CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-011

PHARMACOLOGY REVIEW

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:	**22-011**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/30/05**
PRODUCT:	**TYZEKA™**
INTENDED CLINICAL POPULATION:	**Chronic hepatitis B infected patients with evidence of viral replication and active liver inflammation**
SPONSOR:	**Idenix Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Antiviral Products (HFD-530)**
PHARM/TOX REVIEWER:	**Ita Yuen, Ph.D.**
PHARM/TOX SUPERVISOR:	**James Farrelly, Ph.D.**
DIVISION DIRECTOR:	**Debra Birnkrant, MD**
PROJECT MANAGER:	**Kenny Shade, JD**

Date of review submission to Division File System (DFS): 10/24/2006

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Yes

B. Recommendation for nonclinical studies

None. The studies included in this NDA are adequate for nonclinical safety evaluation.

C. Recommendations on labeling

The language included in the sponsor's labeling for the "Carcinogenesis, Mutagenesis, Impairment of Fertility", "Pregnancy Category B", "Labor and Delivery", and "Nursing Mothers" sections is acceptable except for an editorial change regarding the word "transformation" placed in front of "assay with Chinese hamster ovary cells" in the second to the last sentence in the 2nd paragraph under "Carcinogenesis, Mutagenesis, Impairment of Fertility." The word "transformation" should be deleted since this assay is not a transformation assay as stated. The sponsor has agreed with the change and the label is currently read as follows:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Telbivudine has shown no carcinogenic potential. Long term oral carcinogenicity studies with telbivudine were negative in mice and rats at exposures up to 14 times those observed in humans at the therapeutic dose of 600 mg/day.

There was no evidence of genotoxicity based on in vitro or in vivo tests. Telbivudine was not mutagenic in the Ames bacterial reverse mutation assay using S. typhimurium and E. coli strains with or without metabolic activation. Telbivudine was not clastogenic in mammalian-cell gene mutation assays, including human lymphocyte cultures and an assay with Chinese hamster ovary cells with or without metabolic activation. Furthermore, telbivudine showed no effect in an in vivo micronucleus study in mice.

In reproductive toxicology studies, no evidence of impaired fertility was seen in male or female rats at systemic exposures approximately 14 times that achieved in humans at the therapeutic dose.

Pregnancy Category B

Telbivudine is not teratogenic and has shown no adverse effects in developing embryos and fetuses in preclinical studies. Studies in pregnant rats and rabbits showed that telbivudine crosses the placenta. Developmental toxicity studies revealed no evidence of harm to the fetus in rats and rabbits at doses up to 1000 mg/kg/day, providing exposure levels 6- and 37-times higher, respectively, than those observed with the 600 mg/day dose in humans. There are no adequate and well-controlled studies of telbivudine in pregnant women. Because animal reproductive toxicity studies are not always predictive of human response, telbivudine should be used during pregnancy only if potential benefits outweigh the risks.

Pregnancy Registry: To monitor fetal outcomes of pregnant women exposed to telbivudine, healthcare providers are encouraged to register such patients in the AntiRetroviral Pregnancy Registry by calling 1-800-258-4263.

Labor and Delivery

There are no studies in pregnant women and no data on the effect of telbivudine on transmission of HBV from mother to infant. Therefore, appropriate interventions should be used to prevent neonatal acquisition of HBV infection.

Nursing Mothers

Telbivudine is excreted in the milk of rats. It is not known whether telbivudine is excreted in human milk. Mothers should be instructed not to breastfeed if they are receiving TYZEKA.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The safety profile of telbivudine has been extensively characterized in rats, mice, rabbits, and monkeys. The absorption, distribution, metabolism, and excretion (ADME) profiles of telbivudine in these species are similar to that in humans and made them appropriate for the nonclinical safety evaluation. The toxicological, genotoxic, carcinogenic, allergenic, and reproductive toxicological potentials as well as telbivudine's effects on cardiovascular, neurological, respiratory, gastrointestinal, renal and other systems were evaluated. All of the pivotal toxicology studies employed adequate range of doses that were administered via clinical route of administration (oral) and produced sufficient systemic exposures and safety margins over that at clinical dose of 600 mg/day. In general, telbivudine is found to be well tolerated and produced few or no adverse effect at large multiples of human exposure.

Doses used in a myriad of general toxicology studies ranged from 5 to 3000 mg/kg/day. The highest doses investigated following chronic oral administration of telbivudine (9 months in monkeys and 85 weeks in rats) were 1000 and 2000 mg/kg/day. The exposures at these doses were 8- and 14-fold over that at the clinical dose of 600 mg/kg. At these doses, telbivudine was found to exert no adverse effects on cardiovascular, respiratory, hepatic, and neurological systems even though it can cross blood-brain barrier. It had some effects on gastrointestinal and renal systems.

Gastrointestinal irritation was associated with telbivudine administration. Monkeys and rabbits seemed to be more sensitive to this toxicity than mice and rats. At 1000 mg/kg/day, monkeys had dose-related increases in the incidences of soft/loose feces and emesis without corresponding histopathological findings in any gastrointestinal tissues/organs. The food consumption and body weight gain were not affected. This toxicity is not dose limiting in monkeys. On the other hand, in addition to reduced body weight gain and abnormal feces, one death, one abortion, and three premature deliveries were associated with 1000 mg/kg/day dose in pregnant rabbits. All of these rabbits had abnormal feces, consumed less food, and had weight loss. In addition, one of the rabbits that died had erosion on the stomach mucosal surface, red appearance and red fluid in the intestine, and distended stomach and intestine (with gas). The gastrointestinal irritation is clearly a dose limiting toxicity in rabbits. But this toxicity was associated with high systemic exposure to telbivudine. The AUC value in the pregnant rabbits at 1000 mg/kg/day was 2-3 times higher than those at the highest doses studied in mice, rats, and monkeys, and 37 times higher than that in humans. This toxicity is unlikely to be a dose limiting in humans. About 30% of patients in the both Tyzeka™ and lamivudine treatment arms experienced at least one episode of adverse event in the Gastrointestinal Disorders System Organ Class. Only one patient discontinued the use of Tyzeka™ after 446 days of use because of the unresolved symptoms of nausea and loose stools that started after 389 days of use. The symptoms resolved approximately 43 days after drug discontinuation.

Renal toxicity was not apparent in studies conducted in monkeys, mice, rabbits, or woodchucks. It also did not produce any dose-related renal histopathology in any of the species studied except for chronic progressive nephropathy observed in rats. A marginal increase in incidence and severity of chronic progressive nephropathy was observed in the 6-month toxicity study in this species. This is a common lesion associated with aging in rats. It affects males more than females. Normally, the small increase observed in the 6 month study would not be attributed to drug toxicity and was not considered dose limiting. However, a one-fold increase in the number of deaths attributable to chronic progressive nephropathy were observed in the mid and high dose (1000 and 2000 mg/kg/day) males and in the high dose females in the 2 year rat carcinogenicity study. Many of these deaths occurred after one

year of continuous drug administration. Dosing had to be stopped after 85 weeks of drug administration for the 2000 mg/kg/day dose group because of the high mortality rate in this group. The increased mortality rate attributed to this lesion suggests that chronic administration of telbivudine may potentially exacerbate pre-existing renal impairment or dysfunction in patients.

The no-adverse-effect level (NOAEL) for mouse is 3000 mg/kg/day (22-fold over the exposure in humans at 600 mg/day dose). Since gastrointestinal irritation produced by administration of telbivudine was not dose limiting and was manifested as occasional soft/loose stool and emesis without any other effect, the NOAEL for monkey is 1000 mg/kg/day (8-fold over the human exposure at 600 mg/day dose). However, since this toxicity is dose limiting for pregnant rabbits, the NOAEL in this species is 250 mg/kg/day (11-fold over the human exposure at 600 mg/day dose). The NOAEL for rats is 500 mg/kg/day since deaths attributable to chronic nephropathy was increased at doses 1000 mg/kg/day and higher after 85 weeks of treatment. This NOAEL provides 4-fold safety margin for humans.

Rare cases of myopathy have been reported in several Tyzeka™clinical trials. Creatine kinase (CK) values were elevated in these patients. However, the degree and timing of CK elevation did not always coincide with the onset of myopathy. CK values were monitored in a 14-day intravenous study in monkeys. Skeletal as well as heart muscles were routinely evaluated histologically in all of the general toxicology studies as well as the carcinogenicity studies. No signals suggesting muscle toxicity were observed in any of the studies performed.

The genotoxic potential of telbivudine was investigated in three *in vitro* and one *in vivo* genotoxicity assays. It was found not to be mutagenic or clastogenic with and without metabolic activation. In addition, it was found not to be carcinogenic in the 2 year carcinogenicity study in rats and the 6-month carcinogenicity study in transgenic CB6F1-TgrasH2 mice. The dosing in rats was stopped for the 2000 mg/kg dose groups after 85 weeks of telbivudine administration and study terminated prematurely after 96 of drug administration because of the high mortality rate. The increased mortality rate for this study was dose-related. Many of the deaths were caused by tumors. However, except for chronic progressive nephropathy described in the previous paragraph, no statistically significant or dose related increase in the incidence of tumor and/or tumor type and deaths attributable to any tumor type was observed. Dosing in the transgenic mouse carcinogenicity study was continued to the scheduled time. No evidence of increased incidence of tumor was associated with telbivudine administration. The NOAEL for carcinogenicity is 2000 mg/kg/day with a systemic exposure 14-fold higher than that at the clinical dosage of 600 mg/day.

Telbivudine can cross blood-testes and placenta barrier. Both male and female fertility were not affected at doses as high as 2000 mg/kg (about 14-times human exposure) in rats. *In utero* exposure to telbivudine did not adversely affect embryo and fetal development and were not teratogenic in rats and rabbits at doses as high as 1000 mg/kg/day. It is also secreted into rat milk. Exposure to this drug *in utero* or in milk did not affect pup delivery or neonatal development in rats. The second generations exhibited normal behavior and postnatal development, growth, sexual maturity, and fertility. The No-adverse-effect level (NOAEL) for reproductive toxicity is 1000 mg/kg/day, providing 6 to 37-fold safety margins as compared to clinical dose of 600 mg/day.

The safety of telbivudine was also investigated in a variety of *in vitro* and local tolerance studies. It shows low toxicity in cultured human hepatoma cells, peripheral blood mononuclear cells, none marrow progenitor cells, and numerous cells lines of human and other mammalian origin. It is not toxic to mitochondria. It is also not allergenic or irritating in the mouse local lymph node assay.

In conclusion, except for the gastrointestinal irritation in monkeys and rabbits and the exacerbation of nephropathy in rats, telbivudine has a clean safety profile in animals at exposures that are high multiples of that in humans.

B. Pharmacologic activity

Please see Dr. Sung Rhee's review.

C. Nonclinical safety issues relevant to clinical use

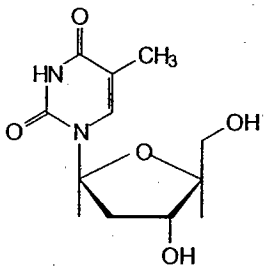
Chronic progressive nephropathy: Chronic progressive nephropathy is a common, age-related lesion especially prevalent in male rats, though also present in female rats. In the six-month toxicology study in this species, there was a marginal increase in the incidence and severity of this lesion in male rats. After 85 weeks of continuous oral administration of telbivudine, the number of deaths attributable to nephropathy was dose-relatedly increased, although the incidence and severity of this lesion did not show such trend. The results suggest that long-term exposure to telbivudine has the potential to exacerbate any pre-existing or underlying renal dysfunction. The clinical data so far have not suggested any renal toxicity in associated with Tyzeka™ administration. In the label, patients with renal impairment are recommended to reduce their Tyzeka™ dosage. Therefore, this toxicity is not included in the label.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	22-011
Review number:	1
Sequence number/date/type of submission:	000/Dec. 30, 2005/Original
Information to sponsor:	Yes ( ) No (X)
Sponsor and/or agent:	Idenix Pharmaceuticals, Inc. 60 Hampshire St. Cambridge, MA 02139 617-995-9800
Manufacturer for drug substance:	Novartis Pharma Stei, Switzerland Novartis Grimsby Ltd., UK
Reviewer name:	Ita Yuen, Ph.D.
Division name:	Division of Antiviral Products
HFD #:	530
Review completion date:	

Drug:

Trade name:	**TYZEKA ™**
Generic name:	Telbivudine
Code name:	L-dT; NV-02B; β-L-2'-deoxythymidine; 2'-deoxy-β-L-thymidine, β-L-thymidine; L-thymidine
Chemical name:	1-(2-deoxy-β-L-ribofuranosyl)-5-methyl-uracil
CAS registry number:	3424-98-4
Molecular formula/molecular mass:	$C_{10}H_{14}N_2O_5$/243.33 daltons
Structure:	

Relevant INDs/NDAs/DMFs: IND 60,459

Drug class: Unnatural nucleoside

Intended clinical population:	Patients with chronic hepatitis B infection, evidence of viral replication, and active liver inflammation
Clinical formulation:	Film-coated tablets containing 600 mg telbivudine, [redacted] microcrystalline cellulose, [redacted], povidone, [redacted] sodium starch glycolate, and [redacted]
Route of administration:	Oral

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

[For (b)(2) applications:

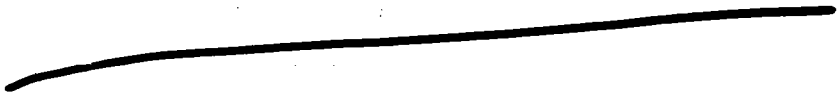
Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-011 are owned by Idenix Pharmaceuticals, Inc. or are data for which Idenix Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-011 that Idenix Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Idenix Pharmaceuticals does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-011.

Studies reviewed within this submission:

Cellular pharmacology of β-L-2'-deoxythymidine: cytotoxicity and mitochondrial function (Study report # RD0006)
Central nervous system safety study of β-L-2'-deoxythymidine (Study # 02-PK-031)
Effects of LdT on cloned hERG channels expressed in mammalian cells (Study # IDIX-04-100)
Telemetry study of β-L-2'-deoxythymidine in conscious cynomolgus male monkeys to assess cardiovascular and respiratory safety pharmacology (Study # 02-TX-030)
An oral (stomach tube) toxicokinetic study of LdT in pregnant rabbits (Study # IDIX-04-109)
Absorption, metabolism, and excretion of radioactivity after a single oral or intravenous administration of [^{14}C]-L-thymidine to rats (Study # 02-PK-010)
Pharmacokinetics of LdT following IV and PO administration to cynomolgus monkeys (Study # NC-NV-02B-001)
Pharmacokinetics and bioavailability of L-dT after IV and PO administration to healthy woodchucks (Study # NC-NV-02B-006)
In vitro permeability of [^{3}H]LDT600 (telbivudine) across Caco-2 cell monolayer (Study # ADME(US) R0301196)
Tissue distribution of radioactivity after a single oral administration of [14C]-L-thymidine to rats (Study # 02-PK-011)
The in vitro binding of L-thymidine to plasma proteins from rat, monkey, and human (Study # 02-CP-004)

Inhibitory potential of L-thymidine towards human hepatic microsomal cytochrome P450 isoenzymes (Study # 02-CP-005)
The effect of L-thymidine on hepatic microsomal cytochrome P450 enzyme activities in rats (Study # 02-CP-007)
Dose range-finding and acute toxicity study of NV-02B administered orally to rats (Study # GAW-104)
Dose escalating toxicity study of NV-02B administered orally to monkeys (Study # GAW-106)
2-week intravenous toxicity study in rats including sighting phase (Study # 0510032)
Twenty-eight day repeated dose toxicity study of NV-02B administered orally to rats (Study # NC-NV-02B-003)
Six-month chronic oral gavage toxicity study in rats with one-month recovery (Study # 02-TX-022)
A 4-week oral dose toxicity and toxicokinetic study of □-L-2'deoxy-thymidine in CB6F1 mice (Study # 02-TX-030)
13-week oral gavage toxicity and toxicokinetic study with L-thymidine (LdT) in mice (Study # 02-TX-028)
Maximum tolerated dose (MTD) followed by a 5 day intravenous (bolus) administration toxicity study in the cynomolgus monkey (Study # 0580155)
14-day intravenous (bolus) administration toxicity study in the cynomolgus monkey (Study # 0580156)
Twenty-eight day repeated dose toxicity study of NC-028 administered orally to monkeys (Study # NC-NV-02B-005)
Nine-month chronic oral gavage toxicity study in cynomolgus monkeys with a two-month recovery (Study # 02-TX-021)
Evaluation of a test article in the *Salmonella typhimurium/Escherichia coli* plate incorporation mutation assay in the presence and absence of induced rat liver S-9 (Study # GVAW-116)
Test for chemical induction of chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells with and without metabolic activation (Study # GVAW-117)
L-deoxythymidine chromosome aberration test (Study # IDIX-04-164)
In vivo test for chemical induction of micronucleated polychromatid erythrocytes in mouse bone marrow cells (Study # GVAW-118)
104-week oral gavage carcinogenicity study with L-Thymidine (LdT) in rats (Study # 02-TX-025)
A 26-week oral dose carcinogenicity and toxicokinetic study of **β-L-2'-deoxythymidine in CB6F1-**TgrasH2 mice (Study # 02-TX-024)
Combined oral (gavage) fertility study and development toxicity study of L-thymidine in rats (Study # 02-TX-023)
Oral (gavage) fertility and general reproduction toxicity study of L-thymidine in male rats (Study # 1314-005)
Oral (gavage) fertility and general reproduction toxicity study of L-thymidine in female rats (Study # 1314-006)
Oral (stomach tube) developmental toxicity study of L-thymidine in rabbits (Study # 1314-002)
Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of l-thymidine in rats, including a postnatal behavioral/functional evaluation (Study # 1314-002)
Assessment of contact allergenic potential with the murine local lymph node assay (LLNA tier I) (Study # 0417004)
Lacteal excretion and placental transfer of radioactivity after a single oral administration of [^{14}C]-L-thymidine to pregnant and lactating rats (Study # 02-PK-012)

Studies <u>not</u> reviewed within this submission:



2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Telbivudine (LdT; β-L-2′-deoxythymidine) is a β -L-2-deoxynucleoside, an enantiomer of the natural D-nucleoside with no chemical modification. It showed antiviral activity against hepadnavirises, including human, woodchuck, and duck hepatitis B viruses. It is activated intracellularly by phosphorylation to the active triphosphate derivative. The detailed pharmacodynamic information can be found in the Dr. Sung Rhee's Microbiology review.

Telbivudine was found to be less toxic to bone marrow cells and mitochondria in *in vitro* assays as compared to other nucleoside and nucleoside analogs. It exhibited no inhibitory activity to human cellular DNA polymerase α, β, or γ at concentrations greater than 10 μM. The effects of telbivudine on cardiovascular and respiratory systems were studied in cynomolgus monkeys at oral doses of 250, 750, and 2000 mg/kg. At the top dose, the C_{max} and AUC values were 9 and 10 times, respectively, those achieved in humans. The effect on central nervous systems was investigated in rats after the administration of single oral doses of 150, 500, and 1000 mg/kg/day. The C_{max} and AUC values at 1000 mg/kg/day provided 9- and 7-fold safety margin at the clinical dose of 600 mg/day, respectively. The effect on the hERG channel was studied in HEK-293 cells at concentrations of 10, 100, 1000, and 10,000 μM cell culture. No effect on the hERG current amplitude was associated with any concentrations up to 10, 000 μM which is 656-fold over the C_{max} value at clinical dose of 600 mg/day.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Please see Dr. Sung Rhee's Microbiology review.

Drug activity related to proposed indication: Please see Dr. Sung Rhee's Microbiology review.

2.6.2.3 Secondary pharmacodynamics

Please see Dr. Sung Rhee's Microbiology review.

2.6.2.4 Safety pharmacology

Neurological effects: No effect. Please see the study description below:
Central nervous system safety study of β-L-2'-deoxythymidine (Study # 02-PK-031 (m4\42-stud-rep\421-pharmacol\4213-safety-pharamcol\snbl-046-02): Eight rats/sex/dose received a single dose of 0 (0.5% carboxymethylcellulose), 150, 500, or 1000 mg/kg LdT. Functional observational battery evaluating home cage observations, handling associated observations, open field observations, and manipulative tests and reflex measures was performed once immediately prior to dosing and once 60 minutes after dosing on all rats. Mortality checks, food consumption, and body weight measurement were also preformed. No effects were associated with the treatment.

Cardiovascular effects: No effect. Please see the study descriptions below:
Effects of LdT on cloned hERG channels expressed in mammalian cells (Study # IDIX-04-100; m4\42-stu-rep\421-pharmacol\4213-safety-pharmacol\idix-04-100): The *in vitro* effects of 10, 100, 1,000, and 10,000 μM telbivudine (LdT) on ionic currents in voltage-clamped human embryonic kidney (HEK-293) cells that stably express the human ether-á-go-go-related gene (hERG) were determined. The positive control for the test was 60 nM terfenadine while the negative control was PBS supplemented with 0.3% DMSO. hERG current amplitude was reduced by 0.2% by the vehicle control as well as by all the 4 concentrations of LdT tested. Terfenadine inhibited hERG current by 80.8%. Thus, telbivudine was found not to have an effect on in vitro hERG current.

Telemetry study of β-L-2'-deoxythymidine in conscious cynomolgus male monkeys to assess cardiovascular and respiratory safety pharmacology (Study # 02-TX-030; m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\snbl-046-01): LdT at 0 (0.5% carboxymethylcellulose-sodium), 250, 750, and 2000 mg/kg was administered orally to 4 conscious telemeterized male cynomolgus monkeys on four separate dosing days. Heart rate, blood pressure, ECG (lead II), respiratory rate, and blood gas (pH, pCO2, pO2, and O2Hb) as well as body temperature, motor activity, clinical observations, food consumption, body weight, behavior assessment, and clinical pathology were evaluated. Except for transient abnormal feces (liquid, soft and/or test article color) in 1 and 3 animals after doses of 750 and 2000 mg/kg, respectively, no other parameters evaluated were affected.

