0
Lymphocytic infiltration	0	0	0	1	1	0	0	0
Salivary glands	5	0	0	5	5	0	0	5
Plasmacytic infiltration					1	0	0	0
Lymphocytic infiltration	2	0	0	2	2	0	0	1
Thyroid	5	0	0	5	5	0	0	5
Lymphocytic infiltration	1	0	0	2	1	0	0	1
Tongue	5	0	0	5				
Lymphocytic infiltration	0	0	0	1				
histiocytic infiltration	0	0	0	1				
Heart	5	5	5	5	5	5	5	5
Steatosis	1	3	1	0	3	0	1	0
Reactive mesothelium					0	0	0	1
Pericardial thickening					0	0	0	1
Mucin accumulation	0	1	0	0				
Lymphocytic infiltration	0	0	1	0				
Lung	5	0	0	5				
Lymphocytic infiltration	1	0	0	0				
Mixed mononuclear infiltration	1	0	0	1				
Duodenum	5	0	0	5				
Lymphocytic infiltration	0	0	0	1				

Mixed mononuclear infiltration	1	0	0	0				
Liver	5	0	0	5	5	0	0	5
Lymphocytic infiltration	0	0	0	1	0	0	0	1
Pigment	0	0	0	1	1	0	0	1
Mucous gland	5	0	0	5				
Lymphocytic infiltration	0	0	0	1				
Mixed mononuclear infiltration	0	0	0	1				
Pancreas	5	0	0	5	5	0	0	5
Lymphocytic infiltration	0	0	0	1	1	0	0	0
Pituitary gland	5	0	0	5				
Lymphocytic infiltration	0	0	0	1				
Ovary					5	0	0	5
Lymphocytic infiltration					0	0	0	1
Prostate	5	0	0	5				
Lymphocytic infiltration	2	0	0	1				
Mixed mononuclear infiltration	0	0	0	1				
Kidney	5	0	0	5	5	0	0	5
Lymphocytic infiltration	0	0	0	1		0	0	1
Pigment, renal tubular epithelium	0	0	0	2	1	0	0	3
Muscle					5	0	0	5
Myofiber necrosis					2	0	0	1
Histiocytic infiltration					0	0	0	1
Myofiber disruption					0	0	0	1
Pleocellular infiltration and neutrophil infiltration					1	0	0	0
Mammary gland					5	0	0	5
Lymphocytic infiltration					3	0	0	0
Urinary bladder	5	0	0	5				
Lymphocytic infiltration	0	0	0	1				
Mucosal detachment	0	0	0	1				

Summary: Beagle dogs were treated with NX1838 (0.3, 1.0, and 3.0 mg/eye) via bilateral intravitreous injection once every 2 weeks for 9 months with a recovery period of 6 weeks. No treatment-related, toxicologically significant abnormal findings were noted in clinical observations, body weights, food consumption, clinical pathology, ophthalmology, necropsy and histopathology examinations. Injection procedure-related findings were observed in all groups that were evidenced by clinical signs (conjunctival injection of blood vessels, bruising, redness, swelling, conjunctival/scleral hemorrhage, squinting, ocular discharge, tearing, corneal/lens opacity, and/or prominent third eyelid), ophthalmology examination findings (tapetal scars, subcapsular cataracts, focal retinal hemorrhages and partial retinal detachments), and cellular infiltration and lens changes in histopathological examinations. Based on study results, the high dose of 3.0 mg/eye was considered as the NOAEL.

Study title: 109-97008-T: A 90-day toxicity study of 2'-fluorouridine and 2'-fluorocytidine –HCl in male F-344 rats with a 90-day reversibility

Key study findings: At high doses (500 mg/kg), both compounds induced a decrease in body weight gain (10%, at 500 mg/kg). Extramedullary hematopoiesis and brown pigment accumulation in spleen were observed in animals receiving these 2 compounds at high dose. Neither compound showed evidence of toxicity similar to that induced by the antiviral drug, FIAU.

Study no.: 109-97008-T
Volume #, and page #: ECTD
Conducting laboratory and location: —

—

GLP compliance: No
QA reports: yes ( ) no (X)

Drug, lot #, and % purity: 2'-fluorouridine – Lot # 1-617-84) and 2'-fluorocytidine-HCl (– Lot #1-617-82)
Formulation/vehicle: Normal sterile saline

Methods

Animals: Male Fisher 344 rats
Route: Intravenous
Dose: 0, 5, 50 or 500 mg/10 ml/kg/day
Dosing Regimen: qd for 90 days
Study design:

Group	Dose (mg/kg x 90 days)	Dose volume	N	Main study	Recovery
0. Normal saline	0	10 ml/kg	18	12	6
1. 2'-f-uridine	5	10 ml/kg	9	6	3
2. 2'-f-uridine	50	10 ml/kg	9	6	3
3. 2'-f-uridine	500	10 ml/kg	9	6	3
4. 2'-f-cytidine	5	10 ml/kg	9	6	3
5. 2'-f-cytidine	50	10 ml/kg	9	6	3
6. 2'-f-cytidine	500	10 ml/kg	9	6	3

The purpose of this study was to determine the potential of 2'-fluorouridine and 2'-fluorocytidine, the assumed degradation products of NX1838, to induce toxicities similar to those observed with the antiviral drug Fialuridine (FIAU), a fluorinated nucleoside analogue developed for the treatment of hepatitis B, which showed severe liver toxicity. The toxicity of FIAU is characterized by severe lactic acidosis, liver failure and shock, pancreatitis, and microvesicular steatosis of the liver. Myopathy and neuropathy were also described. The mechanism was described as a delayed mitochondrial toxicity.

The first day of dosing was designated as Day 0. Toxicity was assessed as shown below.

Toxicity assessment for Study 109-97008-T

Parameter	Procedure
Clinical observations	Daily. Physical examinations were performed weekly.
Body weights	Weekly
Clinical pathology	Blood samples for hematology and clinical chemistry were collected on Days 0, 28, 56, 90.
Gross pathology	Animals designated for necropsy were euthanized on Days 90 and 91. Recovery animals were euthanized on Day 182. A complete necropsy was performed.
Organ weights	Organ weights were obtained for the following in each animal: brain, heart, kidney, adrenals, spleen, testis and liver. The percent of body weight of each organ was calculated.
Histopathology	Tissue samples from all organs examined were collected at necropsy. Histologic evaluation was performed.
Enzymatic and molecular analysis	At necropsy, the following tissues were collected for enzymatic and molecular analysis: liver, kidneys, spleen, muscle, heart, WBC, testes, bone marrow and pancreas.

Results:

Clinical observations: Four animals, two controls, one low dose 2'-fluorouridine and one high dose 2'-fluorocytidine died early in the study of causes unrelated to treatment (injury in the tail and injury during handling). There were no significant findings during the study.

Body weights: Body weights are summarized in the following table. High doses of the compounds decreased the body weight gain by about 20%. After recovery period, the difference between Group 0 and Group 3 was diminished.

Body weight data for rat (g)

Group	Dose (mg/kg)	Day -1	Day 90	% of control on Day 90	Day 181	% of control on Day 181
0	0	142.1	282.8	100	378.3	100
1	5	142.5	282.9	100	382.3	101
2	50	144.2	282.3	99.8	381.1	100.7
3	500	145.3	261.1	92.3	359	94.9
4	5	140.6	284.5	100.6	350.5	92.7
5	50	143.6	275.9	97.6	373.9	98.8
6	500	144.9	257.9	91.2	335.6	88.7

Clinical pathology: No toxicologically significant changes in hematology and clinical chemistry were observed.

Gross pathology: The lobular pattern of the liver was slightly accentuated in 1/6 rats in Group 3, 3/6 rats in Group 5, and 2/6 rats in Group 6. No histologic correlating findings were observed. The thymus size was reduced in 1/6 rats in Group 3 and 5/6 rats in Group 6. No other gross lesions were noted. No abnormal findings were noted in recovery animals.

Histopathology: Splenic lesions and thymic atrophy after 90 days of treatment are presented in the table below. These changes were resolved at the end of the recovery period. No other treatment-related, toxicologically significant findings were noted.

Splenic and thymic lesions after 90 days of treatment

Group	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Number of samples	**11**	**6**	**6**	**6**	**6**	**6**	**6**
Spleen							
↑ extramedullary hematopoiesis	0	0	0	0	1m	2m	6m
Focal brown pigment	0	1m	0	1m	0	0	5m
Thymus							
Atrophy	0	0	0	1m	0	0	1d4o1s

m: minimal; d: moderate; o: moderately severe; s: severe

Organ weights: Absolute splenic weights were decreased in Groups 3 (0.48 g) and 6 (0.45 g) relative to the control animals (0.54 g). The differences were diminished after 90 days recovery period.

Enzymatic analysis: No significant differences in cytochrome *c* oxidase activity and citrate synthase activity in liver and muscle of rats were noted. No decreases in mitochondrial DNA content similar to that caused by AZT and FIAU were noted. The results of the rat bone marrow micronucleus study are summarized in the table below. These 2 test articles were neither cytotoxic, nor clastogenic under the present study conditions.

Results of micronucleus assay

Group	Compound	Dosage (mg/kg)	PCE : NCE	MNPCE (%)
0	Saline	0	0.86	0.21
3	2'-Fluoro-uridine	500	0.98	0.34
6	2'-Fluoro-cytidine	500	0.93	0.25

PCE: polychromatic erythrocyte; NCE: normochromatic erythrocyte;
MNPCE: micronucleated PCE.

In conclusion, in this study, the potential of 2'-fluorouridine and 2'-fluorocytidine to produce toxicities similar to those induced by FIAU were investigated by intravenous injection to male Fisher 344 rats at the doses of 5, 50 or 500 mg/kg/day for 90 days. At high doses, both compounds induced a decrease in body weight gain (>10%, at 500 mg/kg). Extramedullary hematopoiesis and brown pigment accumulation in

spleen were observed in animals receiving these 2 compounds at high dose. Neither compound showed evidence of toxicity similar to that induced by the antiviral drug, FIAU. **[Reviewer's comments**: There is a deficiency for this study. The purpose of this study was to compare the potential of 2'-fluorouridine and 2'-fluorocytidine to induce toxicity similar to those observed with the antiviral drug FIAU. However, FIAU was not included in this study.]

Study title: 109-97009-T: A 90-day toxicity study of 2'-fluorouridine and 2'-fluorocytidine–HCl in woodchucks (Marmota Monox)

Key study findings: Neither compound showed evidence of toxicity similar to that induced by the antiviral drug, FIAU, in woodchucks.

Study no.: 109-97009-T
Volume #, and page #: ECTD
Conducting laboratory and location: —

GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: 2'-fluorouridine, Lot# 1-617-84, purity: —
fluorocytidine-HCl, Lot # 1-617-82, purity: —
Formulation/vehicle: Normal sterile saline

Methods

Animals: Woodchucks (3-4 years old, 3.06-6.04 kg)
Route: Intravenous
Dose: 0, 0.75 or 7.5 mg/10 ml/kg/day
Dosing Regimen: qd for 90 days
Study design:

Group	Dose (mg/kg x 90 days)	Dose volume	Male	Female
0. Normal saline	0	10 ml/kg	2	4
1. 2'-f-uridine	0.75	10 ml/kg	3	3
2. 2'-f-uridine	7.5	10 ml/kg	3	3
3. 2'-f-cytidine	0.75	10 ml/kg	3	3
4. 2'-f-cytidine	7.5	10 ml/kg	3	3

The purpose of this study was to determine the toxicity potential of 2'-fluorouridine and 2'-fluorocytidine, the assumed degradation products of NX1838, to induce toxicity similar to those observed with the antiviral drug FIAU, a fluorinated nucleoside analogue. The first day of dosing was designated as Day 0. Toxicity was assessed as shown below.

Toxicity assessment for Study 109-97009-T

Parameter	Procedure
Clinical observations	Twice daily. Physical examinations were performed weekly.
Body weights	Weekly
Clinical pathology & urinalysis	Blood samples for hematology and clinical chemistry were collected on Days -5, 28, 57, 88 for males and Days –6, 28, 58 and 89 for females. Urine samples were collected at the same time points for urinalysis.
Gross pathology	A complete necropsy was performed on all the animals on Day 90.
Organ weights	Organ weights were obtained for the following in each animal: brain, heart, kidney, pituitary, spleen, thyroid/parathyroid, testis, ovaries and liver. The percent of body weight of each organ

	was calculated.
Histopathology	Tissue samples from all organs examined were collected at necropsy. Histologic evaluation was performed.
Enzymatic and molecular analysis	At necropsy, the following tissues were collected for enzymatic and molecular analysis: liver, kidneys, spleen, muscle, heart, WBC, testes and pancreas.

Results:

Clinical observations: No drug-related abnormalities in physical appearance or behavior were observed. One animal receiving 0.75 mg/kg of 2'-fluoro-cytidine died on Day 69. The cause of death was unknown.

Body weights: No treatment-related differences were noted.

Clinical pathology: No toxicologically significant changes in hematology, clinical chemistry, and urinalysis were observed.

Gross pathology: The increase in reticular pattern in the liver was possibly treatment-related (see table below). No other treatment-related gross lesions were noted.