Pulmonary effects: No effect. Please see the study description above.

Renal effects: No specific safety pharmacology study was conducted. In general, no indication of renal toxicity was observed in general toxicology studies conducted in mice, rats, and monkeys. However, in the rat carcinogenicity study, a dose-related increase in the number of mortality attributable to chronic progressive nephropathy, a common lesion in older rats, was observed at doses greater than 1000 mg/kg/day. The results suggest that chronic administration of telbivudine has the potential to exacerbate underlying renal dysfunction and impairment.

Gastrointestinal effects: No specific safety pharmacology study was conducted for this system. The results from the general toxicology studies suggested that telbivudine may be irritating to the gastrointestinal system. Monkeys and pregnant rabbits seemed to be more sensitive to this effect than rats and mice. In the shorter term toxicology studies, monkeys exhibited soft feces, emesis, and weight loss. However, only the observation of soft feces persisted in the 9 months toxicology study. Body weight gain in the study was not affected. At 1000 mg/kg/day dose, which was administered from gestation day 6 to 18, pregnant rabbits exhibited abnormal feces and reduced weight gain. In addition, one abortion, two early deliveries, and one death were observed. This dose was considered the maximum tolerated dose for the pregnant rabbits. Gastrointestinal function was probably not affected since periods of increases food consumption and/or weight gain were evident in most of the species and in most of the studies. No corresponding histopathological finding in any of the gastrointestinal tissues/organs in was apparent in any of the general toxicology studies.

Abuse liability: Not studied.

Other:

Cellular pharmacology of β-L-2'-deoxythymidine: cytotoxicity and mitochondrial function (Study report # RD0006; NIH NIAID Antiviral Research and Antimicrobial Chemistry Program; non-GLP; Study dates 5/19/99-8/30/99). Cytotoxicity was measured by the uptake of neutral red dye, trypan blue exclusion, or metabolism of 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) in a variety of cells including 2.2.15 (human hepatoma cell line), HepG2, human foreskin fibroblast (HFF), Daudi (Burkitt's B-cell lymphoma), A549 (human lung carcinoma), MDCK (canine kidney epithelial cells), CV-1 (African green monkey kidney fibroblast cells), MA-104 (rhesus monkey kidney epithelial cells), KB (human nasopharyngeal carcinoma), and peripheral blood mononuclear (PBM) cells in the presence of several concentrations of L-dT. 50% cytotoxic concentrations (CC_{50}) in various cell lines tested were greater than 100 μM. The 50% effective concentration for HBV was 0.19 μM.

The *in vitro* myelosuppressive potential of L-dT was determined using a human bone marrow clonogenic assay. Mononuclear cells were harvested from human bone marrow cells that were collected from healthy volunteers and subjected to assays for human granulocyte-macrophage colony forming (CFU-GM) and erythroid burst forming (BFU-E) activity. Zidovudine was used as a positive control. It was found that L-dT was non-inhibitory of CFU-GM and BFU-E at concentrations > 10 μM.

The potential to produce mitochondrial toxicity was assessed *in vitro* by the analysis of lactic acid production, mitochondrial DNA (mtDNA) content, morphology change (e.g., loss of cristae, matrix dissolution and swelling, and lipid droplet formation) of mitochondrial ultrastructure. Zidovudine and fialuridine (FIAU) were used as positive controls. No effect was associated with L-dT concentrations up to 10 μM.

L-dT was also found to exhibit no inhibition of human cellular DNA polymerase α, β, or γ at concentrations greater than 10 μM. The results suggested that L-dT may have a favorable toxicity profile as compared to other nucleosides and nucleoside analogs.

2.6.2.5 Pharmacodynamic drug interactions

No study done. Telbivudine was found to have no inhibitory or inductive activities for any of the cytochrome P450 isoenzymes. It's unlikely that it would have drug-drug interaction with co-administered drug.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetic profile of telbivudine (LdT) was determined in CB6F1 and CD-1 mice, Sprague-Dawley and Long Evans rats, New Zealand white rabbits, woodchucks, and cynomolgus monkeys. Telbivudine was administered orally or intravenously. The vehicle used commonly in the pivotal toxicology/toxicokinetic studies was 0.5% carboxymethycellulose. Normal saline and aqueous glucose solution containing phosphate buffer and 0.5% polyvinylpyrrolidone were the vehicles in nonclinical pharmacokinetic studies and intravenous toxicity studies, respectively. Telbivudine plasma concentrations were determined using HPLC/UV (limit of detection: 0.2 μg/ml) and/or LC-MS/MS (limit of detection: 0.01 μg/ml) methods. Proposed clinical dosage is 600 mg/day by oral administration. This dose was also used in the human ADME and nonclinical pharmacokinetic and ADME study (on a mg/kg basis). The formulations used in these studies were suspensions or solution of telbivudine.

The pharmacokinetic data suggest that telbivudine was well absorbed across the species studied, including human. The oral bioavailability of 10 mg/kg telbivudine was 60%, 59%, 38%, and 40% in rats, monkeys, woodchucks, and humans, respectively. The systemic exposure generally increased in a dose-related manner. Food and state of pregnancy did not affect the pharmacokinetic parameters of telbivudine. There was no gender difference in pharmacokinetic profile. The rate of absorption was moderate to fast (T_{max} values ranged 0.5 to 3 hours) in animals as compared to moderate rate in humans (T_{max} of 3 hours). The permeability results in *in vitro* Caco-2 cell model also suggest moderate absorption without an efflux mechanism. The plasma clearance was similar in rats and monkeys but slower in woodchucks. The $t_{1/2}$ values suggest that mice, rats, and woodchucks eliminated telbivudine more rapidly than monkeys ($t_{1/2}$ 7.5-18 hr) and humans ($t_{1/2}$ 41.1 hr). Accumulation of telbivudine following multiple oral dosing was not apparent in mice and rats but was slight (1.2 to 1.6-fold) in monkeys.

Telbivudine was extensively distributed into tissues following a single oral dose to male Sprague-Dawley and Long Evans rats with the highest concentrations in organs associated with absorption and elimination like small and large intestines, urinary bladder, kidneys, and stomach and the lowest ones in brain (brain/plasma concentrations ratios of 0.03 to 0.3) and spinal cord. Telbivudine was also found to cross blood-testes and placenta barriers and was secreted into rat milk. The milk/plasma AUC ratio was 2.8. These data suggested that the embryos, fetuses, and pups were exposed to telbivudine in the reproductive toxicology studies. Extravascular distribution was also

apparent as suggested by the stead-state volume of distribution values (V_{ss}: 0.833 and 0.909 L/kg in woodchucks and monkeys, respectively).

Binding to plasma proteins was low across species (3.3 to 7.5% in rats, monkeys, and humans). The concentrations tested ranged from 0.4 to 40 µg/ml which encompass the C_{max} values observed in the clinical trials. Telbivudine partitioned (32-43%) into erythrocytes of rats, monkeys, and humans. It did not bind to melanin since the pharmacokinetic profiles were similar in pigmented and nonpigmented skin and eyes of rats.

Telbivudine was eliminated mainly in urine as unchanged parent drug following oral and intravenous routes. It is converted to the active triphosphate derivative intracellularly, however, no metabolite was detected in the systemic circulation or excreta of humans, monkeys, and woodchucks. A minor (< 0.8% of total administered drug), unidentifiable metabolite, M4, was detected in the plasma, urine, and bile of female rats (found in the bile of male rats also). It exhibited no inhibitory or inducing activities to any of the human cytochrome P450 isozymes examined, suggesting that telbivudine is unlikely to interact with other co-administered drug.

2.6.4.2 Methods of Analysis

See under individual study reviews.

2.6.4.3 Absorption

Absorption, metabolism, and excretion of radioactivity after a single oral or intravenous administration of [14C]-L-thymidine to rats (Study # 02-PK-010): The pharmacokinetics, excretion of radioactivity, and metabolite profiling of telbivudine were examined following a single intravenous or oral administration of 10 mg/kg [^{14}C]-L-dT to Sprague Dawley rats. Four groups were included. Two groups, consisted of 21 rats/sex/group, received a single oral or intravenous telbivudine dose. Blood samples were collected at 0.083 (IV only), 0.25, 0.5, 1, 2, 3 (oral only), 4, 8, 12, 16, 20, 24, 48, and 72 hours postdose from 3 rats/sex/time point. Urine was also collected at 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 hour intervals from the three rats/sex that received the intravenous dosing and were designated for blood collection at 72 hours postdose. The 3rd and 4th groups comprised of 3 rats/sex/group received 10 mg/kg radioactively labeled telbivudine orally. Urine, feces, expired air and volatiles were collected at 24-hour intervals through 168 hours from the animals in the third group. Additional urine samples were collected at 0-4, 4-8, 8-12, and 12-24 hour postdose and expired air and volatiles samples collected at 0-8, 8-24 hour intervals postdose. Bile, urine, and feces were obtained from the animals in the 4th group. The sampling time intervals following dose administration were 0-8, 8-24 , and at 24-hour intervals through 72 hours for urine collection, 0-2, 2-4, 4-8, 8-24, 24-48, 48-72 hour-intervals for bile collection, 24-hour intervals through 72 hours for fecal collection. The results are listed as follows:

Sex	Male			Female		
Route of administration	**Oral**	**IV**	**Bile-Cannulated**	**Oral**	**IV**	**Bile-Cannulated**
C_{max} (ng eq/ml)	1520	-	-	1620	-	-
$AUC_{0-\infty}$ (ng eq-hr/ml)	6710	12100	-	7240	11600	-
$t_{1/2}$ (hr)	4.82	4.46	-	3.59	4.70	-
Bioavailability (%)	57.4	-	-	63.5	-	-
Clearance (ml/hr/kg)	-	849	-	-	886	-
% radioactivity recovered						
Total	92.0	-	93.6	91.2	-	94.1
Urine	43.5	74.7	44.4	52.0	86.5	45.0
Feces	48.2	-	48.2	39.1	-	45.0
Expired air/volatiles	< 0.1	-	-	<0.1	-	-

There was no apparent gender difference in all of the pharmacokinetic parameters examined. Based on the 57.4 and 64.5% oral absorption, the results indicated that urinary excretion was the main route of drug elimination following both oral and intravenous administration. One minor unidentifiable metabolite (M4) was observed in the female rats and represented less than 4% of the radioactivity in plasma and < 7.3% of radioactivity in urine sample. Bile samples from the bile-cannulated rats showed 5 HPLC peaks including those of telbivudine and M-4. The total amount of metabolites eliminated in the bile represented less than 0.8% of the total administered dose.

Pharmacokinetics of LdT following IV and PO administration to cynomolgus monkeys (Study # NC-NV-02B-001): Three male, drug non-naïve cynomolgus monkeys received a single intravenous dose of 10 mg/kg [^{3}H]-LdT in saline. Following a 6-week washout period, the same three animals received the same dose of LdT orally. Blood samples were collected at predose, 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 hours postdose. Urine was collected 0-2, 2-4, 4-8, 8-12 hour intervals and then at 12-hour interval until 336 hours postdose. The results are shown in the following table.

	Oral	**Intravenous**
T_{max} (hr)	1.67	
C_{max} (µg/ml)	3.11	10.6
AUC_{0-24h} (µg-hr/ml)	10.0	16.8
$t_{1/2}$ (hr)	-	1.37
Bioavailability (%)	59	-
Total Body Clearance (ml/hr/kg)	-	0.59
Renal Clearance (ml/hr/kg)	-	0.43
V_{ss} (L/kg)	-	0.91
% total radioactivity recovered		
Urine	37	77

Urinary excretion is the main route of elimination. Over 95% of the radioactivity recovered in urine existed as unchanged LdT, indicating no metabolism. No metabolites were recovered in the plasma or urine following oral or intravenous administration in monkeys.

Pharmacokinetics and bioavailability of L-dT after IV and PO administration to healthy woodchucks (Study # NC-NV-02B-006): Three healthy woodchucks, negative for woodchuck hepatitis virus, received a single intravenous dose of 10 mg/kg [^{3}H]-

LdT. Following three weeks of washout period, the same three animals received the same dose of LdT orally. Blood samples were collected at predose, 2 (IV only), 5 (IV only), 15, and 10 minutes and 1, 1.5, 2, 3, 4, 8, and 24 hours postdose. Urine was also collected. The results are presented in the table below:

	Oral	Intravenous
T_{max} (hr)	3.00	-
C_{max} (μg/ml)	2.08	39.2
AUC_{0-24h} (μg-hr/ml)	18.3	52.3
$t_{1/2}$ (hr)	-	3.99
Bioavailability (%)	38.3	-
Total Body Clearance (ml/hr/kg)	-	0.199
V_{ss} (L/kg)	-	0.83

Unchanged LdT accounted for the majority of radioactivity recovered in urine. No metabolites were observed in plasma and urine following both oral and intravenous LdT administration. Bioavailability was lower than those in monkeys and rats.

In vitro permeability of [3H]LDT600 (telbivudine) across Caco-2 cell monolayer (Study # ADME(US) R0301196): The apparent permeability of [^{3}H]-LdT was measured across confluent Caco-2 cell monolayers under steady state at 25 and 100 μM LdT. The steady state flux rates across the Caco-2 cell layer were calculated based upon the average amount of radioactivity transported over the 120 min from the donor to the receiver compartment. The calculated permeability in the apical to basolateral direction was 2.2×10^{-6} cm/sec and 1.3×10^{-6} cm/sec in the basolateral to apical direction. Compared to propranolol (highly permeable; apical to basolateral permeability of 15.8 $\times 10^{-6}$ cm/sec) and mannitol (poorly permeable; apical to basolateral permeability of 0.66×10^{-6} cm/sec), LdT is considered to be medium permeable. The estimated LdT permeability rates were comparable in both directions, indicating that it traverses the monolayer either through passive diffusion or via the para cellular route.

2.6.4.4 Distribution

Tissue distribution of radioactivity after a single oral administration of [14C]-L-thymidine to rats (Study # 02-PK-011): The tissue distribution of radioactivity was assessed by tissue excision and whole body autography following a single oral dose of 10 mg/kg [^{14}C]-LdT to male Long-Evans (pigmented) and Sprague-Dawley (nonpigmented) rats. Blood and tissues were collected from 3 Long-Evans rats/time point at 1, 3, 8, 24, 72, and 168 hours postdose. One rat/time point (both Long-Evans and Sprague-Dawley) were sacrificed at 1, 3, 8, 24, 72, and 168 postdose for whole-body autoradiography. The results are presented in the following table

	Long-Evans					Sprague Dawley			
	Plasma	**Blood**	**Pig. Skin**	**Nonpig Skin**	**Eyes**	**Plasma**	**Blood**	**Nonpig Skin**	**Eyes**
T_{max} (hr)	1	1	1	3	3	-	-	-	-
C_{max} (ng eq/g)	1550	1240	1120	1140	459	1540	1110	982	387
AUC_{0-24h} (ng eq*hr/g)	7320	5500	5110	7110	2690	-	-	-	-
$t_{1/2}$ (hr)	2.99	1.34	1.51	3.48	2.65	-	-	-	-

Tissue distribution of LdT was rapid and extensive. L-dT-derived radioactivity was detected in all tissues by one hour following oral administration. The concentrations of radioactivity declined rapidly. It was not detectable in 19 out of 29 tissues collected at 24 hours postdose and disappeared from all tissue by 72 hours post dose. LdT can cross blood-brain and blood-testes barriers since radioactivity was detected in brain and testes. The similarities between the pharmacokinetic parameters in pigmented and nonpigmented skin and between the tissue concentrations in pigmented (Long-Evans) and nonpigmented (Sprague-Dawley) eyes suggest that LdT does not bind to melanin. As observed in other studies, renal excretion was the main route of drug elimination as confirmed by whole-body autoradiographic data. Drug-derived radioactivity was highest in small and large intestine, urinary bladder, kidneys, prostate, mesenteric lymph node, stomach, and pancreas and lowest in brain and spinal cord.

The in vitro binding of L-thymidine to plasma proteins from rat, monkey, and human (Study # 02-CP-004): In vitro plasma protein binding and erythrocyte-to-plasma portioning of LdT was evaluated in rat, monkey, and human plasma. For plasma protein binding determination, [2-^{14}C]-LdT at concentrations of 0.4, 1.27, 4, and 40 µg/ml was incubated in rat, monkey, and human plasma at 37°C for 15 minutes and filtered by ultrafiltration. The radioactivity before and after ultrafiltration was determined. Erythrocyte-to-plasma partitioning was evaluated by incubating rat, monkey, and human plasma fortified with erythrocytes (1:1; v:v) and [2-^{14}C]-LdT at concentrations of 0.4, 1.27, 4, and 40 µg/ml for 15 minutes at 37°C. The radioactivity was determined in plasma with and without erythrocytes. It was found that the mean plasma protein binding was 7.5, 5.1, and 3.3% in rat, monkey, and human plasma, respectively. The mean erythrocyte-to-plasma concentration ratios were 0.945, 0.986, and 1.01 in rat, monkey, and human, respectively. The results indicate that plasma protein binding of LdT is low in all three species and is independent of LdT concentration over the range of 0.4 to 40 µg/ml. They also suggest that LdT can partition into rat, monkey, and human erythrocytes.

2.6.4.5 Metabolism

Inhibitory potential of L-thymidine towards human hepatic microsomal cytochrome P450 isoenzymes (Study # 02-CP-005): The inhibitory potential of L-dT on cytochrome P450 CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 isoenzymes was assessed by incubating human hepatic microsomes with isoenzyme-selective substrate at a concentration approximating the K_m value in the presence and absence of 0.4-40 µg/ml LdT. No inhibition was observed on any of the cytochrome P450 isoenzymes, with IC_{50} values > 40 µg/ml. In contrast, the isoenzyme-selective inhibitors (fluvoxamine, sulfaphenazole, omeprazole, quinidine, dietheyldithiocarbamate, and ketoconazole) produced significant inhibition (>70%).

The effect of L-thymidine on hepatic microsomal cytochrome P450 enzyme activities in rats (Study # 02-CP-007): Female rats received LdT at oral doses of 0, 1, or 10 mg/kg/day for 7 days. The induction potential of LdT was evaluated by quantitating hepatic total cytochrome P450 content and CYP1A-, CYP2B-, CYP3A-, and CYP4A-

selective activities at 24 and 336 hours after the last dose. Phenobarbital was used as the positive control. The hepatic total cytochrome P450 content and the CYP-isoenzyme activities were not affected at 24 hour time point. The hepatic total cytochrome P450 content was slightly increased as compared to the vehicle control value but the isoenzyme activities were unaffected at the 336 hour time point. The mechanism of this reduction is unknown. The sponsor did not attribute this finding to drug administration. It's concluded that LdT is not an inducer of the cytochrome P450 isoenzymes.

2.6.4.6 Excretion

Lacteal excretion and placental transfer of radioactivity after a single oral administration of [14C]-L-thymidine to pregnant and lactating rats (Study # 02-PK-012): A single oral dose of 10 mg/kg [^{14}C]-LdT was administered to time-pregnant Sprague-Dawley rats on days 13 (group 1) or 18 (group 2) of gestation, or 12 days postpartum (group 3). Blood and selected tissues from the dams and fetuses were collected from 3 dams/time point at 1, 3, 8, and 72 hours postdose from groups 1 and 2. Milk and plasma samples were collected from the 3 animals/time point from the 3rd group at 0.5, 1, 3, 8, 24, 48, and 72 hours postdose. The tissue distribution and lacteal excretion of radioactivity were determined. The results are presented in the following table:

C_{max} values from various maternal tissues and fetuses (ng eq/g)								
Dosing day	**Gestation Day 13**				**Gestation Day 18**			
	Maternal		**Fetal**		**Maternal**		**Fetal**	
	T_{max} (hr)	C_{max} (ng eq/g)	T_{max} (hr)	C_{max} (ng eq/g)	T_{max} (hr)	C_{max} (ng eq/g)	T_{max} (hr)	C_{max} (ng eq/g)
Blood	3	988	3	155	1	830	3	283
Brain	3	45.2			3	31.6	3	188
Kidneys	3	4440			3	2150	3	363
Liver	1	1220			1	1110	3	188
Placenta	3	848			3	512	-	-
Plasma	3	1210			1	1000	-	-
Residual		-			-	-	3	328

Radioactivity was widely distributed in both maternal and fetal tissues following oral administration of radioactive LdT and was detectable through 8 hours postdose. The maternal tissue distribution patterns were similar between dosing in gestation days 13 and 18. Peak tissue/plasma levels generally occurred 3 hours postdose. Highest L-dT-derived radioactivity was detected in kidneys. LdT clearly can cross the placenta since LdT-derived radioactivity was measurable in fetuses. It was also shown to penetrate both maternal and fetal brain. [^{14}C]-LdT-derived radioactivity was detectable in milk through 24 hours postdose. The AUC values were 19,700 and 7030 ng equivalent/g in milk and plasma, respectively. The peak concentration in milk occurred at 3 hours postdose. The mean milk to plasma concentration ratios were less than 1 through 1 hour postdose and greater than 1 from 3 through 24 hours postdose.

2.6.4.7 Pharmacokinetic drug interactions

No studies were done since the LdT was found not to be an inhibitor or inducer of cytochrome P450 isoenzymes. In addition, three phase I drug-drug interaction studies have been conducted for telbivudine coadministered with lamivudine, peg interferon alfa-2a, and adefovir dipivoxil. The pharmacokinetic profiles of the coadministered drugs were not affected.

2.6.4.8 Other Pharmacokinetic Studies

None.