Liver changes at necropsy

Group	**0**	**1**	**2**	**3**	**4**
Males: Number examined	2	3	3	3	3
Discoloration, reticular pattern	1	2	0	1	0
Females: Number examined	4	3	3	3	3
Discoloration, reticular pattern	0	0	1	1	2

Histopathology: Histologic changes in the hearts, testes, thymus, adrenals and kidneys were observed in both control and treated animals. These lesions may be the results of normal seasonal changes that were exacerbated by the treatment. The changes noted in the livers (see table below) were considered treatment-related, and were correlated with the gross pathologic findings. The sponsor indicated that moderate to severe microvesicular and mixed microvesicular/macrovesicular steatoses in both genders were a hallmark of FIAU toxicity in the woodchuck and humans. In woodchucks, FIAU-induced changes were much more severe and occurred at a much greater incidence. They were accompanied by decreases in mitochondrial DNA content and increases in blood lactate. Neither 2'-fluorouridine nor 2'-fluorocytidine-HCl altered cytochrome *c* oxidase and citrate synthase activity, mitochondrial DNA content, or plasma lactate concentrations. Taken with the gender specificity of this histologic change, these results did not support an FIAU-like toxicity in these woodchucks.

Liver lesions after 90 days of treatment

Group	**0**	**1**	**2**	**3**	**4**
Males: number examined	2	3	3	3	3
Vacuolization, diffuse, mixed	0	0	0	1	0
Group severity				1.7	
Vacuolization, periportal, macrovesicular	0	0	0	0	1
Group severity					0.3
Vacuolization, periportal, microvesicular	0	2	1	1	0
Group severity		1.7	0.3	1.0	
Vacuolization, periportal, mixed	1	0	0	0	0
Group severity	1.0				
Females: Number examined	4	3	3	3	3
Infiltration, mononuclear cell, periportal	2	3	3	3	2
Group severity	0.5	1.3	1.3	1.0	1.0
Vacuolization, periportal, macrovesicular	0	0	1	1	0
Group severity			1.0	1.7	
Vacuolization, periportal, microvesicular	0	0	0	0	1

Group severity					0.7
Vacuolization, periportal, mixed	0	0	0	0	1
					1.0

Severity: 1 = minimal, 2 = slight

Organ weights: No significant differences in organ weights between the treatment and control groups were observed.

Enzymatic analysis: No significant differences in cytochrome *c* oxidase activity and citrate synthase activity in liver and muscle of the animals were noted. No decreases in mitochondrial DNA content similar to that caused by AZT and FIAU were noted.

In summary, 2'-fluorouridine and 2'-fluorocytidine-HCl were administered to male and female woodchucks by intravenous injection at doses of 0.75 or 7.5 mg/kg/day for 90 consecutive days. One male animal in the 0.75 mg/kg/day 2'-fluorocytidine-HCl group died after 69 days of unknown causes. Neither compound affected physical appearance, behavior, or body weight. There were no treatment-related, toxicologically significant changes of the clinical pathology or urinalysis measurements. A slight increase in periportal hepatocellular vacuolation and mononuclear cell infiltration was present in females treated with either compound. In conclusion, neither compound showed evidence of the toxicity induced by the antiviral drug, FIAU. **[Reviewer's comments**: There is a deficiency for this study. The purpose of this study was to compare the potential of 2'-fluorouridine and 2'-fluorocytidine to induce toxicity similar to those observed with the antiviral drug FIAU. However, FIAU was not included in this study.]

Histopathology inventory (optional)

Study	109-98003-T	109-98004-T	109-98010-T	0460LE15-001	0472DE15-001
Species	Rabbit	Rat	Monkey	Rabbit	Dog
Adrenals		X	X	X	X
Aorta		X	X		X
Bone Marrow smear		X	X		
Bone (femur)		X	X	X	X
Brain		X	X	X	X
Cecum		X	X	X	X
Cervix		X	X	X	X
Colon		X	X	X	X
Duodenum		X	X	X	X
Epididymis		X	X	X	X
Esophagus		X	X	X	X
Eye		X	X	X(left)	X(left)
Fallopian tube					
Gall bladder			X	X	X
Gross lesions	X	X	X		
Harderian gland		X		X	
Heart	X	X	X	X	X
Ileum		X	X	X	X
Injection site		X			
Jejunum		X	X	X	X
Kidneys	X	X	X	X	X
Lachrymal gland		X		X	X
Larynx					
Liver	X	X	X	X	X

Lungs	X	X	X	X	X
Lymph nodes, cervical					
Lymph nodes mandibular		X	X	X	X
Lymph nodes, mesenteric		X	X	X	X
Mammary Gland		X	X	X	X
Nasal cavity					
Optic nerves		X	X	X	X
Ovaries		X	X	X	X
Pancreas		X	X	X	X
Parathyroid		X	X	X	X
Peripheral nerve					
Pharynx					
Pituitary		X	X	X	X
Prostate		X	X	X	X
Rectum		X	X	X	X
Salivary gland		X	X	X	X
Sciatic nerve		X	X	X	X
Seminal vesicles		X	X	X	
Skeletal muscle		X	X	X	X
Skin		X	X	X	X
Spinal cord		X	X	X	X
Spleen	X	X	X	X	X
Sternum		X		X	X
Stomach		X		X	X
Testes		X	X	X	X
Thymus		X	X	X	X
Thyroid		X	X	X	X
Tongue		X	X	X	X
Trachea		X	X	X	X
Urinary bladder		X	X	X	X
Uterus		X	X	X	X
Vagina		X	X	X	X
Zymbal's gland					

X, histopathology performed; *, organ weight obtained

6.6.6.4 Genetic toxicology

Study title: 109-98001-T: Mutagenicity test on NX11838 in the L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay

Key findings: NX1838 was not considered mutagenic at the TK locus of mouse lymphoma cells in the presence and absence of S9 activation under the present testing conditions.

Study no.: 109-98001-T
Volume #, and page #: ECTD
Conducting laboratory and location: —

—

Date of study initiation: 1/15/1998
GLP compliance: yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity: NX1838, Lot: 11838.49

Methods

Strains/species/cell line: Mouse lymphoma L5178Y cell line

Doses used in definitive study: 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 μg/ml without or with S9 activation

Basis of dose selection: Results of the cytotoxicity in the dose range finding assay (9.81-5000 μg/ml). Under nonactivation conditions, the test article was noncytotoxic (98.6-113.6%). In the presence of metabolic activation, the test article was none to mild cytotoxic (60.5-104.9%).

Negative controls: Phosphate buffered saline (PBS)

Positive controls: Methyl methanesulfonate (MMS, 5 and 10 nl/ml) w/o S9 activation; Methylcholanthrene (MCA, 2.0 and 4.0 μg/ml) w/ S9 activation

Incubation and sampling times: In the initial assay with or without S9 activation and in the confirmatory assay with S9 activation, cells were treated with NX1838 for four hr. In the confirmatory assay without S9 activation, cells were treated with NX1838 for 24 hr. A standard expression period of 2 days was used to allow for mutant recovery, growth and expression of the $TK^{-/-}$ phenotype. The colonies were counted with an automated colony counter.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The doses used in this study were up to 5000 μg/ml. The positive controls produced typical positive results. The mutant colonies from vehicle controls were within the historical control range. The study was valid.

Study outcome: Only up to 48% and 44% of the relative total growth inhibition as cytotoxicity was produced in the presence and absence of S9 activation, respectively. In both initial assay and confirmatory assay, NX1838 did not increase the mutant frequency. NX1838 was not considered mutagenic at the TK locus of mouse lymphoma cells in the presence and absence of S9 activation under the present testing conditions.

Study title: 109-98002-T: Mutagenicity test with NX11838 in the *Salmonella–Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay

Key findings: NX1838 was not considered mutagenic under the present testing conditions.

Study no.: 109-98002-T
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: 1/15/1998

GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: NX1838, Lot: 11838.49

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-98 and TA-100;
Escherichia coli strain WP2uvrA

Doses used in definitive study: 100, 333, 1000, 3330, and 5000 µg/plate

Basis of dose selection: The results of the cytotoxicity in the dose range finding assay (6.67-5000 µg/ml). No cytotoxicity was observed in the dose range finding study.

Negative controls: Buffered saline (PBS)

Positive controls: 4-nitroquinoline-N-oxide; 2-Nitrofluorene (NF); 2-aminoanthracene (AA);
Benzo(a)pyrene; Sodium azide; ICR-191

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)		Dose µg/plate (w/o S9)	
Salmonella typhimurium	TA-1535	AA	2.5	Sodium azide	2.0
	TA-1537	AA	2.5	ICR-191	2.0
	TA-98	Benzo(a)pyrene	2.5	NF	1.0
	TA-100	AA	2.5	Sodium azide	2.0
Escherichia coli	WP2uvrA	AA	25	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: The tester strains were exposed to the test article via the plate incorporation. Following incubation at 37 ± 2°C for 52 ± 4 hr, revertant colonies were counted. All doses of the test article, the vehicle controls and the positive controls were plated in triplicate. The number of revertant colonies for the vehicle controls and NX1838 were counted manually. The number of revertant colonies for the positive controls was counted by automated colony counter.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The doses tested were 5000, 3330, 1000, 333 and 100 µg/plate in the presence and absence of S9 activation. In both initial mutagenicity assay and confirmatory assay, positive control compounds caused positive responses, and the number of revertants per plate of the vehicle controls was within the historical range. The study was valid.

Study outcome: NX1838 showed no cytotoxicity. In both initial mutagenicity assay and confirmatory assay, NX1838 did not increase the number of revertants of *Salmonella typhimurium* (strains TA-1535, TA-1537, TA-98, and TA-100) and *Escherichia coli* (strain WP2uvrA). Therefore, NX1838 was not mutagenic under the present testing conditions.

Study title: 7167-117: *In vitro* transformation of Syrian hamster embryo (SHE) cells by 7-day exposure to pegaptanib sodium

Key findings: Pegaptanib sodium was negative in SHE cell transformation assay under the experiment conditions in this study.

Study no.: 7167-117
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: 3/14/2003
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Pegaptanib sodium, Batch #: 2880-AG-2P 158, purity: —

Methods

Strains/species/cell line: Syrian hamster embryo (SHE) cells

Doses used in definitive study: Initial test: 100, 500, 1000, 2000, and 3000 μg/ml; second test: 10, 25, 50, 100, 500 and 1000 μg/ml

Basis of dose selection: The results of the cytotoxicity in the dose range finding assay.

Negative controls: Complete culture medium

Positive controls: Benzo (a) pyrene (BP), 5 μg/ml

Incubation and sampling times: After 7 days continuous exposure, colonies were counted and scored for transformation.

Analysis:

No. dishes analyzed: 45/concentration (5 dishes/concentration in dose-range finding studies)
Counting method: After fixation with methanol, colonies were stained with GIEMSA solution and analyzed in a blinded fashion — for morphological transformation.
Genetic toxicity endpoints/results: The frequency of morphologically transformed cells
Criteria for colonies showing morphologically transformed phenotype:
--colonies possessing piled-up cells with random orientation (criss-crossing) of the 3-dimentional growth
--colonies with criss-crossing cells and increased cytoplasmic basophilia throughout the colony, and/or
--colonies containing cells with decreased cytoplasm:nucleus ratios compared to normal SHE cells
Statistical methods: One-sided Fisher's exact test
Criteria for acceptance:
--there should be an average of 25-45 colonies/dish in each treatment group.
--the total colony count should be at least 1000/dose.
--positive control should induce a significant increase in the transformation frequency.

Criteria for positive results: A statistically significant increase in morphological transformation frequency for at least 2 dose levels compared to concurrent vehicle control, or a statistically significant increase in one dose and the trend test was significant.
Criteria for negative results: No statistically significant increase in morphological transformation was induced or only one dose showed a statistically significant increase without a positive dose-related response and the highest dose of test article caused a sufficient level of toxicity or the maximum applicable dose was achieved.

Results

Dose range finding toxicity study: A dose-dependent increase in cytotoxicity was noted (see table below). Based on the results, five concentrations covering a dose range from 100 to 3000 μg/ml were used in the initial trial of the transformation assay.

Cytotoxicity of dose range finding study

Pegaptanib sodium concentration (μg/ml)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
0 (medium)	38.4	100
2.5	37.6	98
5	31.4	82
10	32.8	85
25	34.6	90
50	35.0	91
100	34.0	89
250	26.0	68
500	29.0	76
1000	25.0	65
2000	21.0	55

Definitive study: The initial trial of the transformation assay failed due to more than expected cytotoxicity based on the dose-range finding study (see table below).

Cytotoxicity of the initial trial

Pegaptanib sodium concentration (μg/ml)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
Vehicle	30.5	100
BP 5	33.3	109
100	21.6	71
500	20.8	54
1000	11.6	27
2000	17.4	36
3000	15.1	25

Based on the cytotoxicity in the initial trial, concentrations of 10, 25, 50, 100, 500 and 1000 μg/ml were used in the second trial. Results are summarized in the table below. No significant increase in the transformation frequency was noted in any treatment groups. Positive controls developed positive responses. In conclusion, pegaptanib sodium did not induce cell transformation in SHE cell cultures under the experiment conditions in this study.