2.6.4.9 Discussion and Conclusions

The ADME profile of telbivudine was adequately studied in several animal species. It is moderately absorbed in both animals and humans, with oral bioavailability ranged from 38 to 60% in rats, woodchucks, mice, rabbits, monkeys, and humans. The rate of absorption ranged from moderate to fast (T_{max} 0.5-3 hours) in animals and moderate in humans (T_{max} 3 hours). Food, gender, and pregnancy had no effect on the pharmacokinetic profile of telbivudine. The systemic exposure generally increased in a dose-related manner across all species studied. The plasma clearance was similar in rats and monkeys but slower in woodchucks. The $t_{1/2}$ values suggest that mice, rats, and woodchuck eliminated telbivudine more rapidly than monkeys ($t_{1/2}$ 7.5-18 hr) and humans ($t_{1/2}$ 41.1 hr). Accumulation of telbivudine following multiple oral dosing was not apparent in mice and rats but was slight (1.2 to 1.6-fold) in monkeys.

Telbivudine was extensively distributed into tissues and extravascular spaces. It was shown to be excreted into milk and cross the blood-brain, blood-testes, and placenta barreiers. The results of placenta transfer and milk excretion studies indicate that fetuses and suckling pups as well as pregnant rats and rabbits received adequate systemic exposure to telbivudine in the reproductive toxicity studies. Telbivudine did not bind to melanin as demonstrated in study in the pigmented skin and eye, had low binding to plasma proteins, and partitioned to erythrocytes.

In vivo metabolism of telbivudine was not significant or was absent in both animals and humans, though it is anabolized intracellularly to its active 5'-triphosphate derivative. The triphosphate derivative is not detectable in plasma. The unchanged parent compound is the primary drug-derived component excreted in plasma and urine.

Tebivudine is eliminated at a moderate to rapid rate ($t_{1/2}$ 2-8 hr) in mice, rats, and woodchucks, but at lower rate in monkeys ($t_{1/2}$ 7.5-18 hr) and humans ($t_{1/2}$ 41.1 hr). This is consistent with the lack of accumulation in the rodent species and modest accumulation (1.2-1.6-fold) in monkeys and humans. Telbivudine is excreted mainly through the renal route as unchanged drug. No metabolite was detected in the systemic circulation or excreta of humans, monkeys, and woodchucks. A minor (< 0.8% of total administered drug), unidentifiable metabolite, M4, was detected in the plasma, urine, and bile of female rats (found in the bile of male rats also). It exhibited no inhibitory or inducing activities to any of the human cytochrome P450 isozymes examined, suggesting that telbivudine is unlikely to interact with other co-administered drugs.

The nonclinical ADME data suggest that the species (mice, rabbits, rats, and monkeys) used in the toxicology studies are appropriate for the evaluation of the nonclinical safety profile of telbivudine.

2.6.4.10 Tables and figures to include comparative TK summary

Comparative Pharmacokinetics of Telbivudine in Animals and Humans Following Single and Multiple Oral Does of Radiolabeled and Nonradiolabeled Telbivudine

Species[a]	Dose (mg/kg/day)	Dosing Duration	C_{max} (μg/ml)	T_{max} (hr)	AUC_{0-24} (μg-hr/ml)	CL (L/hr/kg)	$t_{1/2}$ (hr)	Dose normalized AUC
CD-1 mice	500	1 day	51.9	0.75	149	-	3.01	0.298
		13 weeks	52.2	0.75	145	-	2.13	0.290
	1000	1 day	84.6	0.75	265	-	1.90[b]	0.265
		13 weeks	81.0	1.0	238	-	2.29	0.238
	3000	1 day	145	1.0	616	-	2.25	0.205
		13 weeks	141	1.0	595	-	3.55	0.198
SD rats	10[c]	1 day	1.57	1.5	6.96	0.868[d]	4.21	0.696
	500	1 day	24.1	2.0	108	-	4.45	0.216
		28 days	20.7	1.5	111	-	4.85	0.222
	1000	1 day	42.8	2.0	194	-	4.40[e]	0.194
		28 days	35.8	1.5	169	-	3.60[e]	0.169
	2000	1 day	66.2	2.0	364	-	3.20	0.182
		28 days	50.5	2.0	398	-	3.95	0.199
Rabbits[f]	50	13 days	12.3	1.0	72.5	-	-	1.45
	250	13 days	38.9	1.0	296	-	-	1.18
	1000	13 days	66.4	1.4	1023	-	-	1.02
Woodchucks	10[c]	1 day	2.08	3.0	18.3	0.199[d]	7.54	1.83
Cynomulgus monkeys	10[c]	1 day	3.11	1.7	10.0	0.586[d]	1.75	1.00
	500	1 day	15.9	1.0	95.4	-	9.55	0.191
		28 days	21.1	1.5	127	-	18.0	0.254
	1000	1 day	27.7	2.0	167	-	7.45	0.167
		28 days	33.1	2.0	232	-	17.3	0.232
	2000	1 day	34.8	2.0	252	-	11.1	0.126
		28 days	44.6	2.0	329	-	15.8	0.165
Humans	600 mg[g]	1 day	2.86	3.0	19.8	-	41.1	2.53
	600 mg[h]	1 day	2.88	2.5	19.0	-	19.9	2.27
		9 days	3.44	3.0	27.5	-	-	3.29

a: Combined male and female data unless otherwise indicated
b: Half-life for females only
c: [^{14}C]-Telbivudine AME study in rats
d: Determined following intravenous administration
e: Half-life for males only
f: Pregnant females only
g: [^{14}C]-Telbivudine AME study in 6 human volunteers with a mean weight of 76.6 kg (dose = 7.83 mg/kg)
h: Repeated dose study in 16 normal volunteers with a mean weight of 71.8 kg (dose = 8.36 mg/kg)

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The nonclinical toxicological profile of LdT has been studied in rats, monkeys and mice. Single dose studies were performed in rats and monkeys at doses of 20 (monkeys

only), 100 (monkeys only), 500, 1000, and 2000 mg/kg/day. The NOAEL was determined to be greater than 2000 mg/kg/day.

A five-day intravenous toxicity study in monkeys indicated that doses of 2, 10, and 40 mg/kg/day doses of LdT were well tolerated and the same doses were used in the two week intravenous toxicity study in the same species. A similar 14-day intravenous toxicity was also carried out in rats at doses of 0, 5, 15, and 45 mg/kg/day. Minimal increases in the incidence and severity of histopathological changes were seen in pancreas, kidneys, and heart of 45 mg/kg/dose group animals in one sex only. Since none of these findings were seen in the longer term studies at higher systemic exposure, they were not considered significant. The intravenous NOAELs in rats was thus considered to be 45 mg/kg/day and in monkeys 40 mg/kg/day.

Twenty-eight days oral toxicity/toxicokinetic studies were performed in rats, monkeys, and CB6F1 mice at oral doses of 0, 500, 1000, and 2000 mg/kg/day. The study done in CB6F1 mice, the parent strain for the transgenic TgrasH2 mouse, was used to support the dose selection for a 6-month transgenic mouse carcinogenicity study. The toxicokinetic data indicated that systemic exposure increased in a dose-related manner without gender difference in pharmacokinetic profile across the three species studied. Slight accumulation of LdT in the CB6F1 mice was observed after 4 weeks of continuous drug administration but was absent in rats or monkeys. Drug accumulation was not apparent in the 6 month carcinogenicity study in its transgenic strain. Slight changes in hematological, clinical chemistry parameters and organ weight parameters were observed in rats, mice and monkeys. However, these changes were seen mostly in one species only and without the corresponding histological observations. None were considered dose limiting or seen in the longer term studies in the same species. One exception is the dose-related increases in the incidence of soft/loose feces and reduced body weight gain in monkeys without histopathological changes in any of the gastrointestinal tissues. This gastrointestinal toxicity was not considered dose-limiting. Therefore, the NOAEL was 2000 mg/kg for rats and CB6F1 mice and 500 mg/kg/day for monkeys, providing a 5- to 24-fold safety margin for the clinical dose of 600 mg/day.

Longer term toxicology studies were also conducted in rats, mice, and monkeys. The longest dosing duration in CD-1 mice was 3 months with toxicokinetic arm at oral doses of 0, 500, 1000, and 3000 mg/kg/day. No gender difference or accumulation of drug was seen. No adverse effect on any parameters monitored was attributed to LdT treatment. The NOAEL for mice was 3000 mg/kg/day, providing a 22-fold safety margin for humans. The longest subchronic toxicology study performed in rats was 6 months with a 3-month interim sacrifice and a 1-month drug-free recovery arms at oral doses of 0, 250, 500, and 1000 mg/kg/day. Except for a very small increase in the incidence of nephropathy in males after 6-month of treatment, no effect was attributable to LdT treatment. At this duration, nephropathy was not considered dose limiting since the increase was too small for a common lesion in this species. NOAEL was considered to be 1000 mg/kg/day with exposure projected to be about 6-fold above that at clinical dose of 600 mg/day. The longest toxicology study conducted in monkeys was 9 months with a 3 month interim sacrifice and a two-month drug-free recovery arm at oral doses of 0, 250, 500, and 1000 mg/kg/day. Dose-related increases in the incidences of soft feces, erythema of skin, and fur loss were observed. These clinical

observations did not have the corresponding histological or clinical pathological changes nor did they affect body weight gain or food consumption. Gastrointestinal toxicity was clearly related to LdT treatment but was not dose-limiting. This toxicity was seen in pregnant rabbits manifested as reduced body weight gain, abnormal feces, death, abortion, and early delivery and was dose-limiting. The NOAEL for monkeys was 500 mg/kg/day, providing 5-fold safety margin for humans.

Genetic toxicology:

The genotoxic potential of LdT was investigated in the Ames test at concentrations up to 5000 µg/plate, in chromosome aberration tests with Chineses Hamster Ovary cells at concentrations up to 5000 µg/ml and with human peripheral blood cells isolated from healthy volunteer at concentrations up to 2422 µg/ml, and in *in vivo* mouse micronucleus assay at oral doses up to 2000 mg/kg/day. Appropriate positive and negative controls were included. LdT was not cytotoxic at all concentrations studied across all *in vitro* and *in vivo* models. It was found not to be mutagenic and clastogenic with and without metabolic activation.

Carcinogenicity:

The carcinogenic potential of LdT was studied in the 2 year carcinogenicity study in rats and the 6-month carcinogenicity study in transgenic CB6F1-TgrasH2 mice at oral doses of 500, 1000, and 2000 mg/kg. The dosing in rats was stopped for the 2000 mg/kg dose groups after 85 weeks of LdT administration and study terminated prematurely after 96 weeks of drug administration because of the high mortality rate. The increased mortality rate for this study was dose-related. Many of the deaths were caused by tumors. However, except for deaths caused by chronic progressive nephropathy, no statistically significant or dose-related increases in the incidence of tumor and/or tumor type and deaths attributable to any tumor type were observed. Dosing in the transgenic mouse carcinogenicity study was continued to the scheduled time. No evidence of increased incidence of tumor was associated with LdT administration at doses up to 2000 mg/kg/day. LdT is considered not carcinogenic.

Reproductive toxicology:

The effect of LdT on fertility and early embryonic development was evaluated in three separate studies. The first study was a combined male and female Segment I reproductive toxicology study in rats at oral doses of 0, 100, 500, and 1000 mg/kg/day which were administered during premating, cohabitation, and early gestation. An increase in the incidence of soft or liquid feces in F_0 males and a decrease in the fertility index for the 500 and 1000 mg/kg F_0 groups were observed. Because of the finding associated with decreased fertility index, two additional Segment I reproductive toxicity studies were done in the same species. LdT-treated males (oral doses of 0, 1000, and 2000 mg/kg/day) was mated with untreated females and LdT-treated females (oral doses of 0 and 2000 mg/kg/day) mated with untreated males. No effects were associated with any of the parameters normally evaluated in a Segment I reproductive toxicology study at oral doses up to 2000 mg/kg/day. Therefore, the NOAEL for male and female fertility and early embryonic development was considered to be 2000 mg/kg/day, providing a 14-fold safety margin for humans.

The effect of LdT on the embryonic and fetal development was assessed in two Segment II reproductive toxicology studies in rats and rabbits at oral doses of 0, 50 or 100, 500, and 1000 mg/kg/day. In addition, maternal, fetal and neonatal (through milk) exposures to LdT were also evaluated in a separate study in pregnant rabbits at the same doses. The results indicated that LdT can cross placenta, blood-testes, and blood brain barriers. Both the fetuses and neonates were exposed to LdT *in utero* or via milk. The study performed in rats was a combined Segments II and III reproductive toxicology study. In rabbits, a total of one death, one abortion, and two premature deliveries were associated with the 1000 mg/kg/day dose. These dams exhibited gastrointestinal irritation manifested as reduced food consumption and body weight gain and abnormal feces which were the same signs observed for this dose group in general. This gastrointestinal toxicity was associated with the high system drug exposure which was 2-3 times higher than those in rats, mice, and monkeys at the 1000 mg/kg/day and 37-fold higher than that in humans. Clearly, 1000 mg/kg/day dose was the maximum tolerated dose in the pregnant rabbits. However, no effects on any of the parameters for embryonic and fetal development were observed at this high dose. Thus, the NOAEL for the F_1 generation was 1000 mg/kg/day for both rats and rabbits, providing a safety margin of 6- to 37-fold in humans.

The results from the combined Segment II/III reproductive toxicology study in rats at oral doses of 0, 100, 250, and 1000 mg/kg also indicated that LdT exerted no adverse effects on delivery, peri- and postnatal development, post-natal behavior, growth, sexual maturity, and fertility of the F_1 generation. The NOAEL for reproductive toxicity in F_1 generation was 1000 mg/kg/day, providing a 6-fold safety margins for the clinical dose of 600 mg/day.

Special toxicology:

The allergenic potential of LdT was evaluated in a murine local lymph node assay at doses of 7.5, 75, and 750 mg/kg. It was found that LdT is not considered an irritant or allergen by this assay.

2.6.6.2 Single-dose toxicity

Study title: Dose range-finding and acute toxicity study of NV-02B administered orally to rats

Key study findings: There were no effects in all parameters measured at oral doses of 0, 500, 1000, and 2000 mg/kg. The no observed adverse effect level (NOAEL) for NV-02 was greater than 2000 mg/kg.

Study no.: GVAW-104

Volume: m4\42-stud-rep\423-tox\4231-single-dose-tox\gvaw-104

Conducting laboratory and location: [redacted]

Date of study initiation: 1/11/00-2/23/00

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: Lot # LT-9-001E; [redacted] pure

Results:

In the range-finding part of study, 1 rat/sex/dose received a single oral dose of 20, 100, 500, 1000, or 2000 mg/kg L-dT. Clinical observation and body weights were recorded on day 1 and prior to necropsy on day 7. Since no clinical observations were associated with drug treatment, single doses of 0 (0.5% carboxymethylcellulose, w/v), 500, 1000, and 2000 mg/kg were given to 5 rats/sex/dose. Clinical observations were made daily, individual body weights were measured on days 1, 8, 14, and prior to necropsy, and clinical pathology analysis was performed on the blood collected prior to necropsy (on day 14). Organ weights and macroscopic examinations on selected tissues were also recorded. There were no effects in all parameters measured that are attributable to the drug treatment. Thus, the no observed adverse effect level (NOAEL) for L-dT following a single oral dose in rats was 2000 mg/kg.

Study title: Dose escalating toxicity study of NV-02B administered orally to monkeys
Key study findings: Single escalating oral doses of 20, 100, 500, 1000 and 2000 mg/kg L-dT were administered to 2 monkeys/sex. Soft feces were seen throughout the study in females. Some hematological changes that were dose related were observed and were probably associated with frequent blood drawing. The NOAEL is greater than 2000 mg/kg
Study no.: GVAW-106
Volume: m4\42-stud-rep\423-tox\4231-single-dose-tox\gvaw-106
Conducting laboratory and location: [redacted]
Date of study initiation: 1/18/00-2/3/00
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Lot # LT-9-001E; [redacted], pure
Results:

Two cynomolgus monkeys/sex were administered orally single escalating doses of 20, 100, 500, 1000 and 2000 mg/kg L-dT (dissolved in sterile water) on each of the days 1, 4, 7, 10, and 14, respectively. The dose volume was 5 ml/kg for the doses of 20, 100, and 500 mg/kg while that for the doses for 1000 and 2000 mg/kg was 10 ml/kg. Clinical observations were performed once daily, body weights recorded on days 1, 4, 7, 10, 14, and 17, blood collected for clinical pathology analysis prior to treatment on days 1, 4, 7, 10, and 14 and prior to necropsy on day 17. Gross pathological examination was done following necropsy on day 17.

Two females exhibited mild to marked soft feces throughout the study. The gradual decrease of erythrocyte counts, hemoglobin, and hematocrit over the course of the study in all animals and low incidences of anisocytosis, poikilocytosis, hypochromasia, and/or polychromasia in the animals on one or more occasions may be related the frequent blood collection. Alkaline phosphatase and potassium levels were mildly decreased in all animals. Other hematological and serum chemistry changes were seen in one animal only and did not exhibit any dose relationship. Gross macroscopic findings did not correlate any of the observed effects and thus the toxicological significance of those is uncertain. The NOAEL in the study is considered to be at 2000 mg/kg.

2.6.6.3 Repeat-dose toxicity

Study title: 2-week intravenous toxicity study in rats including sighting phase
Key study findings: Two animals/sex/dose were used for an initial sighting phase of the study. The doses used in this phase were 2, 20, and 40 mg/kg/day. The purpose for this phase was to evaluate the local and systemic tolerability for the dose selection for the main study. No adverse effects were seen in this phase, thus the doses selected for the main study were 0, 5, 15, and 45 mg/kg/day. Adequate systemic exposure was achieved at this dose range with proportional increases of the C_{max} and AUC values with the dose. There was no gender difference in the pharmacokinetic parameters. The systemic exposure at 45 mg/kg/day was lower than the low oral dose of 500 mg/kg/day in the longer term toxicology and carcinogenicity studies. A variety of macroscopic and microscopic effects were seen including a malformed left kidney, apotosis/single cell necrosis, inflammatory cell infiltration, and acinar cell atrophy in pancreas, tubular dilatation, interstitial dilatation, and pyelonephritis in kidneys, and inflammatory focus found in heart. Most of these incidences were seen at the 45 mg/kg/day dose and at a single sex only. The incidence and severity were minimally increased. The toxicological significance of these findings is questionable since at higher and longer systemic exposure (up to 97 weeks), no treatment-related effects were seen in these organs. The NOAEL for this study is 15 mg/kg/day.
Study no.: 0510032
Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\0510032
Conducting laboratory and location: [redacted]
Date of study initiation: 2/28/2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: LDT600, lot # 0514036, [redacted] by HPLC
Methods
Doses: 2, 20, and 40 mg/kg/day (sighting part) & 0, 5, 15, and 45 mg/kg/day (main study)
Duration of dosing: 4 days for sighting part & 14 days for main study
Species/strain: Sprague Dawley rats, R/SPF CD
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Intravenous, aqueous glucose solution containing 0.5% polyvinylpyrrolidone and a phosphate buffer at 10 mM adjusted to pH 7.4, 0.8 ml/kg/day, bolus infusion via tail vein
Satellite groups used for toxicokinetics or recovery: None
Age: 8 weeks old
Weight: 177-389 g (sighting part); 174-349 g (main study)
Sampling times: Blood samples taken form 2 animals/sex/dose/time point on day 15 at 5 minutes, 0.5, 1, 4, and 24 hours post dosing
Unique study design or methodology: For the sighting part, 2 animals/dose/sex were dosed for 4 days. The same rats were used for doses of 2 and 40 mg/kg/day with 11 days of washout period between the end of one dose and the beginning of the high dose.

Observations and times:

Mortality: Once daily during pretest period, twice daily during dosing period
Clinical signs: Once daily during pretest period, twice daily during dosing period
Body weights: Once during pretest, daily during the dosing period
Food consumption: once during pretest, twice weekly during the dosing period
Ophthalmoscopy: Not done
EKG: Not done
Hematology: At the end of the dosing period
Clinical chemistry: At the end of the dosing period and included creatinine kinase measurements
Urinalysis: At the end of the dosing period
Gross pathology: One the last treatment day or one day after
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes
Peer review: yes (X), no ( )

Results

Mortality: None
Clinical signs: None
Body weights: No effect
Food consumption: No effect
Hematology: No effect
Clinical chemistry: No effect
Urinalysis: No effect
Gross pathology: Left kidney of one of the 15 mg/kg/day female showed malformation. Right kidney of one of the high dose female (45 mg/kg/day) was small. These two macroscopic findings had microscopic correlates.
Organ weights: No effect.
Histopathology:

	Male				Female			
Dose (mg/kg/day)	**0**	**5**	**15**	**45**	**0**	**5**	**15**	**45**
Animals examined	10	10	10	10	10	10	10	10
Pancreas –								
Apoptosis % affected	0	0	10	30	0	10	0	0
Mean severity	0.0	0.0	1.0	1.3	0.0	1.0	0.0	0.0
Inflammatory cell infil.								
% affected	10	0	0	40	10	0	0	30
Mean severity	1.0	0.0	0.0	1.3	1.0	0.0	0.0	1.0
Acinar cell atrophy								
% affected	10	0	10	20	0	10	0	0
Mean severity	1.0	0.0	1.0	1.5	0.0	1.0	0.0	0.0
Kidneys –								
Tubular dilatation								
% affected	10	0	0	50	0	10	30	60
Mean severity	1.0	0.0	0.0	1.0	0.0	1.0	1.0	1.0
Pyelonephritis								
% affected	0	0	0	0	10	0	0	20
Mean severity	0.0	0.0	0.0	0.0	2.0	0.0	0.0	1.5

	Male				Female			
Dose (mg/kg/day)	**0**	**5**	**15**	**45**	**0**	**5**	**15**	**45**
Animals examined	10	10	10	10	10	10	10	10
Heart –								
Inflammatory focus								
% affected	0	20	0	40	0	0	10	20
Mean severity	0.0	1.0	0.0	1.0	0.0	0.0	1.0	1.0

Severity Grade 1 = minimal/very few/very small; Severity Grade 2 = slight/few/small;
Severity Grade 3 = moderate/moderate number/moderate size; Severity Grade 4 = marked/ many/large
Severity Grade 5 = massive/extensive number/ extensive size

Histopathological changes were seen in pancreas, kidneys, and heart. They included apotosis/single cell necrosis, inflammatory cell infiltration, and acinar cell atrophy in pancreas, tubular dilatation, interstitial dilatation, and pyelonephritis in kidneys, and inflammatory focus found in heart. Most of these incidences were seen at the 45 mg/kg/day dose and at a single sex only. The incidence and severity were minimally increased. The toxicological significance of these findings is questionable since at higher and longer systemic exposure (up to 97 weeks), no treatment-related effects were seen in these organs.