Results of 2nd transformation trial

Pegaptanib sodium concentration (μg/ml)	Morphological transformation frequency (%)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
Vehicle	0.350	31.7	100
BP 5	2.588	36.1	114
10	0.855	26.0	82
25	0.498	22.3	70
50	0.645	20.7	65
100	0.497	22.4	53
500	0.115	19.4	37
1000	0	22.8	27
Historical control	0-0.57		

Study title: 0676-1521: *In vivo* for chemical induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells

Key findings: EYE001 was negative in the micronucleus assay under the experiment conditions in this study.

Study no.: 0676-1521
Volume #, and page #: ECTD
Conducting laboratory and location:

Date of study initiation: 5/22/2001
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: EYE001, Batch #: 65L 8001, purity:

Methods

Strains/species/cell line: CD-1 mice, 28-34 g for males and 22-26 g for females, 5/sex/group

Doses used in definitive study: 1, 10, and 100 mg/10 ml/kg, iv, single dose

Basis of dose selection: Not indicated

Negative controls: PBS

Positive controls: Cyclophosphamide (CP) 80 mg/kg, po (gavage), single dose

Incubation and sampling times: Animals were terminated at 24, 48 and 72 hr after dosing and bone marrow samples were prepared. Positive control animals were sacrificed at 24 hr after dosing. The number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) among 200 erythrocytes (PCE + NCE) per animal was determined. The number of micronucleated polychromatic erythrocyte (MPCE) then was determined for 2000 PCE per animal.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The positive controls produced a significant increase in the number of MPCE. The number of MPCE from vehicle controls was within the historical control range. The study was valid.

Study outcome: Results are summarized in the table below. EYE001 at 1, 10 or 100 mg/kg iv caused no decrease in PCE ratio (PCE/(PCE + NCE) and no increase in the number of MPCE. In conclusion, EYE001 showed no clastogenic effects in this in vivo micronucleus assay under the experiment conditions in this study.

Summary of micronucleus assay results

Time (hr)	Dose (mg/kg)	Cell count		MPCE/2000 PCE	Historical control
		PCE	NCE		
Males					
24	Vehicle	112	88	0	0-1.6
24	1.0	115	85	0.2	
24	10.0	107	93	0.6	
24	100.0	118	82	0.2	
24	CP 80	56	114	77.8	8.6-91.4
48	Vehicle	138	62	0	
48	1.0	142	58	0.4	
48	10.0	114	86	0	
48	100.0	100	100	0.8	
72	Vehicle	92	108	0.2	
72	1.0	90	110	0.2	
72	10.0	98	102	0.4	
72	100.0	79	121	0.2	
Females					
24	Vehicle	122	78	0.2	0-1.8
24	1.0	130	70	0.6	
24	10.0	108	92	0.6	
24	100.0	116	84	1.2	
24	CP 80	91	109	56	21.8-84.6
48	Vehicle	142	58	0	
48	1.0	135	65	0	
48	10.0	129	71	0.6	
48	100.0	122	78	0	
72	Vehicle	97	103	0.2	
72	1.0	102	98	0	
72	10.0	86	114	0.6	
72	100.0	79	121	0.2	

The following genetic toxicology studies were conducted with monomer nucleotides.

Study title: 109-97004-T: Mutagenicity test on 2'-fluoro-cytidine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation

Key study findings: Monomer nucleotide 2'-fluoro-cytidine was classified as non-clastogenic under these experiment conditions.

Study no.: 109-97004-T
Compound: 2'-fluoro-cytidine, Lot: 1-617-82 – #8770C
Concentration: 840, 3500, 2450 and 5000 µg/ml
Treatment Time: 19.2 hr (22 hr harvest)
(+) Control: Mitomycin C (MMC, 0.4 µg/ml)
(-) Control: Sterile deionized water and medium

Indicator cell:Human lymphocyte from a healthy male donor
Study Site: [redacted]
GLP: Yes
Study initiation: 5/14/1997

The purpose of this assay was to evaluate the ability of 2'-fluoro-cytidine to induce chromosomal aberrations in cultured human lymphocytes without metabolic activation. Duplicate cultures were used at each concentration.

Results:

Reduction of 17%, 14%, 34%, 45%, 52%, 48% and 62% were noted in the mitotic indices of the cultures dosed with 590, 840, 1200, 1720, 2450, 3500 and 5000 µg/ml, respectively, as compared with solvent control cultures. Chromosomal aberrations were analyzed with 840, 2450, 3500 and 5000 µg/ml. 2'-fluoro-cytidine, without S9 activation, did not induce significant increases in the incidence of aberrations in human lymphocyte cultures relative to vehicle control (see table below), except for a weak increase (6%) at 5000 µg/ml. This was a statistical anomaly due to very low results (0% chromosomal aberration) in vehicle control cultures. Compared to the historical control data (2%), these data would not have been statistically significant. The significant response might be cytotoxicity-related since 62% inhibition in mitotic index was seen at this concentration. Hence, 2'-fluoro-cytidine was classified as non-clastogenic under these experiment conditions.

Results of chromosomal aberration assay with 2'-fluoro-cytidine in human lymphocytes

Group	Treatment	Concentration (µg/ml)	% cell with aberration	Historical control	% mitotic index
Negative control	Medium		0.5	0-2	4.2
Solvent control	Deionized water	50 µl/ml	0	0-2	2.9
Positive control	MMC	0.4	36	10.4-52.0	
2'-fluoro-cytidine		840	0.5		2.5
		2450	1.0		1.4
		3500	1.0		1.5
		5000	6.0 ($P < 0.01$)		1.1

Study title: 109-97005-T: Mutagenicity test on 2'-fluoro-uridine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation

Key study findings: [redacted] was classified as non-clastogenic under these experiment conditions.

Study no.: 109-97005-T
Compound: 2'-fluoro-uridine, Lot: 1-617-84 — #8760B
Concentration: 1720, 2450, 3500 and 5000 µg/ml
Treatment Time: 19 hr (22 hr harvest)
(+) Control: Mitomycin C (MMC, 0.4 µg/ml) w/o S9
(-) Control: Sterile deionized water and medium
Indicator cell:Human lymphocyte
Study Site: [redacted]
GLP: Yes
Study initiation: 5/14/1997

The purpose of this assay was to evaluate the ability of 2'-fluoro-uridine to induce chromosomal aberrations in cultured human lymphocytes without metabolic activation. Duplicate cultures were used at each concentration.

Results:

Reductions of 7%, 10%, 14%, 14%, and 55% were observed in the mitotic indices of the cultures dosed with 840, 1200, 2450, 3500, and 5000 µg/ml, respectively, as compared with the solvent control cultures. Chromosomal aberrations were analyzed with 1720, 2450, 3500 and 5000 µg/ml. 2'-fluoro-uridine, without S9 activation, did not induce increases in the incidence of aberrations in human lymphocyte cultures relative to vehicle control (see table below). Therefore, 2'-fluoro-urindine was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without S9 activation.

Results of chromosomal aberration assay with 2'-fluoro-uridine in human lymphocytes

Group	Treatment	Concentration (µg/ml)	% cell with aberration	Historical control	% mitotic index
Negative control	Medium		0.5	0-2	4.2
Solvent control	Deionized water	50 µl/ml	0	0-2	2.9
Positive control	MMC	0.4	36	10.4-52.0	
2'-fluoro-uridine		1720	0		3.0
		2450	2.0		2.5
		3500	1.5		2.5
		5000	1.5		1.3

Study title: 109-97006-T: Mutagenicity test with 2'-fluoro-cytidine hydrochloride in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay

Key study findings: Monomer nucleotide 2'-fluoro-cytidine caused positive increases in the mean number of revertants per plate with tester strain WP2*uvr*A in the presence and absence of S9 activation.

Study no.: 109-97006-T
Compound: 2'-fluoro-cytidine, Lot: 1-617-82 — #8770C
Concentration: 10, 33.3, 100, 333, 1000, 3330, and 5000 µg/plate with or without S9 activation
(+) Control: 4-nitroquinoline-N-oxide; 2-Nitrofluorene (NF); 2-aminoanthracene (AA); Sodium azide; ICR-191
(-) Control: Sterile deionized water
Bacteria: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-98 and TA-100; *Escherichia coli* strain WP2*uvr*A
Study Site: —
GLP: Yes
Study initiation: 5/14/1997

The ability of 2'-fluoro-cytidine HCl to induce reverse mutations with or without S9 activation was tested in this study. Duplicate cultures were used at each concentration. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)		Dose µg/plate (w/o S9)	
Salmonella typhimurium	TA-1535	AA	2.5	Sodium azide	2.0

	TA-1537	AA	2.5	ICR-191	2.0
	TA-98	AA	2.5	NF	1.0
	TA-100	AA	2.5	Sodium azide	2.0
Escherichia coli	WP2*uvr*A	AA	25	4-nitroquinoline-N-oxide	1.0

Results:

Seven dose levels ranging from 10 µg/plate to 5000 µg/plate were used. Results, summarized in the table below, showed that 2'-fluoro-cytidine caused positive increases in the mean number of revertants per plate with tester strain WP2*uvr*A in the presence (4.3 and 3.3-fold at most, respectively, in 2 experiments) and absence (3.7 and 3.1-fold at most, respectively, in 2 experiments) of S9 activation. No increases with any of the remaining tester strains with or without S9 were noted.

Results of Ames test with 2'-fluoro-cytidine (mean revertants per plate ± SD)

Treatment	Concentration (µg/plate)	TA98	TA100	TA1535	TA1537	WP2*uvr*A (test 1)	WP2*uvr*A (test 2)
			With S9				
Vehicle		31±4	126±1	12±1	7±1	13±6	13±6
2'-F-cytidine	1						13±8
	3.33						14±4
	10	32±4	107±10	16±1	9±1	25±1	18±2
	33.3	35±6	95±11	14±1	10±4	42±11	26±1
	100	17±4	78±0	13±5	6±6	41±18	43±6
	333	28±0	95±11	12±1	6±0	50±9	40±0
	1000	24±3	81±13	11±1	6±6	54±18	37±7
	3330	22±5	71±11	14±1	3±1	49±6	
	5000	28±8	78±0	14±4	6±1	56±9	
Positive cont		919±132	1309±1	158±1	150±3	249±24	367±108
			Without S9				
Vehicle		19±2	78±5	7±1	4±3	15±6	12±2
2'-F-cytidine	1						14±9
	3.33						16±7
	10	14±9	81±17	15±5	5±1	23±1	18±0
	33.3	21±1	84±1	8±4	4±1	44±11	26±8
	100	12±2	89±3	10±7	6±1	43±4	37±2
	333	14±0	74±13	11±4	5±4	56±6	34±0
	1000	10±3	72±4	13±0	5±3	34±8	27±2
	3330	14±5	73±10	10±1	6±1	46±1	
	5000	17±0	65±2	7±5	3±1	48±2	
Positive cont		124±5	519±75	565±26	232±11	266±23	384±4

Study title: 109-97007-T: Mutagenicity test with 2'-fluoro-uridine in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay

Key study findings: Monomer nucleotide 2'-fluoro-uridine caused positive increases in the mean number of revertants per plate with tester strain WP2*uvr*A in the presence and absence of S9 activation.

Study no.: 109-97007-T
Compound: 2'-fluoro-uridine, Lot: 1-617-84 – #8760B
Dose Level: 10, 33.3, 100, 333, 1000, 3330, and 5000 µg/plate with or without S9 activation
(+) Control: 4-nitroquinoline-N-oxide; 2-Nitrofluorene (NF); 2-aminoanthracene (AA); Sodium azide; ICR-191
(-) Control: Sterile deionized water
Bacteria: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-98 and TA-100; *Escherichia coli* strain WP2*uvr*A
Study Site:

GLP: Yes
Study initiation: 5/14/1997

The ability of 2'-fluoro-uridine to induce reverse mutations with or without S9 activation was tested in this study. Duplicate cultures were used at each concentration. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)		Dose µg/plate (w/o S9)	
Salmonella typhimurium	TA-1535	AA	2.5	Sodium azide	2.0
	TA-1537	AA	2.5	ICR-191	2.0
	TA-98	AA	2.5	NF	1.0
	TA-100	AA	2.5	Sodium azide	2.0
Escherichia coli	WP2*uvr*A	AA	25	4-nitroquinoline-N-oxide	1.0

Results:

Seven dose levels ranging from 10 µg/plate to 5000 µg/plate were used. Results, summarized in the table below, showed that the test article caused positive increases in the mean number of revertants per plate with tester strain WP2*uvr*A in the presence (2.7 and 3.5-fold at most, respectively, in 2 experiments) and absence (2.9 and 3.0-fold at most, respectively, in 2 experiments) of S9 activation. No increases with any of the remaining tester strains with or without S9 were noted.