Toxicokinetics:

	Male			Female		
Dose (mg/kg/day)	**5**	**15**	**45**	**5**	**15**	**45**
C_{max} (µg/ml)	7.76	24.49	80.89	10.15	27.69	95.43
AUC_{0-24} (ng-hr/ml)	6.69	18.01	59.83	8.50	22.59	71.18

The C_{max} and AUC values increased dose proportionally over the dose range 5 to 45 mg/kg. There was no gender difference in the pharmacokinetic parameters. Traces of LdT (54.1 ng/ml) were detected in one control female at 0.5 h postdose. Since it only occurred in one animal at one time point, it is not considered to be indicative of poor conduct of the study.

Study title: Twenty-eight day repeated dose toxicity study of NV-02B administered orally to rats

Key study findings: LdT was administered orally to 10 rats/sex/dose at doses of 0, 500, 1000, and 2000 mg/kg/day. The systemic exposure increased linearly with increased doses up to 2000 mg/kg/day. There was no difference in the systemic drug exposure between days 1 and 28, indicating the absence of saturation of absorption. Small changes seen one of in hematological parameters and increases in adrenal weight were considered not to be related to LdT treatment since the increases were small, occurred in one sex only, and did not have any corresponding histopathological effect, and were not observed in the 6-month study in the same species. The NOAEL for the study is 2000 mg/kg/day.

Study no.: NC-NV-02B-003

Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\GVAW-105

Conducting laboratory and location: [redacted]

Date of study initiation: 3/15/2000

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: NV-02B, Lot # LT-0-002E, [redacted] pure by UV

Methods

Doses: 0, 500, 1000, and 2000 mg/kg/day
Duration of dosing: Three to six months
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Oral, dissolved in 0.5%, w/v, carboxy-methylcellulose, 10 ml/kg dosing volume
Satellite groups used for toxicokinetics: 9 rats/sex/dose
Age: 6-7 weeks old
Weight: 178.4-223.2 g for males and 168.0-201.4 g for females
Sampling times: Blood samples collected from 3 rats/dose/sex/time point on days 1 & 28 at 0.5, 1, 2, 4, 8, and 24 hours post dosing for toxicokinetic analysis
Unique study design or methodology: None

Observations and times:

Mortality: Twice daily
Clinical signs: Daily
Body weights: Days 1, 8, 15, 22, 28, & 29
Food consumption: Days 8, 15, 22, and 28
Ophthalmoscopy: Not done
EKG: Not done
Hematology: Prior to necropsy
Clinical chemistry: Prior to necropsy
Urinalysis: Prior to necropsy
Gross pathology: At necropsy
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes
Peer review: yes ( ), no (X)

Results

Mortality: None
Clinical signs: None
Body weights: No effect
Food consumption: No effect
Hematology: The absolute neutrophil count at 2000 mg/kg/day was significantly decreased as compared to the control. There was a dose-related decrease in both absolute and relative neutrophil count in males. No such relationship was apparent in females. No other hematologic parameters were affected. In addition, there was no correlated histological change and these hematological effects were not seen in the 6-month toxicity study or 2 year carcinogenicity study. Thus, there was no toxicological significance for these findings.

	Male				Female			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Absolute neutrophil count ($10^3/\mu l$)	0.737	0.530	0.494	0.416*	0.489	0.551	0.361	0.512
Neutrophil (%)	9.5	7.7	6.8	5.6	7.5	10.0	5.8	8.5

Clinical chemistry: No effect
Urinalysis: No effect
Gross pathology: No effect

Organ weights: The weights of adrenal glands (both absolute and relative) for males were increased dose proportionally, albeit not statistically significant. The same organ weights for females were significantly increased for the low and high dose animals. No corresponding histopathological changes were observed. In addition, no effect on this organ weights was observed in the 6-months toxicity study or the carcinogenicity study in the same species. Thus, this finding probably has no toxicological significance.

	Male				Female			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Adrenal glands (g)	0.0580	0.0630	0.0619	0.0675	0.612	0.722*	0.0669	0.0702*
Adrenal glands/BW	0.0165	0.0180	0.0171	0.0186	0.0263	0.0309*	0.0282	0.0290

Histopathology: No effect

Toxicokinetics: The absorption for LdT was moderately rapid with T_{max} values ranged from 1 to 2 hours. There were, in general, no gender differences in C_{max} and AUC values. No accumulation was observed following multiple dosing since all of the toxicokinetic parameters measured remained similar between days 1 and 28. The AUC values increased dose proportionally between doses of 500 and 2000 mg/kg/day suggesting that saturation of absorption has not been reached in the study.

		Male			Female		
Dose (mg/kg/day)		**500**	**1000**	**2000**	**500**	**1000**	**2000**
T_{max} (hrs)	Day 1	2	2	2	2	2	2
	Day 28	1	2	2	2	2	2
C_{max} (µg/ml)	Day 1	26.1	45.3	63.2	22.1	40.2	69.1
	Day 28	18.3	29.6	47.7	23.1	42	53.2
$AUC_{0\text{-}24h}$ (µg-hr/ml)							
	Day 1	116	212	328	99.0	176	400
	Day 28	89.7	170	364	133	167	431
$t_{1/2}$ (hrs)	Day 1	4.4	4.4	3.1	4.5	-	3.3
	Day 28	6.0	3.6	4.3	3.7	-	3.6

Study title: Six-month chronic oral gavage toxicity study in rats with one-month recovery

Key study findings: Doses of 0, 250, 500, and 1000 mg/kg/day were administered to rats for 3 to 6 months. A group of animals were allowed to recover drug free for 1 month after 6 months of continuous drug administration. Deaths were observed in control, mid, and high dose groups. Some deaths were not clearly attributable to any causes. However, many of them exhibited signs that suggested dosing errors. Therefore, the deaths were not considered to be caused by LdT toxicity. The incidence and severities of nephropathy and lymphoid hyperplasia were increased in the high dose males and females, respectively, as compared to the concurrent controls. The increases were very small and not dose limiting. Maximum tolerated dose was not achieved in this study. Increase in the incidence of lymphoid hyperplasia was not apparent in the rat carcinogenicity study and was probably an incidental finding in this study. Chronic progressive nephropathy, a common condition relating to the aging process, was exacerbated by chronic administration of LdT (85 weeks of drug administration) and caused deaths. In view of this, the NOAEL for this study is 500 mg/kg/day.

Study no.: 02-TX-022

Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\GVAW-125

Conducting laboratory and location: [redacted]
Date of study initiation: 10/3/2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NV-02B, Lot # LT.0.003E ([redacted] pure by UV), LT.0.004E [redacted] pure by UV), LT.0.005E ([redacted] pure by UV), LT.0.006E [redacted] pure by UV), LT.1.001E [redacted] pure by UV), & LT.1.002E [redacted] by UV)
Methods
Doses: 0, 250, 500, and 1000 mg/kg/day
Duration of dosing: Three to six months
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 5 for 3 months & 5 for 6 months
Route, formulation, volume, and infusion rate: Oral, dissolved in 0.5%, w/v, carboxy-methylcellulose, 5 ml/kg dosing volume
Satellite groups used for recovery: 5 additional rats/sex/dose treated for 6 months and allowed 1 month drug-free recovery for control and high dose groups only
Age: 6 weeks old
Weight: 192.4-224.2 g for males and 147.4-173.8 g for females
Unique study design or methodology: None
Observations and times:
Mortality: Twice daily
Clinical signs: Weekly
Body weights: Once during pretest, weekly during the dosing period
Food consumption: Weekly
Ophthalmoscopy: Pretest and during week 12, 24, and prior to necropsy fore recovery animals
EKG: Not done
Hematology: Prior to necropsy at 3, 6, and 7 months
Clinical chemistry: Prior to necropsy at 3, 6, and 7 months
Urinalysis: Not done
Gross pathology: At necropsy at 3, 6, and 7 months
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes
Peer review: yes ( ), no (X)

Results
Mortality:

	Male				Female			
Dose (mg/kg/day)	**0**	**250**	**500**	**1000**	**0**	**250**	**500**	**1000**
Total # examined	25	20	20	25	25	20	20	25
# dead	1	0	2	1	0	0	0	2
Day of death	50	-	161, 171	133	-	-	-	116, 157

The control male died because of dosing accident.

The two mid dose males had mild congestion, minimal hemorrhage (in one only), and edema (in the other) in their lungs. Both also had chronic inflammation in their trachea. Even though the cause of death was not apparent, the microscopic findings in their lungs and trachea suggested dosing accident.

The high dose male was sacrificed moribund and had lost the use of its rear legs. At necropsy, enlarge spleen and liver due to lymphoma were likely to be the cause of death. This death was not considered treatment related since there was no increase in the incidence of lymphoma in the carcinogenicity study in rats.

One of the high dose females was found dead on day 116. It exhibited labored breathing on the day of deaths. It also had episodes of scant feces, rales, and wheezing during earlier treatment period. At necropsy, accumulation of lymphocyte in peribronchial, perivascular and subserodal spaces of the lungs. The high dose female that died on day 157 did not exhibit any clinical signs during the study or before death. However, the necropsy results showed mild to moderate hemorrhage, congestion, and edema in the lungs. The clinical signs and/or histopathological findings in the lungs suggested that the cause of death was probably related to dosing accident.

None of the deaths was likely to be caused by LdT toxicity.

Clinical signs: No treatment effects
Body weights: No treatment effects
Food consumption: No treatment effects
Hematology: No treatment effects
Clinical chemistry: No treatment effects
Gross pathology: No treatment effects
Organ weights: No treatment effects
Histopathology: Nephropathy was slightly increased in the high dose males after 6 months of continuous LdT treatment. The incidence rate and severity were 50% and 1.4, respectively, as compared to 40% and 1.25 for the control rats. The increase was very small and would not be considered dose limiting. However, an increased number of rats died of chronic progressive nephropathy in the carcinogenicity study. The incidence and severity of lymphoid hyperplasia in mandibular lymph node was slightly increased (80% and 1.38) in the high dose females as compared to the concurrent control animals (90% and 1.78). Again, the increases were small for a common lesion. In addition, significant increase for this same lesion was not apparent in the carcinogenicity study. The lesion is not considered dose limiting for this study.

Study title: A 4-week oral dose toxicity and toxicokinetic study of β-L-2'deoxy-thymidine in CB6F1 mice

Key study findings: The present study was conducted to support dose selection for the 6-months transgenic mouse carcinogenicity study. CB6F1 is the parent strain for the transgenic TgrasH2 mice. Oral doses of 0, 500, 1000, and 2000 mg/kg/day were investigated in this study. Small but statistically significant increase in several hematology and clinical pathology parameters as well as the relative prostate/seminal vesicle and uterus weights were associated with 2000 mg/kg/day dose. Most of these changes were small, occurred in one sex only, and lacked any corresponding macroscopic and microscopic findings. The toxicokinetic data indicated that good systemic exposure to LdT was achieved. The AUC and C_{max} values increased in a dose related manner but the increases were less than dose proportional. No gender difference was apparent. The pharmacokinetic values were similar to those of CD-1 cells at the same doses but were about 1 fold higher than those seen in the transgenic TgrasH2 strain. The NOAEL for this study is 2000 mg/kg/day.

Study no.: 02-TX-030
Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\ [redacted] 046-03
Conducting laboratory and location: [redacted]
Date of study initiation: 2/4/2003
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: β-L-thymidine, Lot # 16102001, [redacted] pure by UV
Methods
Doses: 0, 500, 1000, and 2000 mg/kg/day
Duration of dosing: Four weeks
Species/strain: CB6F1 mice
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Oral gavage in 0.5% carboxymethyl-cellulose solution
Satellite groups used for toxicokinetics: 6/sex for control group, 18/sex/dose for the low and mid dose groups, and 21/sex/dose for the high dose; 2 additional animals/sex/dose were also dosed as the replacements for toxicokinetic collection in the event of deaths
Age: 7 weeks old
Weight: 19.8-26.5 g for males, 17.3-21.5 g for females
Sampling times: Blood samples taken from 3 animals/sex/dose/time point on day 1 and week 13 at 0.5, 1, 2, 4, 8, and 24 hours post dosing
Unique study design or methodology: None
Observations and times:
Mortality: Daily
Clinical signs: Daily (all animals)
Body weights: Days -8 and -1 and weekly thereafter (all animals)
Food consumption: Weekly (main study mice only)
Ophthalmoscopy: Once pretest and once prior to scheduled sacrifice (main study mice only)
EKG: Not done
Hematology: Prior to scheduled necropsy
Clinical chemistry: Prior to scheduled necropsy
Urinalysis: Prior to scheduled necropsy
Gross pathology: At termination
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes (control and high dose animals only)
Peer review: yes ( ), no (X)
Results
Mortality: One control male was found dead hanging from the food hopper by the neck. This death was deemed accident and treatment-related
Clinical signs: No treatment effect
Body weights: No treatment effect
Food consumption: No treatment effect
Ophthalmoscopy: No treatment effect
Urinalysis: No treatment effect

Hematology:

Sex	Male				Female			
Dose (mg/animal/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Leukocyte count (X 10^3/μl)	1.31	2.72	1.15	2.83*	1.82	1.45	-	2.88
Lymphocyte count (X 10^3/μl)	0.87	1.66	0.80	2.20*	1.36	1.15	-	2.28
Erythrocyte count (X 10^6/μl)	7.75	9.63	8.50	9.94	9.26	9.53	-	10.17**
Hemoglobin conc. (g/dl)	14.80	14.87	11.33	15.26	14.55	14.73	-	15.80**
Hematocrit (%)	43.90	44.53	38.40	45.34	41.70	42.48	-	44.53**
* P < 0.05	** P < 0.01							

The absolute number of leukocytes and lymphocytes was statistically increased in males while the relative number of these two parameters was not changed. The erythrocyte count, hemoglobin concentration and hematocrit value in the high dose females were significantly increased as compared to the control. Since these increases were small and occurred in a single sex without corresponding microscopic change in the related tissues, they were probably not treatment-related.

Clinical chemistry:

Sex	Male				Female			
Dose (mg/animal/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Albumin (g/dl)	2.44	2.70*	2.74**	2.90**	2.86	3.26**	3.28**	3.06*
Globulin (g/dl)	1.72	1.94*	1.92	2.04**	1.70	1.90*	1.75	1.84
Total protein (g/dl)	4.16	4.64**	4.66**	4.94**	4.56	5.16**	5.05**	4.90*
* P < 0.05	** P < 0.01							

There were dose-related and statistically significant increases in serum albumin, globulin, and total protein levels in males. Statistical increases in these parameters were also seen in the females but they are not dose related. Though these increases were dose-related, they were small with the corresponding histopathological changes in liver and kidneys. Thus, the toxicological significance of these increases is questionable.

Gross pathology: No treatment effect

Organ weights:

Sex	Male				Female			
Dose (mg/animal/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Relative organ wt. (%)								
Prostate/seminal vesicle	0.868	1.029*	0.996	1.061**	-	-	-	-
Uterus	-	-	-	-	0.793	0.634	0.583	0.536*
* P < 0.05	** P < 0.01							

The relative prostate/seminal vesicle weights increased dose-proportionally in males. The relative uterus weights of the high dose females were significantly lower than the control. There were no corresponding macroscopic or microscopic changes associated with these organs/tissues, thus they probably do not have toxicological significance.

Histopathology: No treatment effect

Toxicokinetics: The analysis of the toxicokinetic parameters suggests that L-dT is absorbed rapidly with T_{max} or 0.5 to 1 hour (the 1st and 2nd sampling time). C_{max} and AUC values were generally less than dose-proportional over the range of doses studied. There were generally no gender differences in exposure but some accumulation of LdT over the course of 4 weeks of daily administration. Terminal half-life ranged from 2.07 to 4.76 hours.

		Male			Female		
Dose (mg/kg/day)		500	1000	2000	500	1000	2000
C_{max} (µg/ml)	Day 1	75.5	105	132	64.5	96.4	122
	Day 28	-	76.8	103	44.3	78.3	114
T_{max} (hrs)	Day 1	0.5	0.5	1.0	0.5	0.5	0.5
	Day 28	-	0.5	0.5	1.0	1.0	1.0
AUC_{0-24h} (µg-hr/ml)							
	Day 1	220	357	457	299	267	439
	Day 28	-	371	662	205	315	679
$t_{1/2}$ (hrs)	Day 1	3.71	3.20	2.74	3.91	3.30	4.66
	Day 28	-	3.20	3.35	3.30	2.07	4.76

Study title: 13-week oral gavage toxicity and toxicokinetic study with L-thymidine (LdT) in mice

Key study findings: Doses of 0, 500, 1000, and 3000 mg/kg/day LdT were administered to CD-1 mice for 13 weeks. Four deaths were seen in the study, none was considered to be associated with the LdT administration. LdT is rapidly absorbed systemically following oral administration. The systemic exposure is generally dose proportional but showed somewhat of saturation of absorption at the high dose. No gender differences in any of the toxicokinetic parameters measured. No LdT-induced toxicity was observed in any parameters measured. The NOAEL is considered to be 3000 mg/kg/day

Study no.: 02-TX-028

Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7245-111

Conducting laboratory and location: [redacted]

Date of study initiation: 11/15/2003

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: β-L-thymidine, Lot # LT.1.005E, [redacted] pure by UV

Methods

Doses: 0, 500, 1000, and 3000 mg/kg/day

Duration of dosing: Thirteen weeks

Species/strain: [redacted] :CD-1®(ICR)BR mice

Number/sex/group or time point (main study): 10

Route, formulation, volume, and infusion rate: Oral gavage in 0.5% carboxymethyl-cellulose solution

Satellite groups used for toxicokinetics: 39/sex/dose in which 3/sex/dose were the replacements for toxicokinetic collection in the event of deaths

Age: 8 weeks old

Weight: 24.5-35.7 g for males, 19.6-29.1 g for females

Sampling times: Blood samples taken from 3 animals/sex/dose/time point on day 1 and week 13 at 0.5, 1, 2, 4, 8, and 24 hours post dosing

Unique study design or methodology: None

Observations and times:

Mortality: Twice daily

Clinical signs: Once during pretest and weekly thereafter (all animals)

Body weights: Once during pretest and weekly thereafter (all animals)
Food consumption: Once during pretest and weekly thereafter (main study mice only)
Ophthalmoscopy: Once pretest and during week 12 (main study mice only)
EKG: Not done
Hematology: One week prior to scheduled sacrifice
Clinical chemistry: At termination
Urinalysis: Not done
Gross pathology: At termination
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes (control and high dose animals only)
Peer review: yes ( ), no (X)

Results

Mortality: Four mice were found dead on days 88 and 89. Congestion of lung was found in three out of four mice. Cyst, nephropathy, lymphocytic infiltration, and congestions were observed in the kidneys of most of these mice. However, cause of death was not determined. The narrow time frame of deaths (2 consecutive days) and findings in lung suggest that death is not likely caused by LdT.

Dose (mg/kg/day)	0	500	1000	3000
Male	0	0	1	2
Female	0	0	0	1

Clinical signs: No treatment effect
Body weights: No treatment effect
Food consumption: No treatment effect
Hematology: No treatment effect
Clinical chemistry: No treatment effect
Gross pathology: No treatment effect
Organ weights: No treatment effect
Histopathology: No effect
Toxicokinetics: LdT was absorbed rapidly with T_{max} values ranging from 0.5 to 1 hour. There were no gender differences in C_{max} and AUC values. No accumulation was observed following multiple dosing since all of the toxicokinetic parameters measured remained similar between day 1 and week 13. The AUC values increased dose proportionally between doses of 500 and 1000 mg/kg/day but less than dose proportional between 1000 and 3000 mg/kg/day

		Male			Female		
Dose (mg/kg/day)		500	1000	3000	500	1000	3000
T_{max} (hrs)	Day 1	0.500	1.00	1.00	1.00	0.500	1.00
	Week 13	1.00	1.00	1.00	0.500	1.00	1.00
C_{max} (µg/ml)	Day 1	48.0	78.5	123	55.8	90.6	166
	Week 13	53.7	79.0	141	50.6	83.0	140
AUC_{0-24h} (µg-hr/ml)							
	Day 1	146	267	640	151	262	592
	Week 13	138	224	538	151	252	651
$t_{1/2}$ (hrs)	Day 1	4.40	NA	2.22	1.61	1.90	2.27
	Week 13	2.31	2.14	3.86	1.94	2.44	3.23

Study title: Maximum tolerated dose (MTD) followed by a 5 day intravenous (bolus) administration toxicity study in the cynomolgus monkey

Key study findings: The maximum tolerated dose for this study is 40 mg/kg/day by the intravenous route. There are no adverse findings in this study except for the slight single-cell necrosis associated with minimal subacute inflammation in the liver of the female in the fixed dose phase. The significance of the findings is unclear since there was only one single animal in this dose and sex, the male in this dose group did not show any histopathological sign at this organ, and no control group was included in this study.

Study no.: 0580155

Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\0580155

Conducting laboratory and location: [redacted]

Date of study initiation: 3/23/2005

GLP compliance: No

QA report: yes ( ) no (X)

Drug, lot #, and % purity: LdT600, Lot # 0514036, [redacted] pure by HPLC

Methods

Doses: 2, 10, and 40 mg/kg/day (MTD phase) and 40 mg/kg/day (fixed dose phase)

Duration of dosing: Single dose in the MTD phase and 5 days for the fixed dose phase

Species/strain: Cynomolgus monkeys

Number/sex/group: 1/sex for the MTD phase (same animals used for all three doses) and 1/sex for the fixed dose phase

Route, formulation, volume, and infusion rate: Intravenous in aqueous glucose solution containing 0.5% polyvinylpyrrolidone and a phosphate bugger at 10 mM, pH 7.4, 10 ml/kg dosing volume

Satellite groups used for toxicokinetics: None

Age: 2.4-4 years old

Weight: 3.5-4.9 kg

Sampling times: None

Unique study design or methodology: None

Observations and times:

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Twice predose and once weekly during dosing

Food consumption: Twice daily

Ophthalmoscopy: Not done

EKG: Not done

Hematology: Pretest and prior to necropsy

Clinical chemistry: Pretest and prior to necropsy

Urinalysis: Not done

Gross pathology: At termination

Organ weights: See histopathology table

Histopathology: Adequate Battery: Yes (control and high dose animals only)
Peer review: yes ( ), no (X)

Results

Mortality: None

Clinical signs: None
Body weights: None
Food consumption: None
Hematology: No effect
Clinical chemistry: No effect
Urinalysis: Not studied
Gross pathology: No effect
Organ weights: No effect
Histopathology: Slight single-cell necrosis associated with minimal subacute inflammation was observed in the liver of the female in the fixed dose phase. The significance of the findings is unclear since there is no control to compare to.

Study title: 14-day intravenous (bolus) administration toxicity study in the cynomolgus monkey
Key study findings: Three monkeys/sex/dose were administered intravenously LdT at doses of 0, 2, 10, and 40 mg/kg/day for 14 days. Detailed cardiovascular as well as other toxicological parameters were monitored. The toxicokinetic data suggested adequate systemic exposure to LdT over the doses studied. No gender differences were associated with the C_{max} and AUC values which increased dose proportionally. No parameters measured were adversely affected by the LdT administration.
Study no.: 0580156
Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\0580156
Conducting laboratory and location: [redacted]
Date of study initiation: 4/14/2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: LdT600, Lot # 0514036, [redacted] pure by HPLC
Methods
Doses: 0, 2, 10, and 40 mg/kg/day
Duration of dosing: 14 days
Species/strain: Cynomolgus monkeys
Number/sex/group or time point (main study): 3
Route, formulation, volume, and infusion rate: Intravenous in aqueous glucose solution containing 0.5% polyvinylpyrrolidone and a phosphate bugger at 10 mM, pH 7.4, 10 ml/kg dosing volume
Satellite groups used for toxicokinetics: No additional animals used to toxicokinetic substudy
Age: 2.5-4 years old
Weight: 2.5-4.2 kg
Sampling times: Blood samples taken on days 1 & 13 at 0, 5 minutes, 0.5, 1, 4, and 24 hours post dosing for toxicokinetic analysis
Unique study design or methodology: None
Observations and times:
Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Pretest, weekly, and on the day of necropsy

Food consumption: Twice daily
Ophthalmoscopy: Once predose and at the end of dosing phase
EKG: Once predose and at the end of dosing phase about 2 to 4 hours after dosing to measure blood pressure, heart rate, RR PR, QRS, QT intervals, QTc intervals, R, S, and T
Hematology: Pretest and during week 2
Clinical chemistry: Pretest and during week 2
Urinalysis: Pretest and week 2
Gross pathology: At termination
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes
Peer review: yes ( ), no (X)

Results

Mortality: None
Clinical signs: No effect
Food consumption: No effect
Hematology: No effect
Clinical chemistry: No effect
Urinalysis: No effect
Gross pathology: No effect
Organ weights: No effect
Histopathology: No effect
Toxicokinetics: Adequate systemic exposure was achieved in this study. The C_{max} and AUC values increased dose-proportionally over the dose range of 2 to 40 mg/kg/day. The systemic exposures were slightly lowered at day 14 as compare to those in day 1. No gender difference in the systemic exposure to LdT was apparent.

		Male			Female		
Dose (mg/kg/day)		**2**	**10**	**40**	**2**	**10**	**40**
C_{max} (μg/ml)	Day 1	4.91 ± 0.58	23.20 ± 5.03	90.78 ± 6.83	4.68 ± 0.70	22.10 ± 1.52	85.67 ± 7.55
	Day 13	3.84 ± 0.37	20.48 ± 2.90	69.07 ± 25.6	4.05 ± 0.48	18.07 ± 4.61	56.49 ± 15.8
$AUC0_{-24h}$ (μg-hr/ml)							
	Day 1	7.43 ± 0.99	32.73 ± 9.90	143 ± 7.87	5.88 ± 0.48	35.46 ± 1.67	141 ± 29.2
	Day 13	6.98 ± 0.67	29.50 ±4.97	136 ± 4.78	6.11± 0.29	29.22 ± 1.37	126 ± 28.2

Study title: Twenty-eight day repeated dose toxicity study of NC-028 administered orally to monkeys

Key study findings: Except for the dose-dependent increases in the incidence and severity of soft feces, lower body weight gain, and increased food consumption, all the other effects were not consistently observed, seen in one sex only, or were commonly observed in all treatment groups without any apparent relation to the dose. The NOAEL was probably lower than 500 mg/kg. However, the maximum tolerated dose was probably not achieved in this study. Soft feces in monkeys suggested that L-dT may cause some GI discomfort. Except for this toxicity, L-dT was well-tolerated.

Study no.: NC-NV-02B-005

Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\GVAW-107

Conducting laboratory and location: [redacted]

Date of study initiation: 3/15/2000

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NV-02B, Lot # LT-0-002E, ▬ pure by UV
Methods
Doses: 0, 500, 1000, and 2000 mg/kg/day
Duration of dosing: Twenty-eight days
Species/strain: Cynomolgus monkeys
Number/sex/group or time point (main study): 4
Route, formulation, volume, and infusion rate: Oral gavage in 0.5% carboxymethyl-cellulose solution, 10 ml/kg dosing volume
Satellite groups used for toxicokinetics: None
Age: Young adult to adult
Weight: 2.0-3.2 kg for males, 1.9-2.7 kg for females
Sampling times: Blood samples taken on days 1 & 28 at 0.5, 1, 2, 4, 8, 12, and 24 hours post dosing for toxicokinetic analysis
Unique study design or methodology: None
Observations and times:
Mortality: Twice daily
Clinical signs: Daily
Body weights: Days 1, 8, 15, 22, and 29
Food consumption: Daily
Ophthalmoscopy: Once pretest and prior to necropsy
EKG: Not done
Hematology: Pretest and prior to necropsy
Clinical chemistry: Pretest and prior to necropsy
Urinalysis: Pretest and prior to necropsy
Gross pathology: At termination
Organ weights: See histopathology table
Histopathology: Adequate Battery: Yes (control and high dose animals only)
Peer review: yes ( ), no (X)
Results
Mortality: None
Clinical signs: The incidence of soft feces is shown in the following table. The number of animals and the days with soft feces increased as dose increased and suggesting gastrointestinal intolerance of the drug/drug formulation.

	Male				**Female**			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
# animals affected	3	3	4	4	3	3	4	4
# days affected	8	10	14	28	17	12	17	30

Body weights: Body weight gain was significantly decreased with increasing dose. This observation may be related to the gastrointestinal intolerance of the drug.

	Male				**Female**			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Body wt gain (kg) Days 1-28	0.15	0.25	0.13	0.02*	0.23	0.10*	0.05**	0.03***

Food consumption: Food consumption was decreased dose proportionally in males but not females. This observation may have caused the reduced weight gain associated with higher doses in males.

	Male				Female			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
g/animal/day	14.1	13.0***	12.7***	13.0**	8.1	9.1**	9.6***	9.0**

Hematology: No effect
Clinical chemistry: No effect
Urinalysis: No effect
Gross pathology: No effect
Organ weights: No effect
Histopathology: No effect
Toxicokinetics: LdT was absorbed rapidly with T_{max} values ranging from 1 to 2 hours. There were no gender differences in C_{max} and AUC values. No accumulation was observed following multiple dosing since all of the toxicokinetic parameters measured remained similar between days 1 and 28. The AUC values increased dose proportionally between doses of 500 and 1000 mg/kg/day but less than dose proportional between 1000 and 2000 mg/kg/day suggesting that saturation of absorption was reached at the high dose.

		Male			Female		
Dose (mg/kg/day)		**500**	**1000**	**2000**	**500**	**1000**	**2000**
T_{max} (hrs)	Day 1	1	2	2	1	2	2
	Day 28	2	2	2	1	2	2
C_{max} (µg/ml)	Day 1	15.2 ± 3.7	29.1 ± 4.5	33.2 ± 6.7	16.5 ± 2.6	26.3 ± 5.7	36.4 ± 3.84
	Day 28	20.9 ± 4.64	34.5 ± 5.7	53.7 ± 12.0	21.2 ± 1.5	31.7 ± 7.9	35.4 ± 4.7
$AUC_{0\text{-}24h}$ (µg-hr/ml)							
	Day 1	83.8 ± 23.4	158 ± 45.8	244 ± 66.4	107 ± 11.5	176 ± 41.0	259 ± 56.3
	Day 28	124 ± 25.5	223 ± 30.4	390 ± 105	129 ± 27.2	240 ± 75.3	267 ± 58.8
$t_{1/2}$ (hrs)	Day 1	8.8 ± 1.8	7.2 ± 2.4	10.1 ± 5.1	10.3 ± 3.8	7.7 ± 0.4	12.1 ± 8.9
	Day 28	13.4 ± 2.5	20.5 ± 17.6	15.7 ± 6.4	22.6 ± 12.7	14.0 ± 6.8	15.9 ± 5.6

Study title: Nine-month chronic oral gavage toxicity study in cynomolgus monkeys with a two-month recovery

Key study findings: Doses of 0, 250, 500, and 1000 mg/kg/day were administered to cynomolgus monkeys for 3 to 9 months. A group of animals was allowed to recover for 2 months after 9 months of drug administration. Except for the dose-related increase in the incidence of erythema of skin and soft feces which increased with the length of dosing, no effect was associated 9-month treatment of LdT in cynomolgus monkeys. The NOAEL for this study is 1000 mg/kg/day.

Study no.: 02-TX-021

Volume #: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\GVAW-126

Conducting laboratory and location: [redacted]

Date of study initiation: Nov. 15, 2000

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: NV-02B, lot #'s: LT.0.005E, LT.0.006E, LT.1.001E, LT.1.002E, LT.1.004E, & LT.1.003E, purity by uv: [redacted] (different from those listed in the certificate of analysis for the batch number, amount, & date received)

Methods

Doses: 0, 250, 500, and 1000 mg/kg/day

Duration of dosing: Nine months with 3 months interim sacrifice and 2 months drug-free recovery

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 3 for interim sacrifice and 4 for 9-month sacrifice

Route, formulation, volume, and infusion rate: Oral gavage using 5 ml/kg dosing solution with 0.5% carboxymethylcellulose as vehicle

Satellite groups used for toxicokinetics or recovery: Two additional animals/sex/dose for the control and high dose groups were allowed to recover drug-free for 2 months

Age: Unspecified

Weight: 1.8-2.1 kg

Sampling times: None

Unique study design or methodology: None

Observations and times:

Mortality: Twice daily during dosing period

Clinical signs: Weekly

Body weights: Once during pretest, weekly during the dosing period

Food consumption: Daily

Physical examinations: Pretest, 3 months, 9 months, and 11 months

Ophthalmoscopy: Pretest, weeks 11, 39, and prior to necropsy for recovery animals

EKG: Not done

Hematology: Pretest, 3, 9, and 11 months necropsy on all animals

Clinical chemistry: Pretest, 3, 9, and 11 months necropsy on all animals

Urinalysis: Pretest, 3, 9, and 11 months necropsy on all animals

Gross pathology: Three, nine, and eleven months

Organ weights: See histopathology table

Histopathology: On control and high dose animals only

Adequate Battery: Yes

Peer review: yes ( ), no (X)

Results

Mortality: None

Clinical signs: A dose-related increase in the incidence of soft feces, erythema of skin and fur loss was observed. The incidence rate for soft feces increased with the length of dosing.

Body weights: No effect

Food consumption: No effect

Ophthalmoscopy: No effect

Hematology: No effect

Clinical chemistry: No effect

Urinalysis: No effect

Gross pathology: No effect.
Organ weights: No effect.
Histopathology:

	Males						Females					
	3 Month		9 Month		Recovery		3 Month		9Month		Recovery	
	0	1000	0	1000	0	1000	0	1000	0	1000	0	1000
Spinal Cord – Hemorrhage												
# affected	2	3	4	3	1	2	1	2	3	4	1	1
Mean severity	1.5	1.0	1.0	1.0	1.0	1.5	2.0	1.5	1.0	1.0	1.0	2.0
Axonal swelling												
# affected	0	0	0	1	0	0	0	0	0	0	0	0
Mean severity	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sciatic nerve – Degeneration												
# affected	0	0	0	0	0	0	0	2	0	1	0	0
Mean severity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	1.0	0.0	0.0

Minimal to mild sciatic nerve degeneration was seen at the 3-month necropsy in 2 high dose females. However, after 6 additional month of drug administration, only one high dose female still had this lesion at minimal severity. In addition, it's seen only in females. Thus, it's unlikely that the sciatic nerve degeneration was related to LdT toxicity.

Spinal cord hemorrhage was seen in most of the control and high dose animals of both sexes. The severity ranged from minimal to mild. There was no temporal pattern for the development of this lesion. The results showed that the longer the animals were treated (regardless whether the treatment was with vehicle control or LdT), the more likely spinal cord hemorrhage would develop. The severity of this finding did not increase with the duration of treatment. In males, slightly higher number of high dose animals had spinal cord hemorrhage than the control ones in the three-month data. However, after 9 months of continuous administration, less high dose animals exhibited this finding. After 2 month of drug free recovery, one more high dose animal than the control had this lesion at slightly higher severity (mild vs. mild to moderate) score. This same pattern was not seen in females. The high dose female group had more animals with or higher severity score for this histopathological finding than the concurrent control group. But the severity of this finding actually decreased with longer LdT treatment. The results suggested that this finding was incidental and not related to LdT treatment.

Axonal swelling was seen in one high dose male in the three-month treatment group and one high dose male in the 9-month treatment group. The severity scores for this finding went from minimal to mild for the 3- and 9-month treatment. Since it occurred in one sex only, one animal only, and at minimal to mild severity, this finding is unlikely to be related to LdT treatment.

Histopathology inventory

Study	0510032	GAW-105	02-TX-022	02-TX-28	02-TX-30	580155	580156	GVAW-107	02-TX-021
Species	Rats	Rats	Rats	Mice	CB6F1 mice	Monkeys	Monkeys	Monkeys	Monkeys
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X	X	X	X	X
Bone marrow smear	X (sternum)	X (sternum)	X (femur)	X (sternum)	X (sternum)	X (sternum)	X (sternum)	X (femur)	X (sternum)
Bone	X (femur)	X (sternum)		X (femur)	X (femur)	X (femur)	X (femur)	X (rib)	X (femur)
Brain	X*	X*	X*	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X	X	X	X
Cervix	X*		X	X	X				X
Colon	X	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X	X
Epididymis	X	X*	X*	X*	X*	X*	X*	X*	X*
Esophagus	X	X	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X	X	X
Fallopian tube									
Gall bladder			X	X*	X	X	X	X	X
Gross lesions	X	X	X	X	X	X	X	X	X
Harderian gland	X	X		X	X				
Heart	X*	X*	X*	X	X*	X*	X*	X*	X*
Hyphophysis									
Ileum	X	X	X	X	X	X	X	X	X
Injection site	X						X		
Jejunum	X	X	X	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lachrymal gland	X		X			X	X	X	X
Larynx	X								X
Liver	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lungs	X	X*	X*	X*	X*	X	X	X*	X*
Lymph nodes, cervical									
Lymph nodes, submandibular		X	X		X			X	
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X	X	X	X
Nasal cavities									
Optic nerves	X				X		X		X
Ovaries	X*	X*	X*	X	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X	X	X	X
Parathyroid	X*	X	X*	X*	X*	X*	X*	X	X*
Peripheral nerve	X								
Pharynx									
Pituitary	X*	X*	X*	X*	X*	X*	X*	X*	X*
Prostate	X*	X	X	X*	X*	X*	X*	X	X
Rectum	X	X	X	X	X	X	X	X	X
Salivary glands	X	X*	X*	X*	X*	X	X	X*	X*
Sciatic nerve		X	X	X	X	X	X	X	X
Seminal vesicles	X	X	X	X*	X*	X*	X*	X	X
Skeletal muscle	X	X	X	X	X	X	X	X	X
Skin	X	X	X	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X	X	X	X
Spleen	X*	X*	X*	X	X*	X*	X*	X*	X*
Sternum	X			X	X	X	X		
Stomach	X	X	X	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*	X	X	X	X*
Thyroid	X*	X*	X*	X*	X*	X*	X*	X*	X*
Tongue	X	X	X	X	X	X	X	X	X
Trachea	X	X	X	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*	X	X	X*	X*
Vagina	X	X	X	X	X	X	X	X	X
Zymbal gland									
Knee joint	X								
Lymph node, mandibular	X			X		X	X		X

X, histopathology performed
* organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Evaluation of a test article in the Salmonella typhimurium/Escherichia coli plate incorporation mutation assay in the presence and absence of induced rat liver S-9

Key findings: Concentrations of LdT ranged from 5 to 5000 µg/plate were tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 /*Escherichia coli* strain WP2uvrA in the presence and absence of S9 activation. Under the conditions tested, LdT displayed no mutagenic potential.

Study no.: GVAW-116

Volume: m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\nc-nv-02b-015, pp. 1-104

Conducting laboratory and location: [redacted]

Date of study initiation: 8/23/1999

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot #, and % purity: NV-02B, lot # 7/22/99, [redacted] pure

Methods

Strains/species/cell line: *Salmonella* strains TA98, TA100, TA1535, and TA1537 and *E. coli* tester strain WP2uvrA

Doses used in definitive study: 50, 100, 500, 1000, and 5000 µg/plate L-dT

Basis of dose selection: Range finding test using strains TA100 abd WP2uvrA indicated that relative cloning efficiency ranged from 107% to 82% for LdT concentrations of 5 to 5000 µg/plate in the absence of S-9 activation and was basically 100% for the same LdT concentrations in the presence of S-9 mix. No precipitation of LdT was observed at the highest concentration tested, therefore, the maximum LdT concentration for the definitive test was set at 5000 µg/plate.

Negative controls: DMSO

Positive controls: 2-aminoanthracene (with S9 mix), sodium azide (without S9 mix in TA 1535 and TA 100), 9-aminoacridine (without S9 mix in TA 1537), 2-nitrofluorene (without S9 mix in TA 98), and methyl methanesulfonate (without S9 mix in WP2uvrA) were used as positive controls. All of the substances, except sodium azide and methyl methanesulfonate, but including L-dT were dissolved in dimethyl sulfoxide (DMSO). Sodium azide and methyl methanesulfonate were dissolved in sterile water.

Incubation and sampling times: Incubation of bacteria with test article was done at 37°C for 66.5 hours.

Results

Study validity: Two plates per concentrations were used. At the end of the incubation period, the plates were examined for precipitation and revertant colonies counted three times per plate using automatic colony counter. A response was considered positive if there is a doubling of revertants as compared to the concurrent vehicle control for strains TA98 or TA100 and a 3-fold increase for tester strains TA1535, TA1537, or WP2uvrA in at least one dose.

Study outcome: Two confirmatory assays were performed in addition to the definitive assay using a different lot of *E. Coli* strain WP2uvrA with S9 activation only because the positive control 2-aminoanthracene did not give an appropriate response in the definitive assay. At the top dose of 5000 µg/plate, no reduction in background lawn

was observed for all tester strains with or without S9 activation. Under the conditions described, L-dT was not mutagenic in any of the concentrations tested.