Results of Ames test with 2'-fluoro-uridine (mean revertants per plate ± SD)

Treatment	Concentration (µg/plate)	TA98	TA100	TA1535	TA1537	WP2*uvr*A (test 1)	WP2*uvr*A (test 2)
			With S9				
Vehicle		23±7	107±6	14±6	8±1	21±4	12±1
2'-F-cytidine	1						18±0
	3.33						16±1
	10	17±1	110±16	10±3	8±0	21±6	20±4
	33.3	26±8	101±1	14	8±1	45±2	33±9
	100	27±6	82±9	7±4	7±1	57±4	33
	333	18±4	75±14	12±6	4±2	36±4	42±17
	1000	22±6	93	11±0	10±2	44±2	37±4
	3330	22±11	83±10	15±1	6±1	37±8	
	5000	16±1	65±5	10±4	5±1	33±3	
Positive cont		1008±126	1046±10	107±35	105±11	231±7	396±71
			Without S9				
Vehicle		13±2	74±9	12±0	8±2	19±6	14±4
2'-F-cytidine	1						22±0
	3.33						14±1
	10	18±3	86±4	14±4	5±2	19±1	21±6
	33.3	14±3	77±4	9±3	5±2	39±8	35±6
	100	16±6	90±9	13±8	5±1	55±5	40±1
	333	14±0	71±2	13±1	3±1	41±1	42±3
	1000	9±4	84±16	9±3	5±2	48±4	33±14
	3330	16±4	55±2	12±1	8±5	32±0	
	5000	15±3	62±3	13±1	2±1	43±13	
Positive cont		148±23	631±55	605±30	193±13	227±29	389±12

Study title: 109-98006-T: Mutagenicity test on 2'-O-methylguanosine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation

Key study findings: Monomer nucleotide 2'-O-methylguanosine was negative for inducing chromosomal aberrations in cultured human lymphocytes without S9 activation.

Study no.: 109-98006-T
Compound: 2'-O-methylguanosine, Lot: — #8940A
Concentration: 114, 162, 330 and 470 µg/ml
Treatment Time: 19.3 hr (22.2 hr harvest)
(+) Control: Mitomycin C (MMC, 0.4 µg/ml) w/o S9
(-) Control: Culture medium and DMSO
Indicator cell:Human lymphocyte
Study Site: —
GLP: Yes
Study initiation: 12/29/1997

The purpose of this assay was to evaluate the ability of 2'-O-methylguanosine to induce chromosomal aberrations in cultured human lymphocytes without metabolic activation. Duplicate cultures were dosed with 2'-O-methylguanosine at 27.4, 39.0, 55.8, 79.6, 114, 162, 232, 330, 470, 672, 960, 1370, 1960, 2800, and 4000 µg/ml. Four dose levels, cultures dosed at 114, 162, 330, and 470 µg/ml, were scored for chromosomal aberrations.

Results:

Reductions of 25%, 44%, 50%, 19%, 63%, 69%, 56%, 63%, 75%, 100%, and 94% were observed in the mitotic indices of the cultures dosed with 114, 162, 232, 330, 470, 672, 960, 1370, 1960, 2800, and 4000 µg/ml, respectively, as compared with the solvent control cultures.

Chromosomal aberrations were analyzed at concentrations of 114, 162, 330 and 470 µg/ml. The mitotic index decreased by 63% at the concentration of 470 µg/ml. 2'-O-methylguanosine, without S9 activation, did not induce increases in the incidence of aberrations in human lymphocyte cultures relative to vehicle control. Therefore, 2'-O-methylguanosine was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without S9 activation.

Results of chromosomal aberration assay with 2'-O-methylguanosine in human lymphocytes

Group	Treatment	Concentration (µg/ml)	% cell with aberration	Historical control	% mitotic index
Negative control	Medium		0	0-2	1.7
Solvent control	DMSO	20 µl/ml	0.5	0-2	1.6
Positive control	MMC	0.4	28	10.4-52.0	
2'-O-methylguanosine		114	0		1.2
		162	1.0		0.9
		330	0		1.3
		470	0.5		0.6

Study title: 109-98007-T: Mutagenicity test on 2'-O-methyladenosine measuring chromosomal aberrations in human whole blood lymphocytes without exogenous metabolic activation

Key study findings: 2'-O-methyladenosine was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without S9 activation.

Study no.: 109-98007-T
Compound: 2'-O-methyladenosine, Lot: — #8930A
Concentration: 840, 1200, 1720 and 2460 µg/ml
Treatment Time: 19.3 hr (22.2 hr harvest)

(+) Control: Mitomycin C (MMC, 0.4 µg/ml) w/o S9
(-) Control: Culture medium and DMSO
Indicator cell: Human lymphocyte
Study Site: —
GLP: Yes
Study initiation: 12/29/1997

The purpose of this assay was to evaluate the ability of 2'-O-methyladenosine to induce chromosomal aberrations in cultured human lymphocytes without metabolic activation. Duplicate cultures were dosed with 2'-O-methyladenosine at 33.9, 48.4, 69.1, 98.7, 141, 202, 288, 412, 588, 840, 1200, 1720, 2460, 3510, and 5010 µg/ml. Four dose levels, cultures dosed at 840, 1200, 1720, and 2460 µg/ml, were scored for chromosomal aberrations.

Results:

Reduction of 38%, 31%, 31%, 50%, 44%, 50% and 63% were noted in the mitotic indices of the cultures dosed with 412, 588, 840, 1200, 1720, 2460 and 3510 µg/ml, respectively, as compared with solvent control cultures. Chromosomal aberrations were analyzed with 840, 1200, 1720 and 2460 µg/ml. 2'-O-adenosine, without S9 activation, did not induce increases in the incidence of aberrations in human lymphocyte cultures relative to vehicle control. Therefore, 2'-O-adenosine was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without S9 activation.

Results of chromosomal aberration assay with 2'-O-methyladenosine in human lymphocytes

Group	Treatment	Concentration (µg/ml)	% cell with aberration	Historical control	% mitotic index
Negative control	Medium		0	0-2	1.7
Solvent control	DMSO	20 µl/ml	0.5	0-2	1.6
Positive control	MMC	0.4	28	10.4-52.0	
2'-O-methyladenosine		840	0.5		1.1
		1200	0.5		0.8
		1720	0		0.9
		2460	1.5		0.8

Study title: 109-98008-T: Mutagenicity test with 2'-O-methylguanosine in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay

Key study findings: Under the conditions of this study, 2'-O-methylguanosine did not cause positive increases in the mean number of revertants per plate with any tester strains with or without S9.

Study no.: 109-98008-T
Compound: 2'-O-methylguanosine, Lot: — 8940A
Dose Level: 10, 33.3, 100, 333, 750, 1000, 2000, 3330, and 5000 µg/plate with or without S9 activation
(+) Control: 4-nitroquinoline-N-oxide; 2-Nitrofluorene (NF); 2-aminoanthracene (AA); Benzo(a)pyrene; Sodium azide; ICR-191
(-) Control: DMSO
Bacteria: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-98 and TA-100; *Escherichia coli* strain WP2*uvr*A
Study Site: —
GLP: Yes

Study initiation: 12/16/1997

The ability of 2'-O-methylguanosine to induce reverse mutations with or without S9 activation was tested in this study. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)		Dose µg/plate (w/o S9)	
Salmonella typhimurium	TA-1535	AA	2.5	Sodium azide	2.0
	TA-1537	AA	2.5	ICR-191	2.0
	TA-98	Benzo(a)pyrene	2.5	NF	1.0
	TA-100	AA	2.5	Sodium azide	2.0
Escherichia coli	WP2*uvr*A	AA	25	4-nitroquinoline-N-oxide	1.0

Results:

Seven dose levels ranging from 10 µg/plate to 5000 µg/plate were used. The results showed that 2'-O-methylguanosine did not cause positive increases in the mean number of revertants per plate with any tester strains with or without S9.

Results of Ames test with 2'-O-methylguanosine (mean revertants per plate ± SD)

Treatment	Concentration (µg/plate)	TA98	TA100	TA1535	TA1537	WP2*uvr*A (test 1)	WP2*uvr*A (test 2)
			With S9				
Vehicle		26±7	78±8	10±1	15±4	11±2	11±2
2'-O-methylguanosine	10	36±1	99±1	13±1	10±2	14±4	
	33	27±1	68±1	11±5	13±4	19±6	13±4
	100	27±8	77±18	12±0	17±1	16±1	17±3
	333	28±2	86±7	12±2	13±1	18±6	17±5
	750						16±4
	1000	22±4	92±19	10±0	12±4	23±1	18±4
	2000						16±5
	3330	27±3	84±1	8±1	16±4	9±1	18±6
	5000	39±11	88±10	9±1	17±2	19±2	14±5
Positive cont		394±32	911±24	141±13	164±10	338±11	384±12
			Without S9				
Vehicle		16±6	62±13	11±6	10±1	15±1	
2'-O-methylguanosine	10	15±1	72±0	11±1	12±5	19±1	
	33.3	18±1	70±15	11±2	12±1	15±3	
	100	16±5	58±7	12±2	14±1	21±1	
	333	19±5	69±1	12±1	18±4	17±3	
	1000	15±1	66±28	13±6	14±1	13±3	
	3330	16±4	66±18	14±7	13±4	14±4	
	5000	21±2	66±8	14±2	11±3	14±4	
Positive cont		117±14	418±61	521±18	571±13	406±64	

Study title: 109-98009-T: Mutagenicity test with 2'-O-methyladenosine in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation screening assay

Key study findings: Under the conditions of this study, 2'-O-methyladenosine did not cause positive increases in the mean number of revertants per plate with any tester strains with or without S9.

Study no.: 109-98009-T
Compound: 2'-O-methyladenosine, Lot: ~ ,8930A
Dose Level: 10, 33.3, 100, 333, 750, 1000, 2000, 3330, and 5000 µg/plate with or without S9 activation
(+) Control: 4-nitroquinoline-N-oxide; 2-Nitrofluorene (NF); 2-aminoanthracene (AA); Benzo(a)pyrene; Sodium azide; ICR-191

(-) Control: DMSO
Bacteria: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-98 and TA-100; *Escherichia coli* strain WP2*uvr*A
Study Site: [redacted]
GLP: Yes
Study initiation: 12/16/1997

The ability of 2'-O-methyladenosine to induce reverse mutations with or without S9 activation was tested in this study. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)		Dose µg/plate (w/o S9)	
Salmonella typhimurium	TA-1535	AA	2.5	Sodium azide	2.0
	TA-1537	AA	2.5	ICR-191	2.0
	TA-98	Benzo(a)pyrene	2.5	NF	1.0
	TA-100	AA	2.5	Sodium azide	2.0
Escherichia coli	WP2*uvr*A	AA	25	4-nitroquinoline-N-oxide	1.0

Results:

Seven dose levels ranging from10 µg/plate to 5000 µg/plate were used. Results showed that 2'-O-methyladenosine did not cause positive increases in the mean number of revertants per plate with any tester strains with or without S9.

Results of Ames test with 2'-O-Methyladenosine (mean revertants per plate ± SD)

Treatment	Concentration (µg/plate)	TA98	TA100	TA1535	TA1537	WP2*uvr*A (test 1)	WP2*uvr*A (test 2)
			With S9				
Vehicle		29±6	80±6	13±6	15±1	11±1	16±1
2'-O-methylguanosine	10	24±7	79±3	6±1	12±1	11±3	
	33	23±4	88±6	9±3	14±3	16±2	19±4
	100	25±4	96±8	18±4	14±1	18±1	16±3
	333	28±7	105±0	11±6	13±2	16±1	18±1
	750						20±5
	1000	22±3	97±8	8±4	18±2	24±10	15±5
	2000						15±2
	3330	26±0	90±4	10±1	21±6	11±1	16±6
	5000	26±3	86±1	8±4	19±3	18±1	21±3
Positive cont		425±7	1217±11	155±7	224±25	268±26	550±61
			Without S9				
Vehicle		20±13	88±4	12±0	11±5	12±1	
2'-O-methylguanosine	10	15±3	96±3	8±4	19±4	14±4	
	33.3	19±1	85±3	8±1	14±1	15±7	
	100	20±6	85±8	15±2	12±1	10±5	
	333	21±5	83±2	11±6	16±2	10±6	
	1000	18±1	88±0	11±1	18±4	13±6	
	3330	19±5	80±8	10±2	15±3	12±4	
	5000	22±2	86±7	11±1	13±4	17±2	
Positive cont		89±1	400±7	337±81	642±9	385±78	

Study title: 7167-118: *In vitro* transformation of Syrian hamster embryo (SHE) cells by 7-day exposure to 2'-fluoro-2'-deoxy-uridine

Key findings: Monomer nucleotide 2'-fluoro-2'-deoxy-uridine was negative in SHE cell transformation assay under the experiment conditions in this study.

Study no.: 7167-118
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: 3/6/2003
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 2'-fluoro-2'-deoxy-uridine, Lot #: 7-830-9, purity: —

Methods

Strains/species/cell line: Syrian hamster embryo (SHE) cells

Doses used in definitive study: Initial test: 500-3500 μg/ml; second test: 0.1-10 mM (10 mM = 2462 μg/ml)

Basis of dose selection: The results of the cytotoxicity in the dose range finding assay.

Negative controls: Complete culture medium

Positive controls: Benzo (a) pyrene (BP), 5 μg/ml

Incubation and sampling times: After 7 days continuous exposure, the colonies were counted and scored for transformation.

Analysis:

No. dishes analyzed: 45/concentration (5 dishes/concentration in dose-range finding studies)
Counting method: After fixation with methanol, the colonies were stained with GIEMSA solution and analyzed in a blinded fashion — for morphological transformation.
Genetic toxicity endpoints/results: The frequency of morphologically transformed cells
Criteria for colonies showing morphologically transformed phenotype:
--colonies possessing piled-up cells with random orientation (criss-crossing) of the 3-dimentional growth
--colonies with criss-crossing cells and increased cytoplasmic basophilia throughout the colony, and/or
--colonies containing cells with decreased cytoplasm:nucleus ratios compared to normal SHE cells
Statistical methods: One-sided Fisher's exact test
Criteria for acceptance:
--there should be an average of 25-45 colonies/dish in each treatment group.
--the total colony count should be at least 1000/dose.
--positive control should induce a significant increase in the transformation frequency.
Criteria for positive results: A statistically significant increase in morphological transformation frequency for at least 2 dose levels compared to concurrent vehicle control, or a statistically significant increase in one dose and the trend test was significant.

Criteria for negative results: No statistically significant increase in morphological transformation was induced or only one dose showed a statistically significant increase without a positive dose-related response and the highest dose of test article caused a sufficient level of toxicity or the maximum applicable dose was achieved.

Results

Dose range finding toxicity study: Two dose-range finding studies were conducted. Results are summarized in the table below. Based on results from the 1st study, five concentrations covering a dose range of 500-3500 μg/ml were used in the initial trial of the transformation assay. The cytotoxicity was more than expected. The sponsor then performed a second study with a new batch of test article. The test article was noncytotoxic up to 3000 μg/ml. The sponsor chose 10 mM (2462 μg/ml) as the top concentration in the second transformation trial.

Cytotoxicity of dose range finding studies

	Study 1		Study 2	
2'-fluoro-2'-deoxy-uridine concentration (μg/ml)	Average # of colonies/dish	Relative plating efficiency (RPE, %)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
Vehicle	38.4	100	33.2	100
5	35.2	92		
10	34.4	90		
25	41.0	107		
50	37.0	97		
100	33.6	88	28.2	85
250	35.2	92		
500	37.8	99	32.6	98
1000	34.2	89	29.0	87
1500			33.4	100
1750			38.0	114
2000	26.4	69	37.2	112
2250			32.4	98
2500			33.2	100
3000			28.6	86
4000	7.0	18		

Definitive study: The initial trial of the transformation assay failed due to more than expected cytotoxicity based on the dose-range finding study (see table below).

Cytotoxicity of the initial trial

Pegaptanib sodium concentration (μg/ml)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
Vehicle	30.5	100
BP 5	33.3	109
500	28.2	92
1000	26.3	86
2000	23.9	54
2500	22.3	40
3500	13.3	15

Based on the cytotoxicity in the second dose-range finding study with the new batch of test article, concentrations of 0.1, 0.5, 1, 2.5, 5 and 10 mM were used in the second trial. Results are summarized in the table below. No significant increase in the transformation frequency was noted in any treatment groups. Positive controls developed positive responses. In conclusion, 2'-fluoro-2'-deoxy-uridine did not induce cell transformation in SHE cell cultures under the experiment conditions in this study.

Results of 2nd transformation trial

2'-fluoro-2'-deoxy-uridine	Morphological transformation	Average # of	Relative plating efficiency

concentration (mM)	frequency (%)	colonies/dish	(RPE, %)
Vehicle	0.255	34.8	100
BP 5 μg/ml	1.116	39.8	114
0.1	0	34.2	98
0.5	0.183	38.9	111
1.0	0.324	38.6	111
2.5	0.341	37.6	108
5.0	0.268	34.0	97
10.0	0.068	32.7	94
Historical control	0-0.57		

Study title: 7167-119: *In vitro* transformation of Syrian hamster embryo (SHE) cells by 7-day exposure to 2'-fluoro-cytidine

Key findings: Monomer nucleotide 2'-fluoro-cytidine was negative in SHE cell transformation assay under the experiment conditions in this study.

Study no.: 7167-119
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: 3/6/2003
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 2'-fluoro-cytidine, Batch #: 2-830-4, purity: —

Methods

Strains/species/cell line: Syrian hamster embryo (SHE) cells

Doses used in definitive study: 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 μg/ml

Basis of dose selection: The results of the cytotoxicity in the dose range finding assay.

Negative controls: Complete culture medium

Positive controls: Benzo (a) pyrene (BP), 5 μg/ml

Incubation and sampling times: After 7 days continuous exposure, the colonies were counted and scored for transformation.

Analysis:

No. dishes analyzed: 45/concentration (5 dishes/concentration in dose-range finding studies)
Counting method: After fixation with methanol, the colonies were stained with GIEMSA solution and analyzed in a blinded fashion — for morphological transformation.
Genetic toxicity endpoints/results: The frequency of morphologically transformed cells
Criteria for colonies showing morphologically transformed phenotype:

--colonies possessing piled-up cells with random orientation (criss-crossing) of the 3-dimentional growth
--colonies with criss-crossing cells and increased cytoplasmic basophilia throughout the colony, and/or
--colonies containing cells with decreased cytoplasm:nucleus ratios compared to normal SHE cells
Statistical methods: One-sided Fisher's exact test
Criteria for acceptance:
--there should be an average of 25-45 colonies/dish in each treatment group.
--the total colony count should be at least 1000/dose.
--positive control should induce a significant increase in the transformation frequency.
Criteria for positive results: A statistically significant increase in morphological transformation frequency for at least 2 dose levels compared to concurrent vehicle control, or a statistically significant increase in one dose and the trend test was significant.
Criteria for negative results: No statistically significant increase in morphological transformation was induced or only one dose showed a statistically significant increase without a positive dose-related response and the highest dose of test article caused a sufficient level of toxicity or the maximum applicable dose was achieved.

Results

Dose range finding toxicity studies: In the initial study, concentrations ranging from 2.5 to 2000 μg/ml did not provide useful information due to much higher than expected toxicity. In the second study, concentrations ranged from 0.01 to 10.0 μg/ml. Substantial toxicity was produced at 2.5 μg/ml and higher. Because a new batch of SHE cells had to be used in the rest of the study, a third range-finding study with the new batch was conducted with concentrations of 0.01 to 100 μg/ml. Results from range-finding studies 2 and 3 are summarized in the table below.

Cytotoxicity of dose range finding studies

	Study 2		Study 3	
2'-fluoro-cytidine concentration (μg/ml)	Average # of colonies/dish	Relative plating efficiency (RPE, %)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
Vehicle	30.4	100	31.2	100
0.01	28.2	93	29.1	93
0.025	25.9	85		
0.05	26.9	88	32.5	104
0.10	28.5	94	26.8	85
0.20			29.4	94
0.25	26.8	88		
0.40			20.6	66
0.50	27.7	91		
0.80			15.8	51
1.00	16.4	54	15.5	50
1.60			11.7	38
2.00			6.8	22
2.50	3.1	10		
3.00			3.2	10
5.00	0.2	1	0.7	2
10.00	0	0	0	0
25.0			0	0
50.0			0	0
100			0	0

Definitive study: Based on the cytotoxicity in the dose-range finding studies, concentrations of 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 μg/ml were used in the definitive study. Results are summarized in the table below. No significant increase in the transformation frequency was noted in any treatment group. Positive controls developed positive responses. In conclusion, 2'-fluoro-cytidine did not induce cell transformation in SHE cell cultures under the experiment conditions in this study.

Results of transformation trial

2'-fluoro-cytidine concentration (μg/ml)	Morphological transformation frequency (%)	Average # of colonies/dish	Relative plating efficiency (RPE, %)
Vehicle	0.137	32.4	100
BP 5 μg/ml	1.553	35.8	110
0.05	0.342	32.5	100
0.1	0.373	29.8	92
0.25	0.467	28.6	88
0.5	0.429	31.1	62
1.0	0	25.0	42
Historical control	0-0.57		

Summary of genotoxicity studies:

NX1838 appeared to be nonmutagenic in the Ames test and in L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay. The drug was also negative in *in vivo* micronucleus assay and *in vitro* SHE cell assay.

Genetic toxicology studies were also conducted with monomer nucleotides. In chromosomal aberrations studies with human whole blood lymphocytes in the absence of S9 activation, results were negative for all 2'-fluoropyrimidines and 2'-O-methylpurines. In the Ames test, results for all nucleotides were negative in the *Salmonella* strains. In *E. coli*, Results for the 2'-O-methylpurines were negative, while 2'-fluoropyrimidines yielded a marginal but reproducible positive response.

2.6.6.5 Carcinogenicity

Pegaptanib sodium, an angiogenesis inhibitor, is being developed as an intravitreal injection agent for the indication of age-related macular degeneration. In May 2002, the sponsor submitted a request to waive carcinogenicity studies for pegaptanib sodium.

The request was based on the drug's chemical structure and pharmacological class, chronic nonclinical toxicity study results, and negative genotoxicity study results. Although many factors supported the waiver, the positive results in *E. coli* in the Ames test for 2'-fluoropyrimidines concerned the reviewer. In addition, as a new molecular entity, the systemic exposure of the drug in humans was not very low (AUC = 26.7-31.8 μg-hr/ml). Therefore, the reviewing pharmacologist considered the data were not adequate to support the waiver of carcinogenicity studies, and recommended SHE cell assays with pegaptanib sodium and its metabolites, especially 2'-fluoropyrimidines. The sponsor was informed in September 2002 that the decision of the waiver of the carcinogenicity studies would be made based on the outcomes of the recommended SHE cell assay.

In the IND submission with Serial #: 134, reports of three SHE cell assays with pegaptanib sodium and 2'-fluoropyrimidines were included. The results were all negative. SHE cells have been used extensively in *in vitro* cell transformation studies. SHE cell assay has been proposed as a useful model to assess the carcinogenicity potential of diverse chemicals. The negative results from the SHE cell assays

relieved the reviewer's concern. The ICH guidance states that pharmaceutics administered by the ocular route may not need carcinogenicity studies unless there is a cause for concern or unless there is significant systemic exposure. Based on the drug's property, negative findings of the SHE assay and results of conducted nonclinical studies, the reviewing pharmacologist considered that a waiver of carcinogenicity studies could be granted.

A waiver for carcinogenicity studies was granted on August 8, 2003.

2.6.6.6 Reproductive and developmental toxicology

Reviewer's comments: Only embryofetal development studies were conducted.

Embryofetal development

Study title: 1005-003P: Intravitreous dosage-range developmental toxicity study of EYE001 in rabbits

Key study findings: EYE001 at doses up to 2 mg/eye (intravitreous injection on gestation days 6, 13 and 19) caused no general and developmental toxicity.
Results from this non-GLP dosage-range study provided limited information for reproductive safety assessment.
Study no.: 1005-003P
Volume #, and page #: ECTD
Conducting laboratory and location: —

Date of study initiation: Not indicated
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: EYE001, Lot #s: 241001A, 111002A, N06006F, C22001L003 and N06004F,

Methods

Doses: 0, 0.067, 0.2, 0.67, or 2.0 mg/0.067 ml/eye on gestation days 6, 13 and 19
Species/strain: Pregnant New Zealand white [ ] NZW)SPF] rabbits
Number/sex/group: 5/group
Route, formulation, volume, and infusion rate: Intravitreous injection, PBS, 0.067 ml/eye (The protocol did not mention if one eye or both eyes were treated.)
Satellite groups used for toxicokinetics: No
Study design: See below.
Parameters and endpoints evaluated: See below.

Mortality: Twice daily
Clinical observations: At least once daily
Body weights: Daily
Food consumption: Daily
TK: Gestation days 6 and 19 at 1, 3, 6, 9, 24, 48, 96, and 144 hr after dosing

All rabbits were terminated on gestation day 29 and examined for the number of corpora lutea, implantation sites, and uterine contents. A gross necropsy was performed. Fetuses were examined for weights, sex and gross external alterations.

Results

Mortality (dams): Two rabbits, one each in the 0.067 mg/eye and 2.0 mg/eye groups, were sacrificed on gestation day 8 due to ocular infection. Adverse clinical observations in these animals included small pupil, iris congestion, injection site swelling, and corneal edema. The death was considered as injection route-related, not drug-related.

Clinical signs (dams): Local ocular inflammatory responses were observed that included injection site swelling and red, iris congestion, small pupil and corneal edema. These changes were seen in control and treated animals, and were considered as injection route-related. No other toxicologically significant clinical signs were seen.

Clinical observations in rabbits treated with EYE001 (total incidence/animals affected)

Group	1	2	3	4	5
Dose (mg/eye)	Control	0.067	0.2	0.67	2.0
Injection site, red	15/1	12/2	5/1	0	7/1
Injection site, swelling	6/1	2/1	0	0	2/1
Left eye, iris congestion	0	1/1	0	0	1/1
Left eye, pupil small	0	1/1	0	0	1/1
Left eye, corneal edema	0	0	0	0	1

Necropsy (dams): No drug-related abnormal findings were noted.

Body weight (dams): Body weight and body weight gain were generally comparable among different groups. No biologically relevant findings were noted.

Food consumption (dams): No clear dose-dependent effects were noted among different groups.

Toxicokinetics: See PK section.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Results are summarized in the table below. No drug-related changes in C-sectioning and litter parameters were observed.