Study title: Test for chemical induction of chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells with and without metabolic activation
Key findings: LdT was tested for its ability to induce chromosome aberrations in cultured CHO cells at concentrations of 100, 500, 1000, and 5000 □g/ml. No statistically significant increase in the aberrant cells was observed for any of the concentrations studied. LdT is considered negative for clastogenicity.
Study no.: GVAW-117
Volume: m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\nc-nv-02b-016, pp. 1-83
Conducting laboratory and location: [redacted]
Date of study initiation: 8/23/1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: NV-02B, lot # 7/22/99, [redacted], pure
Methods
Strains/species/cell line: The clone CHO-W-B1 of the cultured Chinese hamster ovary (CHO) cells line
Doses used in definitive study: 100, 500, 1000, and 5000 μg/ml LdT
Basis of dose selection: An aliquot of cells was obtained after cell harvest and counted using an electronic cell counter. The relative cell growth (RCG) for each concentration was calculated by dividing number of cells in test flask by the number of cells in solvent flask then multiplying by 100. The concentration that caused greater than 50% reduction in RCG was selected as the highest test concentration for the Chromosome Aberration Assay. Three additional concentrations were also evaluated. In a dose range finding test, concentrations of LdT ranged from 1 -5000 mg/ml did not cause any cytotoxicity, therefore, a top dose of 5000 μg/ml was used in the definitive test.
Negative controls: DMSO
Positive controls: 0.08 and 0.2 μg/ml mitomycin-C in the absence of metabolic activation; 7.5 and 12.5 μg/ml cyclophosphamide in the presence of S9 metabolic activation
Incubation and sampling times: In the presence and absence of rat liver S9 metabolic activation, cells were exposed to L-dT for 3 hours and incubated for another 15 hours with 0.1 mg/ml Colcemid present during the final 2 hours. All of the cultures were harvested 18 hours after the initiation of treatment.
Results
Study validity: Two replicate cultures per concentration were used. A definitive as well as a confirmatory assays were also performed. Cells were fixed and transferred to microslides. Five hundred cells were scored per replicate culture flask (a total of 1000 cells per concentration). The number of dividing cells was recorded. The mitotic index (MI) for each concentration was calculated by dividing the number of dividing cells from 1000 cells by 10. The relative mitotic index (RMI) was determined by dividing the test concentration MI by the vehicle control MI then multiplying by 100. When a positive response ($p < 0.05$) is indicated, the Cochran-

Armitage test was then performed for evidence of a concentration-related response. A response was considered positive only if the percentage of cells with aberrations was statistically increased over that of the solvent control and if the dose-response trend was also statistically significant.

Study outcome: Under the conditions tested, LdT at concentrations up to 5000 mg/ml was not mutagenic with or without S9 metabolic activation.

Study title: L-deoxythymidine chromosome aberration test

Key findings: LdT was tested for its ability to induce chromosome aberrations in primary lymphocytes at concentrations ranged from 5 to 2422 µg/ml (limit concentration of 0.01M per OECD guideline). No statistically significant increase in the aberrant cells was observed for any of the concentrations studied. LdT is considered negative for genotoxicity.

Study no.: IDIX-04-164 [redacted] # 960507)

Volume #, and page #: m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\960507, pp. 1-69

Conducting laboratory and location: [redacted]

Date of study initiation: 10/7/2004

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot #, and % purity: LdT, NV-02B, L-thymidine; lot # 16102001, [redacted] pure by uv assay

Methods

Strains/species/cell line: Peripheral blood samples taken from healthy, non-smoking, male donors

Doses used in definitive study: 5, 10, 20, 40, 80, 160, 320, 640, 1280, and 2422 µg/ml

Basis of dose selection: As per OECD guidelines, standard limit concentration of 0.01M (2422 µg/ml) was used as the maximum concentration.

Negative controls: DMSO

Positive controls: 0.05, 0.1, and 0.2 µg/ml Mitomycon C in the absence of S9 activation; 8, 12, and 16 µg/ml cyclophosphamide monohydrate in the presence of S9 activation

Incubation and sampling times: Four hour treatment period with or without S9 mix and a 21 hour treatment period in the absence of S9

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Duplicate cultures were used. Cells were harvested, fixed, and dropped onto microslides. At least two slides were prepared for each culture. Five hundred cells were scored per replicate culture flask (a total of 1000 cells per concentration). The number of dividing cells was recorded. The mitotic index (MI) for each concentration was calculated by dividing the number of dividing cells from 1000 cells by 10. The relative mitotic index (RMI) was determined by dividing the test concentration MI by the vehicle control MI then multiplying by 100. A response was considered positive only if the percentage of cells with aberrations was significantly ($p \leq 0.01$) increased over that of the solvent control and if the dose-response trend was also statistically significant. An assay is considered valid of the vehicle/solvent

control results lay within or close to the historical control range, while the positive control produced a significant increase in the incidence of aberrant cells compared with the concurrent control.

Study outcome: LdT did not cause any increase in chromosome aberrations and is therefore considered not genotoxic.

Study title: *In vivo* test for chemical induction of micronucleated polychromatid erythrocytes in mouse bone marrow cells

Key findings: LdT was administered orally to 5 CD-1 mice/sex/dose at single doses of 0 (DMSO), 500, 1000, and 2000 mg/kg. Cyclophosphamide (80 mg/kg) was used as the positive control. L-dT was not cytotoxic to the bone marrow cells and induced no significant increase in micronucleated polychromatic erythrocytes as compared to the vehicle control at any harvest time points. The positive control induced a statistically significant increase in the percent of MPCE. Thus, under the conditions tested, L-dT was not genotoxic.

Study no.: GVAW-118

Volume: m4\42-stud-rep\423-tox\4233-genotox\ 42332-in-vivo\nc-nv-02b-017, pp. 1-78

Conducting laboratory and location: [redacted]

Date of study initiation: 8/23/1999

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot #, and % purity: NV-02B, lot # 7/22/99, [redacted] pure

Methods

Strains/species/cell line: CD-1 mice

Doses used in definitive study: 0, 500, 1000, and 2000 mg/ml LdT (4 ml/kg)

Basis of dose selection: A range finding test where a single dose of 0 (DMSO), 10, 50, 100, 500, 1000, and 2000 mg/kg was administered to 3 mice/sex/dose. No effects on body weight or clinical signs were associated with any of the doses. A high dose of 2000 mg/kg was selected as the high dose for the definitive micronucleus study.

Negative controls: DMSO

Positive controls: 80 mg/kg cyclophosphamide (10 ml/kg)

Incubation and sampling times: Mice treated with positive control were sacrificed 24 hours following treatment while the rest of the mice were sacrificed either 24 or 48 hours after dose administration.

Results

Study validity: Five mice/sex/dose/time point were treated with vehicle and positive controls as well as 500, 1000, and 2000 mg/kg LdT. Bone marrow cells were harvested from femur bone and washed before placed on the slides. The proportion of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) among 200 erythrocytes (PCE + NCE) per animal was determined. Then, the number of micronucleated polychromatic erythrocytes (MPCE) was determined for 2000 PCE per animal. The criteria for a valid assay include: 1) average MPCE per 2000 PCE should not exceed 10 for vehicle control; 2) positive control should have statistically significant increase in the number of MPCE as compared to the vehicle control; 3) at least 5 animals from each sex must be alive at the time of sacrifice for each dose level. The results of the study met all of the criteria. The assay is considered positive

for genotoxicity if: 1) a positive dose response trend and a statistical significant increase in the number of MPCE were observed at one or more dose levels over that of the vehicle control; 2) two consecutive test doses produced a statistically significant increase in the number of MPCE in the event that there was no positive dose-response trend.

Study outcome: L-dT was not cytotoxic to the bone marrow cells and induced no significant increase in micronucleated polychromatic erythrocytes as compared to the vehicle control at any harvest time points. The positive control induced a statistically significant increase in the percent of MPCE. Thus, under the conditions tested, L-dT was not genotoxic up to a concentration of 2000 mg/ml.

2.6.6.5 Carcinogenicity

Study title: 104-week oral gavage carcinogenicity study with L-Thymidine (LdT) in rats
Key study findings:

LdT was not considered oncogenic at oral doses of 500, 1000 (for at least 95 weeks), and 2000 (for 85 weeks) mg/kg/day. The maximum tolerated dose was 1000 mg/kg/day based on the increased mortality at 2000 mg/kg/day through week 85. The major causes of deaths were neoplasms. However, no single type of tumor contributed significantly to the cause of death. There was an increased number of deaths associated with chronic progressive nephropathy at the 1000 and 2000 mg/kg/day dose groups. The severity of this histopathological change was also slightly increased in these two groups. Thus, LdT may have contributed to the progression of this spontaneous disease. This finding suggests that dose reduction may be recommended for patients who are renally impaired or have preexisting conditions that may be more susceptible to renal impairment.

Adequacy of the carcinogenicity study and appropriateness of the test model: Yes (see Appendice I & II for CAC minutes on study protocol and final reports, respectively).

Evaluation of tumor findings: Statistically significant increases were seen in the incidences of pancreas acinar cell adenoma in the high dose (2000 mg/kg/day) males and adrenal medulla pheochromocytomas and mammary gland fibroadenoma in the high dose females. The positive tumor findings were associated with a dose that clearly exceeded the maximum tolerated dose since dosing had to be terminated early. Excluding these tumor findings from the statistical analysis, no significant finding was associated with any tumor types.

Study no.: 02-TX-025
Volume #, and page #: m4\42-stud-rep\423-tox\4234-carcigen\42341-lt-stud\7245-103
Conducting laboratory and location: [redacted]

Date of study initiation: 11/15/2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: ß-L-deoxythymidine (L-deoxythymidine, LdT, NV-02B), lot #'s LT.1.005E ([redacted] pure by U.V.), LT.R.001E ([redacted] pure by U.V.), LT.R.003/E [redacted] by U.V.), LT.R.002/E [redacted] LT.1.006E [redacted], 16102001 ([redacted] by U.V.)

CAC concurrence: Yes

Methods

Doses: 0, 500, 1000, and 2000 mg/kg/day

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

Species/strain: :CD®(SD) IGS BR Sprague-Dawley rats

Number/sex/group (main study): 65

Route, formulation, volume: Oral gavage, dissolved in 0.5% carboxymethylcellulose,

Frequency of dosing: Once a day

Duration of dosing: 85 weeks for the high dose groups, and 95 weeks for the rest of the groups

Satellite groups used for toxicokinetics or special groups:

Age: Approximately 7 weeks old

Animal housing: Individually housed in suspended, stainless-steel cages or polycarbonate cages when indicated by health conditions.

Restriction paradigm for dietary restriction studies: None

Drug stability/homogeneity: The homogeneity of the dosing formulation was determined in duplicate on weeks 1, 13 and 71. The results were within 90% to 100% of the target level. The concentration of the dosing formulation was verified every 12 weeks until week 97. The concentrations of the formulation were within 10% of the target concentration.

Dual controls employed: No

Interim sacrifices: None

Deviations from original study protocol:

1. Dosing for all surviving high dose (Group 4) animals (both males and females) was stopped on July 24, 2003 (Week 86, Day 602).
2. The following language was added on July 24, 2004 in Protocol Amendment No. 2: "If any of the control or mid dose groups, (Groups 1 or 3), male or female, reaches n=16, then all remaining groups (Groups 1-4) for that gender will be terminated. The remaining groups of the other gender (except for Group 4) will continue to be dosed until n=16 or 104 weeks, whichever comes first. If any of the low dose groups (Group 2), male or female, reaches n=16, then only that specific male or female low dose group will be terminated. The other groups (except for Group 4) will continue to be dosed until n=16 or 104 weeks, whichever comes first."
3. Terminal sacrifice date was changed to Oct. 28, 2003 for all rats which meant that the dosing duration for control, low, and mid dose groups was shortened to 97 weeks instead to 104 weeks in the original protocol.
4. A peer review of 100% of the tumors and those hyperplasias, hypertrophies, and cellular alterations that were graded as moderate or greater severity will be performed by Novartis pathologists.

Observation times

Mortality: Twice daily

Clinical signs: Detailed examined done weekly; grossly visible or palpable mass recoreded for time of onset, location, size, appearance, and progression.

Body weights: Prior to treatment, weekly for weeks 1-14, and every 4 weeks thereafter

Food consumption: Weekly for weeks 1-13 and every 4 weeks thereafter

Hematology: Blood samples collected at scheduled sacrifice; parameters evaluated to include red blood cell count, white blood cell count, and differential blood cell count.

Histopathology: Performed on all organs/tissues of all animals listed in the "histopathology inventory table for carcinogenicity studies" at the end of this section.

Peer review: yes (X), no ( )

Results

Mortality:

	Number of deaths							
	Male				Female			
Dose (mg/kd/day)	0	500	1000	2000	0	500	1000	2000
Weeks 1-26	2	0	2	5	1	2	0	1
Weeks 27-52	3	5	4	5	2	4	3	2
Weeks 53-78	18	26	24	21	24	21	24	25
Weeks 79-85	11	7	5	10	6	9	5	13
Weeks 85-95/96	5	11	14	4	10	11	11	9
Weeks 1-85	29	38	35	41	33	36	32	41
Weeks 1-95/96	40	49	49	45	43	47	43	50
P-value (one-sided) – Weeks 1-95/96								
Cox-Tarone test	-	0.033*	0.115	0.102	-	0.304	0.472	0.170
Gehan-Breslow test	-	0.038*	0.142	0.043*	-	0.289	0.429	0.199
	Cause of death							
Tumor –								
Pituitary adenoma/carcinoma	16	18	12	13	31	23	28	35
Mammary gland adenoma/carcinoma	0	0	0	0	7	12	10	6
Other tumors†	4	6	9	3	2	5	2	3
Nephropathy	4	2	8	9	0	0	0	3
Accident	2	1	5	2	0	0	1	2
Undetermined	11	19	12	13	3	4	1	1
Urinary hemorrhage /necrosis/inflame.	1	3	3	1	0	0	0	0
Others‡	2	0	0	4	0	2	1	0

* Significance at 5% level

† Deaths caused by other tumors included malignant adrenal medulla pheochromocytoma, schwanoma/ endocardial schwanoma, undifferentiated spleen sarcoma, hepatocellular carcinoma, adrenal cortical carcinoma, endocrine carcinoma, granulocytic leukemia, fibrosarcoma, oligodendroglioma, thyroid follicular cell carcinoma, mesothelioma, prostatic carcinoma, histiocytic sarcoma, lymphoma, myxosarcoma, malignant kidney mesenchymoma, uterine carcinoma, astrocytoma, squamous cell carcinoma, malignant ameloblastic odontoma, endometrial stromal sarcoma.

‡ Only one rat/sex/dose died from causes including, urinary bladder calculi, pododermatitis, sepsis, cystic kidney disease, pulmonary hemorrhage, bronchopneumonia, brain malacia/hemorrhage

Because the number of surviving animals in the high dose groups was approaching 25, dosing was stopped at week 85 for this group. Sacrifice of all groups was performed on week 96 when the number of surviving animals reached 16 for the high dose groups. Most of the deaths occurred during weeks 53-78. The mortality rate has reached statistical significance (5% one-sided) in only the high dose male group using Gehan-Breslow test but not Cox-Tarone test, suggesting that deaths due to treatment occurred earlier in the study.

The main cause of deaths from all groups was neoplasm. The most prevalent tumor associated with death was pituitary adenomas/carcinomas (only a couple of cases of carcinomas) in both males and females. A high incidence of mammary gland fibroadenomas/carcinomas as the cause of death was also seen in females. No treatment-related increases in the total incidences of tumor-related deaths or individual

incidence sorted by tumor types were apparent. A small number of deaths was caused by various tumors listed in the table. All these neoplasms occurred sporadically throughout the control and treated groups without any apparent dose-response relationship.

The only cause of death that showed increased trend with dose was chronic progressive rat nephropathy. The mortality rates attributable to this non-neoplastic lesion were 6%, 3%, 12%, & 14%, respectively, for control, 500, 1000, and 2000 mg/kg/day in males and 0%, 0%, 0%, and 5%, respectively for control, low, mid, and high dose in females. The severity of these findings also showed dose-related increases.

Other causes of death were related to accident, miscellaneous neoplasms and inflammatory lesions, or undetermined ones that cannot be attributable to any microscopic lesions. These causes occurred in all groups and distributed sporadically throughout the control and treated groups. They were not considered treatment related. The maximum tolerated dose was 1000 mg/kg/day for this study based on the high mortality rate in the 2000 mg/kg/day groups.

Clinical signs:

	Male				Female			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Hypoactive	12	12	13	13	14	19	15	22
Liquid feces	2	3	2	2	0	2	1	5
Nonformed feces	5	5	4	18	0	2	0	1
Red eye discharge	11	13	14	7	20	16	18	27
Swollen perineal area	6	8	7	6	5	9	15	13
Thin	14	15	15	10	19	17	25	26

Increased incidence of liquid or nonformed feces was seen in the high dose groups. Higher incidences of hypoactivity, red eye discharge, swollen perineal area, and thin appearance were seen in females. There was no apparent pattern to when the observations were recorded. Most of the observation resolved within a few days and did not persist throughout the study. None of the observations had any correlated macroscopic and/or microscopic findings. Therefore, they are not considered treatment related.

Body weights: No effect

Food consumption: No effect

Hematology: No effect

Gross pathology:

		Male				Female			
Dose (mg/kg/day)		**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Adrenal cortex –	Unequally sized	2	2	3	1	7	6	6	10
Kidney –	Dilated pelvis	2	3	5	9	0	4	1	1
Mammary gland –	Mass	0	0	0	0	28	37	29	36
Ovary –	Cyst	0	0	0	0	3	2	7	9
Pituitary –	Enlarged	22	23	17	17	43	47	44	50
	Mottled	11	10	10	10	25	25	28	33
Seminal vesicle –	Small	4	5	8	7	0	0	0	0

Increases in the incidence of the macroscopic findings listed above except for those in pituitary occurred in one sex (mostly females) only. Dose-related increases in the number of observable masses in the mammary gland and ovarian cyst

correlated to the microscopic findings of mammary adenoma/carcinoma and follicular cyst in the ovary, respectively. A slightly high number of enlarged pituitary glands was seen in the high dose females which also correlated to the slightly higher number of pituitary adenoma/carcinoma in the same group, though the increase did not reach a significant level. Higher number of mid and high dose males had small seminal vesicle without associated microscopic finding in the same organ. Thus, this macroscopic finding seems to pose no toxicological relevance.

Histopathology:

Non-neoplastic:

		Male				Female			
Dose (mg/kg/day)		**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Brain –									
Ventral compression	# affected	22	20	14	16	38	44	44	50
	Mean severity	2.95	2.95	2.86	3.00	2.95	2.68	2.87	2.80
Adrenal cortex –									
Thrombosis	# affected	0	2	1	1	3	1	4	6
	Mean severity	0.00	2.50	2.00	1.00	2.67	1.00	2.25	3.00
Heart –									
Cardiomyopathy	# affected	65	64	64	62	52	53	62	57
	Mean severity	1.86	1.75	2.25	2.06	1.52	1.53	1.52	1.63
Liver –									
Focal cystic degen	# affected	7	7	13	13	0	0	2	2
	Mean severity	1.43	1.00	1.08	1.08	0.00	0.00	1.00	1.00
Pigment	# affected	6	5	3	5	7	11	10	12
	Mean severity	2.17	1.83	2.00	1.60	1.43	1.45	1.30	1.67
Focal fatty change	# affected	0	2	3	3	0	0	1	0
	Mean severity	0.00	2.50	2.33	2.00	0.00	0.00	1.00	0.00
Muscle –									
Degeneration	# affected	2	3	5	0	1	1	1	0
	Mean severity	1.50	2.33	1.40	0.00	4.00	2.00	1.00	0.00
Kidney –									
Nephropathy	# affected	63	64	65	64	60	63	62	63
	Mean severity	2.6	2.5	3.0	2.8	1.3	1.3	1.4	2.0
Ovary –									
Follicle cyst	# affected	-	-	-	-	11	5	16	17
	Mean severity	-	-	-	-	2.00	1.60	2.69	2.53
Sciatic nerve –									
Degeneration	# affected	28	12	20	15	12	12	6	6
	Mean severity	1.21	1.00	1.30	1.07	1.17	1.33	1.00	1.50
Spleen –									
Pigment	# affected	28	28	32	37	52	42	44	50
	Mean severity	1.86	1.93	1.65	1.81	1.96	2.00	2.20	2.38
Sternum marrow –									
Myeloid hyperplasia	# affected	3	2	3	0	2	1	6	8
	Mean severity	2.33	2.00	2.33	0.00	2.50	3.00	2.33	2.50
Severity score: 1=minimal; 2=slight; 3=moderate; 4=moderate severe; 5= severe									

Chronic progressive nephropathy in kidneys was a common histopathological change in almost all animals including those in the control group. It also caused a dose related increase in deaths (4, 2, 8, 9 deaths for the control, 500, 1000, and 2000 mg/kg/day male groups, respectively, and 0, 0, 0, 3 deaths in the control, low, mid, and high dose females). Severity scores increased in a dose-related fashion and were slightly higher in the males than in the females. The deaths that were caused by

nephropathy occurred mostly after 75 weeks into the study. LdT treatment did not adversely affect the timing of death. However, since the number of deaths caused by nephropathy showed a dose-related increase in males and nephropathy was generally worse in male rats, there is a potential that treatment with LdT may aggravate preexisting renal dysfunction.

A large number of rats had ventral brain compression. The incidence increased with dose, but the mean severity score for this finding did not, indicating that this particular microscopic finding was probably a result of a high number of rats with pituitary adenoma/carcinoma which did not reach statistical significant level with LdT treatment. Ovarian follicular cyst correlated with the similar macroscopic finding in the same organ. Again, since no reproductive toxicity was associated with LdT treatment, the significance of this finding is questionable.

In the clinical trials, a few patients with no pre-existing conditions relating to muscle dysfunctions came down with myopathy after a year of continuous use of LdT. Thus, parameters that may be related to myopathy, like cardiomyopathy, muscle degenerations, and sciatic nerve degeneration were examined. No dose related increases in the incidence rate or severity scores were observed. It's unclear from the nonclinical toxicology results what the etiology of treatment associated myopathy is.

All the other non-neoplastic histopathological findings listed above include adrenal cortex thrombosis, focal cystic degeneration and fatty changes in liver, pigmentation in liver and spleen, and myeloid hyperplasia in bone marrow. In general, the increase in the incidence rate was small, severity scored increased less than a grade as compared to the control, and the changes affected one sex only. Thus, these findings were considered incidental unrelated to LdT treatment.

Neoplastic:

	Male				Female			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
# animals examined	65	65	65	65	65	65	65	65
Adrenal medulla –								
Pheochromocytoma	6	3	7	7	1	4	1	6
P-value (one-sided)	0.183	0.387	NA	NA	0.056	0.175	NA	0.018*
Malignant pheochromocytoma	3	0	3	0	0	0	0	0
P-value (one-sided)	0.231	0.248	NA	0.113	NA	NA	NA	NA
Pheochromocytoma/malignant								
Pheocytochromocytoma	8[a]	3	10	7	1	4	1	6
P-value (one-sided)	0270	0.206	0.195	NA	NA	NA	NA	NA
Mammary gland –								
Hyperplasia	-	-	-	-	0	0	2	2
Fibroadenoma	-	-	-	-	19	26	22	26
P-value (one-sided)	-	-	-	-	0.086	0.087	0.368	0.033*
Carcinoma	-	-	-	-	19	19	7	15
P-value (one-sided)	-	-	-	-	0.218	NA	0.368	0.369
Fibroadenoma/carcinoma	-	-	-	-	29[b]	38[b]	30[b]	35[b]
P-value (one-sided)	-	-	-	-	0.140	0.078	0.464	0.054

	Male				Female			
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
# animals examined	65	65	65	65	65	65	65	65
Pancreas –								
Acinar cell hyperplasia	5	3	4	6	0	0	1	1
Acinar cell adenoma	0	3	0	5	0	0	1	0
P-value (one-sided)	0.029	0.076	NA	0.012*	NA	NA	NA	NA
Thyroid –								
"C" cell hyperplasia	5	5	5	9	4	10	7	6
"C" cell adenoma	7	7	7	6	5	6	5	5
"C" cell carcinoma	1	0	1	1	1	0	0	1

a One rat had both the benign and malignant forms of pheochromocytoma and was counted once for statistical analysis.
b Many rats had both fibroadenoma and carcinoma and were counted once for statistical analysis
* Significance level at 5.0% level
NA – Not analyzed since the number was not two more or less than the control values

The most prevalent neoplasms in the study included pituitary adnomas, pheochromocytomas, thyroid c-cell adenomas, and mammary gland fibroadenomas and carcinomas. They are common tumor types seen in Sprague-Dawley rats. A positive dose response was observed in males for the acinar cell adenoma (p=0.0289) of pancreas, with significantly increased incidence in the 2000 mg/kg/day groups (p=0.0122). The 1000 mg/kg/day group had 0 incidence, the same as in control group. The trend was not considered significant for common tumors. In addition, even though the incidence of tumor was slightly higher than the historical range at the conducting lab (0-5% at [redacted]), it's well within the [redacted] published range of 1.43-11.43%. As a consequence, the positive trend and increases in the high dose group are not considered treatment related.

In females, no positive dose response was observed in any of the tumors. However, the incidences of benign pheochromocytoma of adrenal medulla (p=0.0175) and mammary gland fibroadenoma (p=0.0334) in the 2000 mg/kg/day were significantly higher than that of the control group. The positive tumor findings were associated with a dose that clearly exceeded the maximum tolerated dose since dosing had to be terminated early. Excluding these tumor findings from the statistical analysis, no significant finding was associated with any tumor types. Thus, LdT is not considered carcinogenic.

Study title: A 26-week oral dose carcinogenicity and toxicokinetic study of β-L-2'-deoxythymidine in CB6F1-TgrasH2 mice

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: Yes (see Appendice III & II for CAC minutes on study protocol and final report, respectively).

Evaluation of tumor findings: LdT was administered orally to TgrasH2 transgenic mice at doses of 500, 1000, and 2000 mg/kg/day. A positive control, 75 mg/kg/day N-methyl-N-nitrosourea was included in the study. While the positive control induced tumors of various types at multiple organs, high number of deaths caused by tumors, and many adverse effects resulting from the tumors, LdT did not affect any parameters monitored. The toxicokinetic results verified that the animals were exposed to LdT

systemically. The exposure at 2000 mg/kg/day was about 14 fold over that in the humans taking 600 mg LdT once a day.

Study no.: 02-TX-024

Volume #, and page #: m4\42-stud-rep\423-tox\4234-carcigen\42341-lt-stud - 046-011

Conducting laboratory and location: [redacted]

Date of study initiation: 10/30/2003

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: ß-L-2'-Deoxythymidine (LDT600-NXA.001), lot # 03830003, - pure by HPLC;

CAC concurrence: Yes

Methods

Doses: 0, 500, 1000, and 2000 mg/kg/day; 75 mg/kg N-methyl-N-nitrosourea (positive control)

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

Species/strain: CB6F1/Jic-TgrasH2@TAC (rasH2) mice

Number/sex/group (main study): 25

Route, formulation, volume: Oral gavage, dissolved in 0.5% sodium carboxymethyl-cellulose; dosing volume of 10 ml/kg

Frequency of dosing: Once a day

Duration of dosing: 26 weeks

Satellite groups used for toxicokinetics or special groups: Toxicokinetic arm with 21 mice/sex/dose at doses of 500, 1000, and 2000 mg/kg/day; blood samples collected at 0.5, 1, 2, 4, 8, and 24 hours postdosing on days 1 and 182 (a predose sample collected on this day) from 3 animals/time point/sex/dose (TK animals only)

Age: Approximately 9 weeks old

Animal housing: Individually housed in polycarbonate solid bottom cages

Restriction paradigm for dietary restriction studies: None

Drug stability/homogeneity: The bulk LdT was determined be stable (within 95% of the stated amount) for up to 156 week. The homogeneity of the dosing solutions was analyzed at weeks 1, 13, and 26. All were within 10% of the nominal concentrations.

Dual controls employed: No

Interim sacrifices: None

Deviations from original study protocol:

1. Weight of pituitary gland was not collected because of its small size and excessive damage from handling.
2. A histopathological monitoring review was added and performed by Dr. Judith Markovits, Novartis Pharmaceuticals Corporation, East Hanover, NJ.
3. A dosing solution was determined to be 123% of the nominal concentration and was used to dose animals for 4 days before a new solution was prepared. The short duration and small amount of overdose probably did not adversely affect the results of the study.

Observation times

Mortality: Once daily

Clinical signs: Once daily; grossly visible or palpable mass recorded for time of onset, location, size, appearance, and progression.
Body weights: Prior to treatment and weekly thereafter
Food consumption: Weekly
Gross pathology: Week 27
Organ weight: At necropsy for adrenals, brain, epididymides, heart, kidneys, liver, lung/bronchi, ovaries, prostate/seminal vesicle, salivary glands (submandibular/ sublingual), spleen, testes, thymus, and uterus
Histopathology: Performed on the organs/tissues (listed under the "histopathology inventory for carcinogenicity studies" table at the end of this section) of all vehicle controls, 2000 mg/kg/day group, and positive control groups and Harderian glands, spleen, urinary bladder, and lung for low dose animals, and all of these tissues plus thymus, mesenteric lymph node, left mandibular lymph node, and stomach for mid dose animals.
Peer review: yes (X), no ( )

Results

Mortality:

	Male					Female				
Dose (mg/kg/day)	**0**	**500**	**1000**	**2000**	**+ control**	**0**	**500**	**1000**	**2000**	**+ control**
# of mice										
Main study	25	25	25	25	25	25	25	25	25	25
TK study	0	21	21	21	0	0	21	21	21	0
# of Death										
Main study	1	0	0	2	20	5	0	2	2	16
TK study	-	0	1	2	-	-	0	1	0	-
Death day										
Main study	178	-	-	95, 111	*	121, 134,	-	20, 64	51, 66	**
TK study	-	-	156	124, 141	-	157, 167, 177	-	180	0	-
Tumor-related death										
Main study	1	0	0	1	2	1	0	0	0	15
TK study	-	0	1	1	-	-	0	0	0	-

* Deaths occurred at days 85, 88, 103, 104, 105, 111, 119, 122, 129, 136, 138, 143, 144, 150, 154, 156, 162, 171, and 175.
** Deaths occurred at days 70, 80, 85, 96, 112, 114, 123, 130, 133, 136, 152, 154, 161, 168

There was a slightly increased number of deaths in the male high dose group. However, no difference was seen in the number of deaths attributable to tumors across the treatment groups. The positive control groups showed 80 and 64% mortality rates in males and females, respectively. At least a half of the deaths were attributable to palpable masses.

Clinical signs: No treatment effect
Body weights: No treatment effect
Food consumption: No treatment effect
Gross pathology: No treatment effect, though many macroscopic with correlated microscopic findings were seen in the positive control groups
Organ weights: No effect, though the relative liver, spleen, heart (males), kidney (males), salivary gland (female) weights in the positive control groups were increased.
Histopathology:

Non-neoplastic: No differences in the non-neoplastic findings were seen between the vehicle control and the high dose groups in both sexes

Neoplastic: No differences in the neoplastic findings were observed between the vehicle control and the high dose groups in both sexes. The positive control animals had multiple tumor types in various organs/tissues.

Toxicokinetics:

	Male			Female		
Dose (mg/kg/day)	**500**	**1000**	**2000**	**500**	**1000**	**2000**
T_{max} (hr)						
Day 1	0.5	0.5	0.5	0.5	0.5	0.5
Day 182	0.5	1.0	1.0	0.5	1.0	1.0
C_{max} (µg/ml)						
Day 1	58.8	89.8	103	72.1	117	129
Day 182	45.1	62.1	79.2	43.9	97.8	131
AUC_{0-24h} (hr*µg/ml)						
Day 1	126	166	244	149	227	347
Day 182	102	176	382	106	178	384
AUC_{0-24h}/dose						
Day 1	0.252	0.166	0.122	0.299	0.227	0.174
Day 182	0.204	0.176	0.191	0.212	0.178	0.192

The pharmacokinetic profile for LdT did not change with 26 weeks of dosing as compared to the 4 week treatment duration. The C_{max} and AUC values still increased less than dose-proportionality, though the values were about one fold less those in the previous 4 week dose ranging study that used the wild-type strain for the rasH2-transgenic m. There were generally no gender differences in exposure nor accumulation or reduction of AUC values over the duration of the study. The results indicated that the animals in the study did have systemic exposure to LdT. The exposure at 2000 mg/kg/day was about 14 fold over that in the humans taking 600 mg LdT once a day.

Histopathology Inventory for Carcinogenicity Studies

Study	02-TX-025	02-TX-024
Species	Rats	Mice
Adrenals	X	X*
Aorta	X	X
Bone Marrow smear	X (femur & sternum)	X (sternum)
Bone	X (femur)	X (femur w/ knee joint)
Brain	X	X
Cecum	X	X
Cervix	X	
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions/tumors	X	X
Harderian gland		X
Heart	X	X*
Hyphophysis		
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X	X*
Lachrymal gland		X
Larynx		
Liver	X	X*
Lungs	X	X*
Lymph nodes, cervical		
Lymph nodes, mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavities		
Optic nerves		X
Ovaries	X	X*
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X	X
Prostate	X	X*
Rectum	X	X
Salivary glands	X	X*
Sciatic nerve	X	X
Seminal vesicles	X	X*
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X	X*
Sternum	X	X
Stomach	X	X
Testes	X	X*
Thymus	X	X*
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X*
Vagina	X	X
Zymbal gland		X
Bronchus		X
Clitoral glands	X	X
Preputial glands	X	X

* organ weight obtained

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Combined oral (gavage) fertility study and development toxicity study of L-thymidine in rats

Key study findings: The study design is a combined Segments I and II reproductive toxicity study. Doses of 0, 100, 500, and 1000 mg/kg/day L-dT were administered to both male and female rats during premating, cohabitation, and gestation. Except for the statistical significant increase in the incidence of soft or liquid feces in F_0 males and decreased in the fertility index for the mid and high dose F_0 groups, no other parameters examined were affected. The observation of soft or liquid feces occurred in males only thus its toxicological significance is unclear. The decrease in fertility index may also have limited significance since the number is within the testing facility's historical control range and no other mating/fertility parameters were affected.

Study no.: 02-TX-023

Volum: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\1314-001

Conducting laboratory and location: [redacted]

Date of study initiation: 4/10/01

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot #, and % purity: L-dT/NV-02B, lot # LT.1.003E, [redacted] pure by uv

Methods

Doses: 0, 100, 500, 1000 mg/kg/day

Species/strain: [redacted] CD®(SD)IGS BR VAF/Plus®

Number/sex/group: 25

Dosing duration: Males: 28 days before cohabitation until sufficient females were Caesarean-sectioned; Females: 15 before cohabitation until gestation day 17

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in aqueous 0.5% carboxymethylcellulose solution, with dosing volume of 10 ml/kg

Satellite groups used for toxicokinetics:

Study design: Within each dose group, one male rat was assigned using consecutive order for cohabitation per one female rat. Females were considered mated and started the clock of gestation day 1 if spermatozoa was observed in a vaginal smear and/or a copulatory plug *in situ*. They will be assigned individual housing. The maximum cohabitation period allowed was 21 days but all females in this study mated within 14 days.

Parameters and endpoints evaluated:

Mortality (F_0 genretaion): Twice daily

Clinical observation (F_0 generation): Weekly during acclimation period and daily during dosing period made within 60 minutes of dosing

Body weights (F_0 generation): Weekly during acclimation period, daily during dosing and postdosing (female rats only)

Food consumption (F_0 generation): Weekly during dosing period except during cohabitation for males; weekly to cohabitation and on gestation days 0, 7, 14, 18, and 21 for females

Estrous cycling (dam): Daily starting on 14 days before dosing until mating was confirmed.

Necropsy: F_0 Males: Performed after completion of cohabitation period and after sufficient females were Caesarean-sectioned.
Organ weight: Right testis, left testis, left epididymis, right epididymis, seminal vesicles and prostate
Sperm motility: Samples collected from left vas deferens and evaluated by the Hamilton Thorn IVOS
Sperm concentration: Determined from a homogenate of left cauda epididymis.
Histopathology: The leftover left epididymis, right epididymis, prostate, and seminal vesicles of the control and high dose animals only

F_0 Females: Caesarean-section: Performed on gestation day 21
Gross necropsy: Thoracic, abdominal, and pelvic viscera
Histopathology: *Uterus*: Pregnancy, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses; *Ovaries*: Number of corpora lutea; *Placenta*: Examined for abnormalities in size, color, or shape

Fetuses: Clinical observation, body weight, litter number, uterine distribution, sex, gross external alterations, soft tissue alterations in one-half of fetuses, and skeletal alterations in the other half

Results

Mortality: One mid dose male and one control female died from intubation error.

Clinical signs: A statistically significant increase in the incidence of soft feces was seen in the males only. The significance of this observation is unclear since it was observed only in males.

	Male				Female			
Dose (mg/kg/day)	**0**	**100**	**500**	**1000**	**0**	**100**	**500**	**1000**
Soft or liquid feces	11/5	3/3	15/12	19/13*	0	0	0	0
Number of days with observation/# animals with the observation								

Body weight: No effect

Food consumption: No effect

Estrous cycling: No effect

Necropsy: No effect

Organ weights: No effect

Sperm motility: No effect

Sperm concentration: No effect

Histopathology: No effect

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

Mating index: No effect

Fertility index: Decreased fertility index (number of pregnancies/number of rats mated) was seen in the 500 (76%) and 1000 mg/kg/day (72%) groups as compared to the control (92%). However the number is within historical control of the testing lab.

Corpora lutea: No effect
Pre-implantation loss: No effect
Post-implantation loss: No effect
Litter sizes: No effect
% Resorption/litter: No effect
Number of live fetuses: No effect
Sex distribution: No effect
Fetal body weights: No effect
Fetal gross external alterations: No effect
Fetal soft tissue alterations: No effect
Fetal skeletal alterations: No effect

Study title: Oral (gavage) fertility and general reproduction toxicity study of L-thymidine in male rats
Key study findings: In the previous fertility and development, decreased fertility index was associated with L-dT doses higher than 500 mg/kg/day. No other reproductive toxicity was seen in that study. In order to investigate if the effect came from drug exposure in males, only male rats were dosed with 1000 and 2000 mg/kg/day L-dT. Except for a slight increase in food consumption in the 2000 mg/kg/day male group, no other effects were associated with the oral administration of L-dT.
Study no.: 02-TX-021
Volume: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\1314-005
Conducting laboratory and location: [redacted]
Date of study initiation: 4/9/02
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L-dT, lot # LT.R.003E, [redacted] pure by uv
Methods
Doses: 0, 1000, and 2000 mg/kg/day
Species/strain: [redacted] :CD®(SD)IGS BR VAF/Plus®
Number/group: 25 males/dose received vehicle or L-dT
Dosing duration: 28 days before cohabitation until sufficient females were Caesarean-sectioned.
Route, formulation, volume, and infusion rate: Oral gavage, dissolved in aqueous 0.5% carboxymethylcellulose solution, with dosing volume of 10 ml/kg
Satellite groups used for toxicokinetics: None
Study design: Within each dose group, one male rat was assigned using consecutive order for cohabitation per one female rat. Females were considered mated and started the clock of gestation day 0 if spermatozoa was observed in a vaginal smear and/or a copulatory plug *in situ*. They will be assigned individual housing. Male rats that did not mate a female within the first 14 days of cohabitation were assigned an alternate female rat and remained in cohabitation for a maximum of three additional days.
Parameters and endpoints evaluated:
Mortality: Twice daily

Clinical observation: Weekly during acclimation period and daily during dosing period made within 60 minutes of dosing for males; weekly during acclimation period, prior to mating, and on gestation days 0, 7, 10, and 13

Body weights: Weekly during acclimation period, daily during dosing, and at sacrifice for males; weekly during acclimation, prior to mating, on gestation days 0, 7, 10, and 13, and at sacrifice for females

Food consumption: Weekly during dosing period except during cohabitation for males; On gestation days 0, 7, 14, 18, and 21 for females

Estrous cycling: Daily starting on 14 days before dosing until mating was confirmed

Necropsy: Males: Performed after completion of cohabitation period and after sufficient females were Caesarean-sectioned.

Organ weight: Right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid), and prostate

Sperm motility: Samples collected from left vas deferens and evaluated by the [redacted]

Sperm concentration: Determined from a homogenate of left cauda epididymis.

Histopathology: The leftover left epididymis, right epididymis, prostate, and seminal vesicles of the control and high dose animals only

Females: Caesarean-section: Performed on gestation day 13

Gross necropsy: Thoracic, abdominal, and pelvic viscera

Histopathology: *Uterus*: Pregnancy, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses; *Ovaries*: Number of corpora lutea; *Placenta*: Examined for abnormalities in size, color, or shape

Results

Mortality: None

Clinical signs: No dose-related or statistically significant changes

Body weight: No effect

Food consumption: A slight but statistically significant increase in food consumption was observed in the high dose males throughout the dosing period.

Estrous cycling: No effect

Necropsy: No effect

Organ weights: No effect

Sperm motility: No effect

Sperm concentration: No effect

Histopathology: No effect

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

Mating index: No effect

Fertility index: No effect

Corpora lutea: No effect

Implantation: No effect

Dams with nonviable embryos: No effect

% nonviable embryos/litter: No effect
Normal placenta: No effect

Study title: Oral (gavage) fertility and general reproduction toxicity study of L-thymidine in female rats
Key study findings: In a previous combined fertility and developmental reproductive toxicity study, decreased fertility index was associated with LdT doses of 500 mg/kg/day and higher. A fertility study was performed where only males were exposed to L-dT at doses of 1000 and 2000 mg/kg/day. No effect on fertility or mating indices or parameters was observed. A similar study was performed here where only females were exposed to L-dT at 2000 mg/kg/day. Except for food consumption and the corresponding body weight increases and a slight increase in the estrous stage during the precohabitation period, no other parameters were affected by the L-dT administration. The results of this and the previous studies indicated that the observed decrease in fertility index in the early study was probably spurious and no concern should be raised.
Study no.: 02-TX-022
Volume: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\1314-006
Conducting laboratory and location: [redacted]
Date of study initiation: 4/9/02
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L-dT, lot # LT.R.003E, [redacted] pure by uv
Methods
Doses: 0 and 2000 mg/kg/day
Species/strain: [redacted] CD®(SD)IGS BR VAF/Plus®
Number/group: 25 females/dose; males used as breeders only
Dosing duration: 15 days before cohabitation (maximum 14 days) to gestation day 7
Route, formulation, volume, and infusion rate: Oral gavage, dissolved in aqueous 0.5% carboxymethylcellulose solution, with dosing volume of 10 ml/kg
Satellite groups used for toxicokinetics: None
Study design: Female rats assigned to cohabitate with breeder male, one per female. Females were considered mated and stated the clock of gestation day 0 if spermatozoa was observed in a vaginal smear and/or a copulatory plug *in situ*. If the female rats did not mate within the first 7 days of cohabitation, an alternate male rat that had mated was assigned and remained in cohabitation for a maximum of 7 additional days. Female rats that did not mate after the completion of the 14-day cohabitation period were considered to be at gestation day 0 and assigned to individual housing.
Parameters and endpoints evaluated:
Mortality: Twice daily
Clinical observation: Weekly during acclimation period and daily during dosing period made within 60 minutes of dosing, and daily during postdosing period
Body weights: Weekly during acclimation period, daily during dosing and postdosing period
Food consumption: Weekly to cohabitation and on gestation days 0, 7, 8, 10, and 13

Estrous cycling: Daily starting on 12 days before dosing, for 5 days during dosing prior to cohabitation, and until confirmation of mating
Necropsy: Caesarean-section: Performed on gestation day 13
Gross necropsy: Thoracic, abdominal, and pelvic viscera
Histopathology: *Uterus*: Pregnancy, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses; *Ovaries*: Number of corpora lutea; *Placenta*: Examined for abnormalities in size, color, or shape