Reproductive parameters in rabbits treated with EYE001 (mean ± SD)

Group	1	2	3	4	5
Dose (mg/eye)	Control	0.067	0.2	0.67	2.0
Pregnant rabbits	5	4	5	5	5
C-section on gestation day 29	5	3	5	5	4
Corpora lutea/dam	9.2± 1.6	9.0± 1.0	8.8± 1.6	11.4± 2.1	13.8± 2.1
Implantations/dam	8.8± 1.3	7.7± 1.5	7.0± 1.9	10.4± 0.9	10.5± 0.7
Litter size	8.2± 1.1	7.3± 2.1	6.8±1.8	10.0± 0.7	10.2±1.5
Live fetuses	8.2± 1.1	7.3± 2.1	6.8±1.8	9.8± 0.8	10.2±1.5
Dead fetuses	0	0	0	0.2±0.4	0
Early resorption	0.6±0.5	0.3±0.6	0.2±0.4	0.2±0.4	0.2±0.5
Late resorption	0	0	0	0.2±0.4	0
Placenta appeared normal (%)	100	100	100	100	100

Offspring (malformations, variations, etc.): Sex and weight data of offspring are summarized in the table below. No abnormal findings in gross external examination were noted.

Offspring data (mean ± SD)

Group	1	2	3	4	5
Dose (mg/eye)	Control	0.067	0.2	0.67	2.0
Litters with one or more live fetuses	5	3	5	5	4
Live fetuses	41	22	34	49	41
Live male fetuses	19	12	19	17	17
Live male fetuses/litter (%)	47.8±18.0	58.1±19.1	52.6±18.9	34.4±9.5	40.5±20.0
Live fetus body weight (g/fetus)	40.81±5.70	48.93±3.67	47.07±2.62	39.37±1.85	43.60±1.01
--Male fetus	41.47±5.61	49.91±3.95	46.84±3.23	39.30±1.17	44.67±2.57
--Female fetus	40.40±5.70	46.87±2.59	46.38±2.85	39.23±2.91	42.80±2.44
Dead or resorbed conceptuses/litter	6.5±6.1	5.6±9.6	2.5±5.6	5.6±5.2	2.1±4.2

In summary, pregnant NZW rabbits were treated by intravitreal injection with EYE001 at 0.067, 0.2, 0.67, and 2.0 mg/eye on gestation days 6, 13 and 19. Animals were sacrificed on gestation day 29 and a C-section was performed. Ocular inflammatory responses including injection site swelling and red, iris congestion, small pupil and corneal edema were noted in several animals across groups and were considered as injection route-related. No drug-related toxicity was noted in general systemic and reproductive toxicology parameters. In conclusion, intravitreal injection of EYE001 in rabbits at doses up to 2.0 mg/eye showed no developmental toxicity in this study.

Study title: 1005-004: Intravenous developmental toxicity study of EYE001 in mice

Key study findings: At 40 mg/kg/day, fetal body weights were significantly reduced for both male and female fetuses, and a reduction in the average number of ossified forepaw phalanges and ossified hindpaw phalanges also occurred. An increase in heart rate and a decrease in RR, PR and QT intervals were seen in MD and HD pups in the ECG examination performed on lactation day 13, 14, 15, 16 or 17, but not on day 10. The toxicological significance was not known. No other Cesarean-sectioning or litter parameters were affected. The dose of 6.5 mg/kg was considered as the NOAEL.

Study no.: 1005-004
Volume #, and page #: ECTD
Conducting laboratory and location: —

—

Date of study initiation: 3/20/2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: EYE001, Lot #: C22001L003

Methods

Doses: 0, 1.0, 6.5, or 40 mg/5 ml/day from gestation day 6 to day 15 (For pup ECG/heart pathology evaluation groups, mice were treated from gestation day 6 to day 17.)

Species/strain: Mated — CD-1(ICR)BR female mice, 23-33 g

Number/sex/group: 21/group

Route, formulation, volume, and infusion rate: Intravenous injection, PBS, 5 ml/kg

Satellite groups used for toxicokinetics: 4/group for pup ECG/heart pathology examinations and 36 mice in HD group for TK assay

Study design: See table below. One hundred fifty virgin female mice were placed into cohabitation with 150 breeder male mice, one male mouse per female mouse. Female mice with a copulatory plug *in situ* were considered to be at gestation day (GD) 0 and assigned to individual study groups. All main study animals were sacrificed on GD 18, Cesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Each fetus was weighed and examined for sex and gross external alterations.

Approximately one-half of fetuses in each litter were examined for soft tissue alterations. The remaining fetuses were examined for skeletal alterations.

Group	Dosage (mg/kg)	N (main study) Treated on GS days 6-15	N (TK)	N (pup ECG/heart evaluation) Treated on GS days 6-17
1	Vehicle	21		4
2	1	21		4
3	6.5	21		4
4	40	21	36	4

Parameters and endpoints evaluated:

Mortality: Twice daily
Clinical observations: At least once daily
Body weights: Daily
Food consumption: Not performed
TK: Group 4 TK animals, blood samples were collected on gestation days 6 and 15 at 5 and 20 min, 1, 3, 9, and 24 hr after dosing (3 mice/timepoint). Additionally, fetuses and amniotic fluid were collected from mice sacrificed on GD 15 or 16.
Gross pathology: All surviving main study mice were sacrificed on GD 18, Cesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus of each mouse was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions.

Mice assigned to pup ECG/heart pathology evaluation: Dams assigned to the pup ECG/heart pathology evaluation were evaluated for natural delivery parameters and observations of maternal behavior. Pups in each litter were counted once daily and evaluated for viability twice daily. Clinical observations and body weights were recorded. Electrocardiogram tracings were recorded for all pups on day 10 of lactation/postpartum; however due to equipment problems, ECGs were performed a second time on either lactation/postpartum day 13, 14, 15, 16 or 17. F0 generation female mice assigned to the pup ECG/heart pathology evaluation were sacrificed on lactation/postpartum day 15 or 20, and the carcasses were discarded without further evaluation. All surviving F1 generation pups were sacrificed on lactation/postpartum day 15 or 20 and examined for gross lesions. The heart and great vessels from each pup were weighed, examined for any apparent external defects and retained. The heart, great vessels and gross lesions from five male pups and five female pups of the control and the high dosage groups were examined histologically.

Results

Mortality (dams): No mortality occurred during the study period.

Clinical signs (dams): No drug-related, toxicologically significant clinical signs were seen. One 1 mg/kg/day and three 40 mg/kg/day mice delivered before C-sectioning on GD 17 or 18. The deliveries were not considered related to the test article but related to early mating (on the day before mating was

confirmed) since mice in the highest dosage group were three of the four heaviest (53, 55, and 56 g, respectively, vs. group mean of 51.1 g) in this group on GD 16. The low dose mouse was 59 g vs. the group mean of 56.3 g on GD 17. Examinations on these early delivery animals showed no general and reproductive toxicity findings.

Necropsy (dams): No drug-related abnormal findings were noted in necropsy.

Body weight (dams): Dosages of the test article as high as 40 mg/kg/day did not affect body weights or body weight gains during the gestation or lactation periods.

Toxicokinetics: See section 2.6.4.4. Distribution.

Terminal and necroscopic evaluations C-section data (implantation sites, pre- and post-implantation loss, etc.): Results are summarized in the table below. Totally 18, 18, 17 and 18 mice were pregnant in Groups 1, 2, 3, and 4, respectively. Because of the early delivery, C-sectioning results were based on 18, 17, 17 and 15 pregnant mice in the four respective groups. The litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions, percent dead or resorbed conceptuses per litter and percent live male fetuses were comparable among the four dosage groups and did not significantly differ. All placentae were normal.

A dosage of 40 mg/kg/day significantly reduced ($p < 0.05$) fetal body weights. Fetal body weights were reduced 4% to 5% for both male and female fetuses at this dose. However, the fetal body weights were within the historical control range.

Reproductive parameters in mice treated with EYE001 (mean ± SD)

Group	1	2	3	4	Historical control
Dose (mg/kg)	Control	1	6.5	40	
Mice tested	21	21	21	21	
Pregnant mice	18	18	17	18	
C-section on gestation day 18	18	17	17	15	
Corpora lutea/dam	14.5 ± 2.3	15.6± 2.0	15.2± 2.4	14.7± 2.0	9.8-15.6
Implantations/dam	13.7± 1.7	14.5± 1.5	14.2± 1.5	13.3± 1.6	8.4-13.6
Litter size	13.1± 1.6	13.4± 1.6	13.5± 1.9	12.9± 1.4	
Live fetuses/litter	13.1± 1.6	13.4± 1.6	13.5± 1.9	12.9± 1.4	7.8-12.6
Dead fetuses	0	0	0	0.1± 0.2	0-0.7
Live male fetuses/litter (%)	50.2± 12.8	50.6± 11.7	46.1± 19.4	49.9± 15.3	
Live fetus body weight (g/fetus)	1.36± 0.08	1.34± 0.07	1.36± 0.08	1.29± 0.07 (↓5%)	
--Male fetus	1.39± 0.08	1.36± 0.07	1.38± 0.08	1.31± 0.08 (↓6%)	
--Female fetus	1.34± 0.08	1.31± 0.08	1.35± 0.08	1.26± 0.06 (↓6%)	
Dead or resorbed conceptuses/litter	4.4± 4.2	8.0± 7.0	5.4± 7.9	3.3± 5.1	
Early resorption	0.4± 0.5	0.9± 0.9	0.7± 1.1	0.3± 0.6	0.2-3.2
Late resorption	0.2± 0.4	0.3± 0.8	0± 0.2	0.1± 0.2	0-0.8
Mice with any resorptions N (%)	10 (55.6)	12 (70.6)	7 (41.2)	5 (33.3)	(16.7-87.5%)
Mice with viable fetuses N(%)	18 (100)	17 (100)	17 (100)	15 (100)	
Placenta appeared normal (%)	100	100	100	100	
% live male fetuses/litter	50.2± 12.8	50.6± 11.7	46.1± 19.4	49.9± 15.3	43.2-59.0
Live fetal body weight (g)	1.36± 0.08	1.34± 0.07	1.36± 0.09	1.29± 0.07*	1.25-1.43
--male fetuses	1.39± 0.08	1.36± 0.07	1.38± 0.08	1.31± 0.08*	1.28-1.45
--female fetuses	1.34± 0.08	1.31± 0.08	1.35± 0.08	1.26± 0.06*	1.22-1.40
% dead or resorbed conceptuses/litter	4.4± 4.2	8.0± 7.0	5.4± 7.9	3.3± 5.1	2.4-12.3

* significantly different from the control values ($p \leq 0.05$)

Offspring (malformations, variations, etc.): Positive findings are summarized in the table below. No fetal gross external, soft tissue or skeletal malformations or variations were considered as drug-related. Alterations that occurred were considered unrelated to the test article because the findings were within the historical control ranges, and there was no dose-dependence.

In the HD group, the number of ossified forelimb phalanges was significantly reduced and the number of ossified hindpaw phalanges was also reduced. These values for the HD group were comparable with historical control values. These delays in ossification are commonly observed in the presence of decreased fetal weights, as occurred in this study. All other fetal ossification site averages were comparable among the groups.

Fetal alteration data (mean ± SD)

Group	1	2	3	4	Historical
Dose (mg/kg)	**Control**	**1**	**6.5**	**40**	
Litters evaluated	**18**	**17**	**17**	**15**	
Fetuses evaluated	236	227	229	194	
Litters with fetuses with any alteration N (%)	15 (83.3)	12 (70.6)	12 (70.6)	12 (90.0)	
Fetuses with fetuses with any alteration N (%)	36 (15.2)	38 (16.7)	34 (14.8)	34 (17.6)	
Fetuses with any alteration observed (%/litter)	15.0± 10.6	16.5± 15.01	15.0± 14.8	17.7± 13.2	
Gross external examination					
Hindlimb: rotated medially	0	0	1/1	0	0-4/0-1
Head: exencephaly	0	1/1*@	1/1&	1/1$	0-1/0-1
Eyes: lids open	0	1/1@	0	0	0-1/0-1
Fetal soft tissue examination					
Fetuses evaluated	**113**	**110**	**108**	**92**	
Brain: misshaped	0	0	1/1&	0	0-1/0-1
Vessels: umbilical artery descended to the left of the urinary bladder	1/1	0	0	0	0-9/0-7
Fetal skeletal alterations					
Fetuses evaluated	**123**	**117**	**121**	**102**	
Skull: frontals, contained an interfrontal	10/8	11/7	16/4	8/4	0-51/0-19
Skull: frontals, incompletely ossified	0	1/1@	0	1/1$	0-1/0-1
Skull: parietals, not ossified	0	1/1@	0	0	0-1/0-1
Skull: interparietal, not ossified	0	1/1@	0	1/1$	0-1/0-1
Skull: supraoccipital, not ossified	0	1/1@	0	1/1$	0-1/0-1
Skull: parietals, incompletely ossified	0	0	0	1/1$	
Cervical vertebrae: cervical rib present at 7th cervical vertebra	22/12	25/9	18/9	25/9	1-25/1-14
Ribs: thickened	0	0	0	1/1	
Sternal centra: irregular ossification	4/4	6/4	0	2/2	
--Sternal centra: asymmetric	1/1	6/4	0	2/2	0-4/0-4
--Sternal centra: incompletely ossified	1/1	0	0	0	0-1/0-1
--Sternal centra: fused	2/2	1/1	0	0	0-9/0-6
Ossification sites/fetus/litter					
Forelimb					
--phalanges	12.03± 0.54	11.96± 0.54	12.06± 0.45	11.49± 0.63**	10.47-11.77
Hindlimb					
--phalanges	11.42± 1.34	11.42± 1.12	11.74± 0.95	10.74± 0.93	10.09-11.47

*fetus incidence/litter incidence; ** significantly different from the control values ($p \leq 0.01$); @, $, and & indicated several findings were seen in the same animals.

Natural delivery and litter observations: Totally 4, 4, 4 and 3 dams that were assigned to the pup ECG/heart pathology evaluation were pregnant and delivered litters in the 0 (Control), 1, 6.5 and 40 mg/kg/day dosage groups, respectively. Natural delivery observations were unaffected by the drug treatment. All reproductive parameters (see table below) were comparable among the four dosage groups and did not significantly differ. One mouse in the 6.5 mg/kg/day dosage group delivered a litter consisting of 11 pups of unknown viability at birth (Cannibalization precluded the determination of viability).

Reproductive parameters in mice for pup ECG/heart pathology examination (mean ± SD)

Group	1	2	3	4
Dose (mg/kg)	Control	1	6.5	40
Mice assigned	4	4	4	4
Pregnant mice	4	4	4	3
Delivered litters	4	4	4	3
Duration of gestation (day)	19.5± 0.6	19.5± 0.6	19.2± 1.0	19.7± 0.6
Gestation index (%)	100	100	100	100
Mice with stillborn pups	0	0	0*	0
Mice with no liveborn pups	0	0	0*	0
Mice with all pups dying on days 1-4 postpartum	0	0	0*	0
Mice with all pups dying on days 5-20 postpartum	0	0	0*	0
Delivered litters with one or more liveborn pups	4	4	3	3
Pups delivered/litter	11.5± 5.3	11.8± 5.8	12.7± 2.3	13.0± 1.7
--Liveborn	11.5± 5.3	11.8± 5.8	12.7± 2.3	12.7± 1.2
--unknown vital status	0	0	0	1
Pups found dead				
--day 1	0	0	0	0
--days 2-4	0	1	0	0
--days 5-12	0	0	0	2**
Surviving pups/litter on day 12 postpartum	11.5± 5.3	11.5± 5.7	12.7± 2.3	12.0± 1.0
Viability index (%)	100(46/46)	97.9(46/47)	100(38/38)	100(38/38)
Lactation index (%)	100(46/46)	100(46/46)	100(38/38)	94.7(36/38)**
% male pups				
--day 1	53.9± 15.5	49.2± 13.7	68.1± 19.0	50.8± 13.0
--day 12	53.9± 15.5	48.2± 14.1	68.1± 19.0	50.9± 17.6
Pup weight/litter (g)				
--day 1	1.6± 0.3	1.8± 0.4	1.7± 0.1	1.6± 0.2
--day 4	2.7± 0.7	2.9± 0.8	2.7± 0.2	2.6± 0.2
--day 12	6.0± 1.4	6.6± 3.1	5.9± 1.0	6.0± 0.6

* One dam had 11 pups in which cannibalization precluded the determination of viability. ** One pup was missing on day 11 and one pup died on day 12 postpartum.

For clinical observations in pups for ECG/heart pathology evaluation, no treatment-related abnormal findings were noted. All pups appeared normal at necropsy.

ECG analysis, heart and great vessel weights and histopathology: The result of ECG tracings from pups evaluated at a frequency response rate of 100 mm/sec over several days of lactation indicated no evidence of prolongation of the QT interval or QTc in male or female pups at any dosage. However, female pups showed a statistically faster heart rate (MD and HD groups, $p \leq 0.05$), shorter RR interval, shorter PR interval and shorter QT interval (HD groups, $p \leq 0.05$) on ECG recorded approximately two weeks postpartum (LDs 13 to 17). The heart rate seemed also high in male MD and HD pups. No effects were seen on heart rate when ECG was examined on LD 10 pups only. The toxicological significance of the increased heart rate was not known.

Pups' mean ECG values (mean± SD)

	Males				Females			
Group	1	2	3	4	1	2	3	4
HR (bpm)	596±120	584±162	671±119	676±62	532±123	581±116	638±128	657±64
RR (msec)	106±29	112±37	93±19	90±9	121±40	107±22	98±22	92±9
PR (msec)	35±6	39±11	32±6	30±1	38±8	36±6	33±8	31±3
QT (msec)	44±11	41±12	36±10	36±8	46±10	41±8	38±12	38±6
QTc(msec)	43±7	39±6	37±7	38±7	42±4	39±6	38±8	39±6

The average weight of the heart and great vessels for the F1 generation pups was similar among groups. The histopathological examination of the ventricles, atria and blood passages, as assessed in tissue sections, appeared normal for all pups.

In summary, pregnant CD-1 mice were treated by daily intravenous injection with EYE001 at 0, 1.0, 6.5 or 40 mg/kg on gestation days 6 through 15. Main study animals were sacrificed on gestation day 18 and a C-section was performed. Dam and fetus data were examined for reproductive toxicity evaluation. Dams assigned to the pup ECG/heart pathology evaluation study (4/group) were evaluated for natural delivery parameters and observations of maternal behavior. Electrocardiogram tracings were recorded for all pups on day 10 of lactation/postpartum and on either LD 13, 14, 15, 16 or 17.

EYE001 at iv doses up to 40 mg/kg/day did not cause any maternal toxicity. However, at 40 mg/kg/day, fetal weights were significantly reduced for both male and female fetuses. A reduction in the average number of ossified forepaw phalanges and a reduction in the average number of ossified hindpaw phalanges were also noted. These changes were within the historical control ranges. In addition, no body weight differences were seen in naturally delivered pups. Therefore, these findings might not be toxicologically significant. No other Cesarean-sectioning or litter parameters were affected. No fetal gross external, soft tissue or skeletal malformations were caused by EYE001. [**Reviewer's comments**: An MTD in dams was not reached. The dams could have tolerated higher doses.]

Natural delivery and litter observations were unaffected and no clinical or necropsy observations in the F1 generation pups were attributable to the treatment. Pups in MD and HD groups showed an increase in heart rate and a decrease in RR, PR, and QT intervals in ECG examination performed on LD 13, 14, 15, 16 or 17, but not on LD10. The toxicological significance was not known.

2.6.6.7 Local tolerance

No specific studies were submitted.

2.6.6.8 Special toxicology studies

Study title: 109-98013-T: Incorporation of 2'-FU into DNA of rats following long-term administration of NX1838

Key study findings: Monomer nucleotide 2'-FU was incorporated into cellular DNA of all tissues examined following subchronic iv administration to rats.

Study no.: 109-98013-T
Volume #, and page #: ECTD
Conducting laboratory and location —
—

GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: NX1838,
Formulation/vehicle:

Methods

Doses: 0 (PBS) and 10 mg/ml/kg, qd x 13 weeks at a dose volume of 1 ml/kg
Species/strain: Sprague-Dawle[illegible].CD®BR VAF/Plus® rats
Number/sex/group or time point (main study): 3

Route, formulation, volume, and infusion rate: Intravenous, PBS
Age: 7 weeks old
Weight: 219-239 g for males and 154-176 g for females

Study design: The purpose of this study was to determine if the administration of NX1838 resulted in the incorporation of 2'-fluorouridine (2'-FU) into cellular DNA when administered to rats by the iv route. Selected tissues from Study 109-98004-T were used for DNA isolation and 2'-FU quantitation. DNA was isolated from selected tissues using the — The DNA was —

— The amount of 2'-FU incorporated into DNA was quantified by dividing the number of picomoles of 2'-FU per µl of DNA hydrolysate (determined by LC/MS) by the micromoles of thymidine per µl of DNA hydrolysate (determined by HPLC).

Results:

Results, summarized in the table below, showed that 2'-FU was present in all tissues examined.

Incorporation of 2'-FU into DNA of rats treated with NX1838 (iv) (pmol/µmol dT, mean ± SD)

Treatment	Kidney	Liver	Muscle	Spleen	Testes	Spiked liver
Control	BLQ	BLQ	BLQ	2.2, 2BLQ*	BLQ	BLQ
10 mg/kg	22.6±2.2	83.2±29.4	33.3±5.6	11.6±1.7	10.0±1.6	

BLQ: — *: Two animals were BLQ.

Administration of NX1838 to rats at a dose of 10 mg/kg daily for 90 days by the intravenous route resulted in incorporation of the 2'-FU into cellular DNA. Incorporation amounts were similar to the levels achieved following administration of 5 mg 2'-FU/kg/day for 90 days. It seemed that NXI838 was degraded in the body to component nucleosides that could subsequently be incorporated into the DNA.

Study title: 109-98014-T: DNA and RNA incorporation of 2'-Fluorouridine into rats and woodchucks following long-term administration

Key study findings: Monomer nucleotide 2'-Fluorouridine was incorporated into cellular DNA and RNA of all tissues examined following subchronic iv administration.

Study no.: 109-98014-T
Volume #, and page #: ECTD
Conducting laboratory and location: —

GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: 2'-fluorouridine (2'-FU)

Methods

For detailed study methods, please see Studies 109-97008-T and 109-97009-T.

Doses: 0, 5, 50 and 500 mg/kg/day for rats and 0, 0.75 and 7.5 mg/kg/day for woodchucks, intravenous injection, qd x 90 days
Species/strain: Male Fisher 344 rats and woodchucks

Study design: The purpose of this study was to determine if 2'-fluorouridine was incorporated into cellular DNA and RNA when administered to rats and woodchucks by the iv route. Toxicology studies in woodchucks and rats indicated that the 2'-pyrimidines would not cause delayed toxicity similar to FIAU when administered by an intravenous route. There was still interest, however, as to whether the 2'-fluoropyrimidines could be incorporated into DNA and RNA.

Selected tissues from Studies 109-97008-T and 109-97009-T were used for DNA/RNA isolation and 2'-FU quantitation. DNA was isolated from selected tissues using the —— RNA was isolated from liver using the —— The amount of 2'-FU incorporated was quantified by dividing the number of picomoles of 2'-FU per µl of DNA or RNA hydrolysate (determined by LC/MS) by the micromoles of thymidine (for DNA) or guanosine (for RNA) per µl of DNA/RNA hydrolysate (determined by HPLC).

Results:

Results, summarized in the table below, showed that 2'-FU was present in all tissues examined.

Incorporation of 2'-FU into DNA and RNA of Rat and Woodchuck tissues (pmol/µmol dT and pmol/µmol G)

	DNA					RNA
Treatment	Kidney	Liver	Muscle	Spleen	Testes	Liver
Rats, N	3	3	3	3	3	3
Control	BLQ	BLQ	BLQ	2.2, 2BLQ*	BLQ	BLQ
5 mg/kg	18.2±1.4	49.1±5.1	53.7±4.57	24.6±2.0	22.3±1.4	4.2±0.8
50 mg/kg	ND	128.6±17.1	ND	ND	ND	ND
500 mg/kg	153.8±72.9	209.3±34.3	170.4±16.6	165.3±15.2 (n=2)	43.7±13.9	43.4±6.3
Woodchucks, N	3	3	3	2	3	3
Control	ND	BLQ	ND	ND	ND	ND
0.75 mg/kg	ND	35.3±12.5	ND	ND	ND	ND
7.5 mg/kg	ND	407.7±124.3	ND	ND	ND	ND

BLQ: —— *: Two animals were BLQ; ND: Not analyzed.

Administration of 2'-FU to rats and woodchucks daily by intravenous route resulted in incorporation of the compound into DNA and RNA. The sponsor indicated that the levels of incorporation in DNA were 10-20 fold lower (in rats) and 5-fold lower (in woodchucks) than observed in studies with fialuridine where animals were dosed with similar amounts of compounds.

Study title: 109-97001-T: Immunogenicity of NX1838 in an *in vitro* lymphocyte stimulation assay

Key study findings: In contrast to DNA-based oligonucleotides, the RNA NX1838 aptamer and RNA oligonucleotides induce little or no immune stimulation of human and mouse lymphocytes *in vitro*.

Study no.: 109-97001-T
Volume #, and page #: ECTD

Conducting laboratory and location:

GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: NX1838
Three oligonucleotides with a common nucleotide sequence containing a single central CpG sequence motif were synthesized as control oligonucleotides: 1) NXcpg1-DNA, an unmodified DNA oligonucleotide, 2) NXcpg1-Phos DNA, a phosphorothioate-modified DNA oligonucleotide, and 3) NXcpg1-RNA, an RNA oligonucleotide.
Formulation/vehicle: PBS
Indicating cells: Human peripheral blood lymphocytes (PBLs) from 4 healthy human donors and murine spleen lymphocytes from 2 C3H/He mice

Methods

Concentrations: 0.000128 μM to 40 μM

Study design: The purpose of this study was to determine the immunogenicity of NX1838, an RNA oligonucleotide containing modified nucleotides, using an *in vitro* lymphocyte stimulation assay. Bacterial DNA and synthetic DNA oligonucleotides are potent inducers of immune responses in humans and mice, stimulating B lymphocyte proliferation, immunoglobulin production and cytokine secretion. Unmethylated CpG dinucleotides are the most potent inducers, and phosphorothioate-modified DNA oligonucleotides are more stimulatory than unmodified DNA oligonucleotides. In this study, test compounds containing CpG structure were dissolved in PBS, and 10 μl of a test compounds was added to the bottom of 96-well microtiter plates in triplicate. Human or murine lymphocytes (1×10^5 cells/well in 90 μl medium) were added to the microwells and incubated for 72 hr (human) or 40 hr (murine). Immune stimulation was measured by a standard ^{3}H-thymidine-uptake assay.

Results:

NXcpg1-PhosDNA at the concentrations between 0.016 and 0.4 μM induced potent stimulation of human peripheral blood lymphocytes. The unmodified DNA oligonucleotide NXcpg1-DNA also induced potent stimulation but at higher concentrations. In contrast to these DNA oligonucleotides, NX1838 and NXcpg1-RNA induced background or near background stimulation. In murine lymphocytes, NXcpg1-ProsDNA showed similar stimulating effects as in human lymphocytes, and NX1838 and NXcpg1-RNA exhibited only background stimulation.

In conclusion, different from DNA oligonucleotides, NX1838 and NXcpg1-RNA induced little or no immune stimulation of human and murine lymphocytes *in vitro*.

Study title: 109-98005-T: Immunogenicity of NX1838 in BALB/c mice, Sprague-Dawley rats, Dutch-belted rabbits

Key study findings: Antibodies against NX1838 were not detected in mice, rats and rabbits after intravenous, intravitreous and subcutaneous administrations.

Study no.: 109-98005-T
Volume #, and page #: ECTD
Conducting laboratory and location: —

—

GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: NX1838
Formulation/vehicle: PBS

Methods

Animals: Female BALB/c mice, 4-6 weeks old, 5/group
Sprague-Dawley rats (Using serum samples obtained from the animals employed in Study 109-98004-T)
Dutch belted rabbits (Using serum samples collected from the animals employed in Study 109-98003-T)

Study design: The purpose of this study was to evaluate the immunogenicity of NX1838 when administered to a variety of animal species by various routes. Detailed study design is summarized in the table below. Serum samples (at a 1:100 dilution) were assayed by an ELISA for the presence of serum anti-NX1838 immunoglobulins.

Immunizations in mice, rats and rabbits

	Group 1	Group 2	Group 3	Group 4	Sample collection
Mouse 5•/ group	100 µl of saline, sq at Weeks 0, 2, 4, 6 and 8.	NX1838 50 µg, sq at Week 0; 25 µg at Weeks 2, 4, 6 and 8.	NX1838 50 µg + BSA 50 µg, sq at Week 0; NX1838 25 µg + BSA 25 µg at Weeks 2, 4, 6 and 8.	NX1838 100 µg iv at Weeks 0, 1, 2, 3, 4, 5, 6, 7 and 8.	Serum samples were collected at Weeks 0 (before dosing), 3, 5, 7 and 9.
Rat 10/sex/ group	PBS 1 ml/kg, iv, qd x 3 months	NX1838 0.1 mg/kg, iv, qd x 3 months	NX1838 1.0 mg/kg, iv, qd x 3 months	NX1838 10 mg/kg, iv, qd x 3 months	Serum samples were collected on Day 90 (24 hr following the final dose).
Rabbit 5/sex/ group	PBS 50 µl, intravitreous inj., qow x 6.	NX1838 0.1 mg, intravitreous inj., qow x 6.	NX1838 0.3 mg, intravitreous injection, qow x 6.	NX1838 1 mg, intravitreous injection, qow x 6.	Serum samples were collected 1 week after the last injection.

Results:

Mouse: No anti-NX1838 antibody was detected in the serum of any dose groups. The finding of IgG bound to native bovine serum albumin indicated that the mice were immunocompetent and that reagents used in the study were functional.

Rat: No response to NX1838 was detected in any dose groups. The same sera, at 1:10,000 dilution, were assayed for the presence of rat immunoglobulins and a maximal response was noted.

Rabbit: No serum samples were positive for NX1838 specific immunoglobulins. The same sera, at 1:10,000 dilution, were assayed for the presence of rabbit immunoglobulins and a maximal response was noted.

In conclusion, no antibody against NX1838 was detected in mice, rats and rabbits after intravenous, intravitreous and subcutaneous administrations.

Special toxicity study summary:

In the immunogenicity study, no antibody against NX1838 was detected in mice, rats and rabbits after intravenous, intravitreous or subcutaneous administrations. Unlike DNA-based oligonucleotides, NX1838 and RNA-based oligonucleotides induced little or no immune stimulation in human or mouse lymphocytes *in vitro*. 2'-fluorouridine was incorporated into cellular DNA and RNA of all tissues examined following subchronic iv administration of NX1838 and 2'-FU to SD rats, Fisher 344 rats and woodchucks.

2.6.6.9 Discussion and Conclusions

No toxicologically significant findings were observed in systemic toxicity studies.

In ocular toxicity studies in different animal species, injection-procedure-induced ocular lesions were seen in all studies in both control and treated animals. All these changes were reversible. The sponsor indicated that in clinical studies, the most frequently reported adverse events were ocular, transient and for the most part related to the injection procedure. These events occurred in ≥ 10% of patients who received 0.3 mg Macugen.

NX1838 was negative in genotoxicity studies. Genetic toxicity studies were also conducted with monomer nucleotides. In chromosomal aberrations studies with human whole blood lymphocytes in the absence of S9 activation, results were negative for all 2'-fluoropyrimidines and 2'-O-methylpurines. In the Ames test, results for all nucleotides were negative in the *Salmonella* strains. In *E. coli*, results for the 2'-O-methylpurines were negative, while 2'-fluoropyrimidines yielded a marginal but reproducible positive response.

In an embryofetal development study in pregnant CD-1 mice, EYE001 at iv doses up to 40 mg/kg/day did not cause any maternal toxicity. However, at 40 mg/kg/day, fetal weights were significantly reduced for both male and female fetuses. A reduction in the average number of ossified forepaw phalanges was also noted. These changes were within the historical control ranges. In addition, no body weight differences were seen in naturally delivered pups. Therefore, these findings might not be toxicologically significant. No other abnormal findings were noted. The reviewer believes that in this study the dams could have tolerated higher doses. An MTD in the dams was not reached. However, the high dose used in mice (40 mg/kg or 120 mg/m^2) was 650 time human dose (0.3 mg/eye, 0.005 mg/kg or 0.185 mg/m^2) using surface area. The safety margin is relatively great. At the dose of 40 mg/kg/day, mean pegaptanib plasma concentrations at 5 min post injection on gestation day 15 were about 2000 µg/ml and the mean AUC_{tau} was approximately 8000 µg.hr/ml. In humans, at the maximum dose administered of 3 mg/study eye given every 4 weeks, mean C_{max} values were about 90 ng/ml (or 0.09 µg/ml) and AUC_{tau} values were approximately 25 µg.hr/ml. Thus, pegaptanib maximum plasma concentrations and AUC values after daily IV dosing in pregnant CD-1 mice were greater than 20000-fold and 300-fold those seen in humans receiving intravitreous injections of 3 mg/eye, respectively. Considering the proposed clinical dose is only 0.3 mg/eye and dosing interval is 6 weeks, a higher safety margin is assured.

The pharmacologic activity of the drug suggests a potential risk and does not appear to benefit embryo/fetal development. Macugen was $VEGF_{165}$ ($VEGF_{164}$ in rodents) specific. Macugen binds to $VEGF_{165}$ with high affinity and specificity, thereby inhibiting $VEGF_{165}$ binding to its VEGF receptors. In humans, there are several VEGF isoforms. It is reported[1] that when pegaptanib sodium, a $VEGF_{164}$-specific neutralizing aptamer, was administered, it suppressed the leukocyte adhesion and pathological neovascularization, whereas it had little or no effect on physiological neovascularization. In parallel

experiments, genetically altered $VEGF_{164}$-deficient ($VEGF_{120/188}$) mice exhibited no difference in physiological neovascularization when compared with wild-type controls. VEGF isoforms other than $VEGF_{164}$, in combination, may be sufficient to promote normal physiological neovascularization.

Considering the great safety margin in the mouse study, multiple publications[1-4] showing that genetically altered $VEGF_{164}$ deficient mice had normal vasculature, and indication of the drug, the reviewer believes that it is acceptable to maintain the "Teratogenic Effects" in "Pregnancy Category" as a B.

(1). Susumu Ishida, et al. 2003. $VEGF_{164}$-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. *J. Exp. Med.* 198:483–489.
(2). Tomobiko Usui, et al. 2004. $VEGF_{164(165)}$ as the pathological isoform: differential leukocyte and endothelial responses through VEGFR1 and VEGFR2. *Invest. Ophthalmol. Vis. Sci.* 45:368–374.
(3). Barry Robert, et al. 2000. Coexpression of neuropilin-1, Flk1, and $VEGF_{164}$ in developing and mature mouse kidney glomeruli. *Am. J. Physiol. Renal. Physiol.* 279: F275–F282.
(4). Ingeborg Stalmans, et al. 2002. Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. *J. Clin. Invest.* 109:327–336

There is a concern that 2'-fluoropyridimes could be liberated after enzyme degradation of NX1838 and subsequently incorporated into DNA. Such incorporation could lead to toxicity similar to those observed during the clinical trials of fialuridine, a 2'-fluoropyrimidine.

Fialuridine (FIAU) is a fluorinated nucleoside analog developed for the treatment of hepatitis B. FIAU was efficacious in clinical trials in reducing viral titers following 30 days of treatment. Because viral titers began to rise following withdrawal of treatment, additional trials of longer duration were conducted. During these longer trials, seven individuals experienced severe toxicity characterized by severe lactic acidosis, liver failure and shock, despite being off treatment for up to 17 days. Five individuals died and two survived most likely because of liver transplants. The characteristics of the toxicity were subsequently described as hepatic failure, pancreatitis, and microvesicular steatosis of the liver. Myopathies and neuropathies were also described. The mechanism was described as a delayed mitochondrial toxicity. 2'-Fluoropyridimes have structural similarities and differences to FIAU. All three have a fluorine substitution at the 2'-position of the sugar. FIAU contains an arabinose sugar with 2'-OH substituted with a fluorine. The 2'-fluoropyridimes used in the aptamers contain a ribose sugar with the 2'-OH substituted with fluorine. FIAU contains a uracil base with an iodine at the 5-position, while the 2'-fluoropyridimes used in the aptamers contain either uracil or cytosine with no substitution at the 5 position. Whether these structural differences between FIAU and the 2'-fluoropyrimidines are sufficient to prevent them from producing the long-term toxic effects caused by FIAU was not known. Nonclinical studies demonstrated that monomer nucleotide 2'-Fluorouridine was incorporated into cellular DNA and RNA of all tissues examined following subchronic (3 months) iv administration. Based on the structure of 2'-Fluorouridine (OH in 3' position), the chain will not be terminated as in AZT but continue to grow. Regarding mitochondrial toxicity, no differences in mtDNA content of heart, spleen, liver, testes, and kidney were observed between treatment groups and the control group in rats and woodchucks after 3-month treatment. It is proposed that the toxicity of FIAU in woodchuck was similar to that in humans. Although there was no direct evidence if 2'-Fluorouridine inhibited mitochondrial DNA polymerase γ (as the case of FIAU) , and these two 3-month studies were conducted without inclusion of FIAU, clinical studies with Macugen for 2 years showed no apparent

systemic issues and no delayed toxicity. The toxicity similar to FIAU does not seem to be a concern for the treatment with Macugen.

2.6.7. TOXICOLOGY TABULATED SUMMARY

No tabulated summary was provided by the sponsor.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Pharmacology/toxicology-related issues were addressed in this application. Macugen is a selective VEGF antagonist. Nonclinical PK studies showed that following intravitreal administration, the drug concentrations were high in vitreous humor. Nonclinical toxicity studies showed no toxicologically significant, drug-induced toxicological events. However, injection procedure-induced events were seen in ocular tissues. From pharmacology/toxicology standpoint, an "approvable" was recommended with some minor labeling modifications.

Unresolved toxicology issues (if any): No

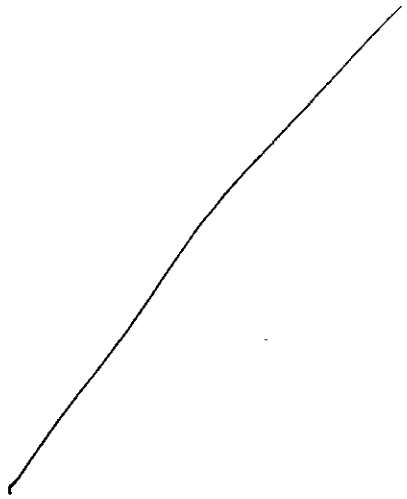
Recommendations:

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

Suggested labeling:

Minor modifications of labeling are recommended in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

Carcinogenesis, Mutagenesis, Impairment of Fertility



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/

Zhou Chen
9/10/04 01:16:18 PM
PHARMACOLOGIST

Josie Yang
9/10/04 01:41:53 PM
PHARMACOLOGIST