Results
Mortality: None
Clinical signs: No dose-related or statistically significant observations
Body weight: Body weight gains were significantly higher in the 2000 mg/kg/day group as compare to the control during days 1-8 in the precohabitation period. No effect was seen during the gestation period.
Food consumption: The 2000 mg/kg/day group also had higher feed consumption corresponding to the increased body weight gain observed in the same period.
Estrous cycling: The number of precohabitation estrous stages was significantly increased (1.7±0.4 vs. 1.4±0.5 in control) in the 2000 mg/kg/day dose group. Since all other mating and fertility parameters were unaffected, the change is not considered biologically relevant.
Necropsy: No effect
Organ weights: No effect
Histopathology: No effect
Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
Mating index: No effect
Fertility index: No effect
Corpora lutea: No effect
Implantation: No effect
Litter size: No effect
% nonviable embryos/litter: No effect

Embryofetal development

Study title: Oral (stomach tube) developmental toxicity study of L-thymidine in rabbits
Key study findings: Time-mated New Zealand white rabbits were orally administered L-dT at 0, 50, 250, and 1000 mg/kg/day. Signs of maternal toxicity were evident at 1000 mg/kg/day, including abnormal feces, alopecia, ungroomed coat, decreased food consumption and body weight gain, and abortion. The fetuses from the aborted or early sacrificed dams did not show any alterations in soft tissues, skeletons or ossification states. There were also no effects on any fetal parameters monitored. The no effect level for fetuses was 1000 mg/kg/day and 50 mg/kg/ for dams.
Study no.: 02-TX-019
Volume: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352- embryo-fetal-dev\1314-002
Conducting laboratory and location: [redacted]
Date of study initiation: 6/1/01
GLP compliance: yes

QA reports: yes (X) no ( )
Drug, lot #, and % purity: L-dT, lot # LT.1.003E, [redacted] pure & LT.1.004E, [redacted] pure by uv
Methods
Doses: 0, 50, 250, and 1000 mg/kg/day
Species/strain: Time-mated female rabbits/Hra:(NZW)SPF
Number/ group: Twenty
Dosing duration: Gestation days 6 to 18
Route, formulation, volume, and infusion rate: Oral stomach tubing, dissolved in aqueous 0.5% carboxymethylcellulose solution, with dosing volume of 10 ml/kg
Satellite groups used for toxicokinetics: None
Study design: The mated rabbits were shipped to the testing facility to arrive on gestation days 1, 2, and 3 and administered vehicle or L-dT on gestations days 6-18.
Parameters and endpoints evaluated:
Mortality: Twice daily
Clinical observation: Weekly during acclimation period and daily during dosing period made within 60 minutes of dosing
Body weights: Gestation day 0, the day of arrival, daily during dosing and postdosing
Food consumption: Daily
Necropsy: Females: Caesarean-section: Performed on gestation day 29
Gross necropsy: Thoracic, abdominal, and pelvic viscera
Histopathology: *Uterus*: Pregnancy, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses; *Ovaries*: Number of corpora lutea; *Placenta*: Examined for abnormalities in size, color, or shape
Fetuses: Clinical observation, body weight, litter number, uterine distribution, sex, gross external alterations, soft tissue alterations, and skeletal alterations

Results
Mortality (dams): One dam received 1000 mg/kg/day L-dT died on gestation day 14 of intubation error. This dam had 10 conceptuses whose viability could not be determined at the time of dam's death.
Abortions:

Animal #	Dose (mg/kg/day)	Abortion on GD* #	Dam's clinical signs	Body wt Status	Food consumption	# Fetuses	Fetal status
6261	250	23	None	0.5 kg wt loss on GD 23	Little feed consumption	1 early & 4 late resorptions	N**
6287	1000	28	Ungroom coat on GD 8-19; localized alopecia on GD 26-27; soft/ liquid /scant/no feces on GD 8, 15-18, 20-27; ungroomed coat	1 kg wt loss on GD 27	Little feed consumption on GD 27	7 dead fetuses	N
6291	1000	29	Delivery on GD 29; soft/liquid/scant/no feces on GD 19-29; ungroomed coat	0.4 kg wt loss on GD 15-29	Little feed after GD 14	8 live pups	N

Animal #	Dose (mg/kg/day)	Abortion on GD* #	Dam's clinical signs	Body wt Status	Food consumption	# Fetuses	Fetal status
6292	1000	29	Delivery on GD 29; soft/liquid/scant/dry/no feces on GD 19-29	0.5 kg wt loss on GD 18-29	Little feed on GD 18-29	10 live pups	N
*GD = gestation day ** N = Normal							

Four abortions were observed in the high dose group. Most of the abortions occurred towards the end of dosing period. Three out of the four dams had abnormal feces throughout most of the dosing period. All of the dams consumed little feed before or around the time of abortion. The data suggested that the 1000 mg/kg/day caused frank maternal toxicity that led to abortion.

Clinical signs (dams):

Dose (mg/kg/day)	**0**	**50**	**250**	**1000**
# examined/group	20	20	20	20
Scant feces	2/2†	0/0	6/2	62/10**
Soft/liquid feces	6/2	6/3	2/1	16/5
No feces	0/0	0/0	0/0	13/5**
Dried feces	0/0	0/0	0/0	10/4**
† Number represents total #'s of observations/# of rabbits with the observation * $p < 0.05$ ** $p < 0.01$				

The incidence of abnormal feces was significantly higher in the 1000 mg/kg/day group. There were also increased incidences of alopecia and ungroomed coat. But these observations occurred in the two dams that were sacrificed early. It's clear that maternal toxicity was evident in the 1000 mg/kg/day group.

Body weight (dams):

Dose (mg/kg/day)	**0**	**50**	**250**	**1000**
# weighed/group	20	20	20	17†
Wt change (Kg)				
GD 15-19	0.06 ± 0.05	0.07 ± 0.04	0.04 ± 0.07	-0.04 ± 0.11*
GD 6-29	0.42 ± 0.16	0.47 ± 0.15	0.43 ± 0.15	0.25 ± 0.35
† Exclude those that were sacrificed early * $p < 0.05$				

There was weight loss between gestation days 15-19 in dams that received 1000 mg/kg/day L-dT even after excluding the numbers from those 3 dams that were sacrificed early. This dose is clearly a maternal toxic dose.

Food consumption (dams):

Dose (mg/kg/day)	**0**	**50**	**250**	**1000**
# averaged/group	20	20	20	17†
Food consumption (g/day)				
GD 15-19	170.4 ± 16.2	175.4 ± 12.2	151.5 ± 61.6	96.5 ± 70.0*
GD 6-29	154.2 ± 21.8	157.9 ± 15.7	151.0 ± 26.0	122.4 ± 43.9
† Exclude those that were sacrificed early * $p < 0.05$				

Reduced food consumption occurred during the same gestation days as the decreased body weight gain and may be the cause of the observation.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): No effect

Offspring (malformations, variations, etc.): No effect

Study title: An Oral (stomach tube) toxicokinetic study of LdT in pregnant rabbits
Key study findings:

The present study is a toxicokinetic study in pregnant rabbits. The study design was similar to that of the previous Segment II reproductive toxicity study in the same species. However, no detailed evaluation of the fetuses was done. Toxicokinetic parameters in the dams as well as the drug concentrations in the fetuses were determined. The analytical assay used was a LC-MS/MS method with limit of detection at 0.01 μg/ml. In order to get enough drug for analysis, LdT was extracted from pooled fetuses from the same dam. One high dose dam which had reduced food consumption and body weight gain as well as an episode of liquid/soft feces died. This animal also had necropsy findings in stomach and intestine suggesting gastrointestinal irritation. Body weight gains and food consumption was reduced for the high dose group as compared to the control group from gestation day 15 to 19, however, the reduction did not reach statistically significant level. The results together with those from the previous study suggested that 1000 mg/kg/day dose is probably the maximum tolerated dose and induced frank maternal toxicity.

The toxicokinetic results suggest that LdT crossed the placenta barrier and the fetuses were exposed to LdT *in utero*. The pharmacokinetic profile in rabbits is similar to those in mice, monkeys and rats. However, the AUC value at 1000 mg/kg/day was 5-6 times higher than those in rats and monkeys at the same dose and 37-fold higher than at clinical dose of 600 mg/day. Without the data from nonpregnant rabbit, it's unclear if bioavailability of LdT increases during pregnancy. The NOAEL for the dams is 250 mg/kg/day.

Study no.: IDIX-04-109
Volume: m4\42-stud-rep\422-pk\4222-absorp\gva00010
Conducting laboratory and location: [redacted]
Date of study initiation: 7/16/2004
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L-dT, lot # 16102001, [redacted] pure & LT.1.004E, [redacted] pure by uv
Methods

Doses: 0, 50, 250, and 1000 mg/kg/day
Species/strain: Time-mated female rabbits/Hra:(NZW)SPF
Number/dose: Five
Dosing duration: Gestation days 6 to 18
Route, formulation, volume, and infusion rate: Oral stomach tubing, dissolved in aqueous 0.5% carboxymethylcellulose solution, with dosing volume of 10 ml/kg
Satellite groups used for toxicokinetics: This study is a toxicokinetic study
Study design: The mated rabbits were shipped to the testing facility to arrive on gestation day 1 and administered vehicle or L-dT on gestations days 6-18.
Parameters and endpoints evaluated:

Mortality: Twice daily
Clinical observation: Once pretest, daily during dosing and postdosing periods

Body weights: Gestation day 0, the day of arrival, daily during dosing and postdosing
Food consumption: Daily
Necropsy: Gestation day 19
Gross examination: Pregnancy status and live/dead fetuses
Toxicokinetics: Blood samples were taken from dams on gestation day 18 at predose, 0.5, 1, 2, 4, 8, and 24 hours postdose. Live fetuses were collected on gestation day 19, pooled, weighed, and processed for drug concentration determination

Results

Mortality (dams): One dam received 1000 mg/kg/day L-dT died on gestation day 19 before blood collection. This dam had soft/liquid feces on gestation day 7. It generally lost weight after gestation day 6. Food consumption was extremely reduced after gestation day 11. At necropsy, gastrointestinal irritation was manifested as erosion on the stomach mucosal surface, red appearance and red fluid in the intestine, and distended stomach and intestine (with gas). This rabbit was pregnant.

Body weight (dams): The high dose group lost 20 g of body weight was compared to a gain be 10 g in the control group between gestation days 15-19 though this change did not reach statistical significance. The body weight gains for all groups were comparable on gestation days 6 to 9 or 0 to 19. The results were similar to those in the previous study.

Food consumption (dams): The food consumption for the high dose was consistently lower as compared to the control group. However, the decrease did not reach statistical significance at any periods.

Necropsy: No effect

Toxicokinetics: The C_{max} and AUC values as well as drug concentration in maternal plasma and fetal tissue increased at dose-related manner. The increase was less than dose proportional for C_{max} values but greater than dose proportional for the AUC values and fetal tissue LdT concentrations. The systemic exposure at 1000 mg/kg/day was about 5-6-fold higher than those at the same dose in rats and monkeys. Because there is no pharmacokinetic data available for the nonpregnant rabbits, it's unclear if the bioavailability for LdT increases during pregnancy. The results indicated that LdT can cross placenta. They also indicate that the fetuses were exposed to LdT *in utero*.

	Maternal Plasma				Fetal Tissue
Dose (mg/kg/day)	T_{max} (hr)	C_{max} (µg/ml)	C_{min} (µg/ml)	$AUC_{0\text{-}24h}$ (hr-(µg/ml)	**Concentration** (µg/g)
500	1.0 ± 0.0	12.34 ± 2.03	1.59 ± 0.44	72.48 ± 13.53	1.29
250	1.0 ± 0.6	38.85 ± 9.89	7.29 ± 1.21	296.15 ± 29.76	3.79
1000	1.4 ± 0.8	66.4 ± 17.7	20.17 ± 8.46	1022.89 ± 307.45	25.34

Prenatal and postnatal development

Study title: Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of l-thymidine in rats, including a postnatal behavioral/functional evaluation

Key study findings: The present study is a combined Segments II/III reproductive toxicity study. Doses of 0, 100, 250, and 1000 mg/kg/day LdT were administered to mated females from gestation days 7 through lactation day 20. Selected offspring (F_1 generation) was evaluated for sexual maturation, and behavior development, and

mating behavior. Their offspring (F_2 generation) was then evaluated for gross malformation. No effect on any of the parameters evaluated was associated with LdT treatment at doses up to 1000 mg/kg/day which is the NOEL.

Study no.: 02-TX-020

Volume: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42353-pre-postnatal-dev/1314-003

Conducting laboratory and location: [redacted]

Date of study initiation: 6/19/01

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot #, and % purity: L-dT/Nv-02B, lot # LT.1.004E, [redacted] pure by UV assay

Methods

Doses: 0, 100, 250, and 1000 mg/kg/day given to F_0 generation females only

Species/strain: Female [redacted] :CD®(SD)IGS Br VAF/Plus® rats as the F_0 generation

Number/sex/group: 25/group for F_0 generation rats; 25/sex/group for F_1 generation rats

Dosing duration: Gestation day 7 to lactation day 20 or gestation day 24 (rats that did not deliver a litter)

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in aqueous 0.5% carboxymethylcellulose solution, with dosing volume of 10 ml/kg

Satellite groups used for toxicokinetics: None

Study design: Mating of F_0 generation was done on site. After confirmation of mating (gestation day 0), female rats were placed in individual housing and received dosing from gestation day 7 to lactation day 20 or gestation day 24 (rats that did not deliver a litter). Dams in the process of delivering pups did not receive dosing for that day. F_1 generation pups were allowed to grow and develop and did not receive directly any dosing but may have been exposed to L-dT *in utero* or via maternal milk during the lactation period. Developmental milestones, like sexual organ maturation, learning, long- and short-term memory, overt coordination, and swimming ability were evaluated in each fetus. In addition, 25 F_1 generation rats/sex/dose were selected to mate at approximately 90 days of age, one male per one female rat, based on a random unit table. Sibling mating was excluded.

Parameters and endpoints evaluated:

F_0 generation: *Mortality*: Twice daily

Clinical observation: Weekly during acclimation, on gestation day 0, daily before dosing and within 60 minutes of dosing, and on the day of sacrifice

Body weights: Weekly during acclimation, on gestation day 0, and daily during dosing period, and on the day of sacrifice

Food consumption: Gestation days 0, 7, 10, 12, 15, 18, 25 (if necessary), lactation days 1, 4, 7, 10, and 14

Parturition: Abnormal behaviors, duration of gestation, litter sizes (all pups delivered), and pup viability at birth

Maternal behavior: Lactations days 1, 4, 7, 14, and 21

Necropsy: Gross examination of thoracic, abdominal, and pelvic viscera on postpartum day 21; Uterus: number and distribution of implantation sites, number of pups per litter

F_1 generation: *Mortality*: Twice daily

Number of pups/litter: Once daily
Clinical observation: Once weekly during postweaning period, and twice weekly during gestation period
Body weight: Lactation days 1, 4, 7, 14, and 21, weekly during postweaning period until sacrifice for the F_1 males; weekly during postweaning period and on gestation 0, 7, 10, 14, 17, and 21 for the F_1 females
Food consumption: Weekly during postweaning period except during cohabitation for the F_1 males; weekly during postweaning period (except during cohabitation) and on gestation days 0, 7, 10, 14, 17, and 21
Sexual maturity: Vaginal patency for females starting at day 28 postpartum and preputial separation for males starting at day 39 postpartum
Behavior evaluation: Passive avoidance test for learning, short-term retention, and long-term retentions starting at day 24±1 days postpartum in one male and one female rats/litter (tested twice/rat); Water-filled M-maze for overt coordination, swimming ability, learning, and memory test beginning on postpartum day 70 in one male and one female rat/litter (tested twice/rat); descriptions of passive avoidance and M-maze tests were transcribed directly from study report and included in Appendix III.
Mating and fertility: Twenty-five/sex/dose selected for cohabitation, one male paired with one female, starting at approximately 90 days of age. The maximum duration of cohabitation was 21 days.
Necropsy: Necropsy was performed on all fetuses except those selected for mating on lactation 21. The selected males for mating were sacrificed after the completion of 21 day cohabitation, necropsy performed, and testes and epididymides weighed and fixed for histopathology evaluation. The selected females for mating were sacrificed on gestation day 21 and Caesarean-section performed. Gross necropsy, uterus examined, number and distribution of corpora lutea, implantation sites, live and dead fetuses, and early and late resorption.

F_2 generation: Body weight, sex determination, and gross external alterations were evaluated.

Results

F_0 in-life:

Mortality: None

Clinical observation: Increased incidences of soft/liquid feces and urine-stained abdominal fur were associated with L-dT treatment.

Dose (mg/kg./day)	0	100	250	1000
Soft or liquid feces	70/13	85/20	93/18	77/21
Urine-stained abdominal fur	2/2	5/4	7/3	14/7
Number of observation/total number of rats with the observation				

Body weights: No effect

Food consumption: No effect
Parturition: No effect
Maternal behavior: No effect
Necropsy: No effect
Number of pups/litter: No effect
F_1 physical development:
Mortality: No treatment effect. One male rat in 100 mg/kg/day was sacrificed as the result of injury
Clinical observation: The observations were recorded weekly from postweaning week 1 to the scheduled sacrifice. Number of animals and incidence rate for constriction of tail and dehydration were increased in high dose females and males, respectively. They are probably not related to LdT treatment since the increases were small and occurred in a single sex only.
Body weight: No effect
Food consumption: No effect
Sexual maturation: No effect
F_1 behavioral evaluation: No effect
F_1 reproduction: No effect
F_2 findings: No treatment effect

2.6.6.7 Local tolerance

No study was performed.

2.6.6.8 Special toxicology studies

Study title: Assessment of contact allergenic potential with the murine local lymph node assay (LLNA tier I)
Key study findings: The allergenic potential of LdT was evaluated in a murine local lymph node assay. Doses of 7.5, 75, and 750 mg/kg LdT was administered to the murine ears and their effects on ear and auricular lymph node weights, as well as cell count and lymphocyte phenotyping were determined. Except for the statistically significant decrease in ear weight at the high dose group, no other parameters were affected. Irritation and inflammation usually causes ear weight increase not reduction. Thus, the toxicological significance of this finding is unclear. LdT is not considered an irritant or allergen by this assay.
Study no.: 0417004
Volume: m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\0417004, p. 1-36
Conducting laboratory and location: [redacted]
Date of study initiation: 2/10/2004
GLP compliance: yes for Swiss GLP
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 1-beta-L-ribofuranosyl-2'-deoxythymidine, L-thyminidine, LdT, L-dT, LDT600-NXA, lot # USNA003367, [redacted] by HPLC

Formulation/vehicle: Dissolved in DMSO
Methods
Doses: 25 µl/ear/animal of 0 (DMSO), 0.3% (~ 7.5 mg/kg), 3% (~75 mg/kg), or 30% (~ 750 mg/kg) LdT dissolved in DMSO or 0.5% dinitrochlorobenzene (positive control)
Study design: Six female BALB/c mice/dose received controls (DMSO and dinitrochlorobenzene) or LdT (7.5-750 mg/kg) epicutaneously on dorsum of both ears for 3 consecutive days. The animals were sacrificed 24 hours after the last application. Body weights, ear weights, and auricular lymph node weights, cell count, and lymphocyte phenotyping (cell counts) were performed. Indices for ear weight, lymph node weight, and cell count were calculated by dividing mean values from LdT treatment groups and the positive control group by the corresponding mean value of the vehicle control. Threshold values derived from an analysis of historical data are: ear weight index – 1.05; lymph node weight – 1.2; lymph node cell count – 1.3. Values were considered positive if they were above or below these thresholds and statistically significance occurred in one of the parameters (ear weight, lymph node weight, or lymph node cell count).
Results: The positive control elicited increases in ear weights, ear lymph weights, and lymph node weights, in accordance to the expected mode of action of a contact allergen. Ear weight index for the high dose group was slightly but statistically significantly lower (0.94 vs. historical value of 1.05) than the vehicle control. However no other statistically significant changes were associated with other parameters monitored. Since an irritant usually increases ear weight (as seen in control group), it's unclear what the toxicological significance of decreased ear weight index in the absence of other changes is. Therefore, LdT is not considered a contact allergen

2.6.6.9 Discussion and Conclusions

The safety profile of telbivudine has been extensively characterized in rats, mice, rabbits, and monkeys. The absorption, distribution, metabolism, and excretion (ADME) profiles of telbivudine in these species are similar to that in humans and made them appropriate for the nonclinical safety evaluation. The toxicological, genotoxic, carcinogenic, allergenic, and reproductive toxicological potentials as well as telbivudine's effects on cardiovascular, neurological, respiratory, gastrointestinal, renal and other systems were evaluated. All of the pivotal toxicology studies employed adequate range of doses that were administered via clinical route of administration (oral) and produced sufficient systemic exposures and safety margins over that at clinical dose of 600 mg/day. In general, telbivudine is found to be well tolerated and produced few or no adverse effect at large multiples of human exposure.

The doses investigated in a myriad of general toxicology studies ranged from 5 to 3000 mg/kg/day. The highest doses investigated following chronic oral administration of telbivudine (9 months in monkeys and 85 week s in rats) were 1000 and 2000 mg/kg/day which give 8 to 14 fold of safety margins over the systemic exposure associated with the 600 mg/day clinical dose. A variety of changes in the toxicological parameters were seen in all studies. However, most of these changes were isolated, seen in one study but not in another in the same species, or were marginal or small without corresponding

macroscopic and/or microscopic alterations. One exception is the gastrointestinal irritation. In monkeys, reduced dose-related body weight gain, abnormal feces, and emesis were observed without other correlated systemic toxicity (including histopathological changes). This toxicity was not considered dose limiting. On the other hand, gastrointestinal irritation was attributable to reduced body weight gain and abnormal feces, one death, one abortion, and two premature deliveries in pregnant rabbits at 1000 mg/kg/day. The rabbits that died, aborted, and delivered early showed the same signs of gastrointestinal irritation. This toxicity was clearly dose-limiting in rabbits and may be related to the high systemic exposure (37-fold higher than in humans at 600 mg/day dose). Rodents seemed to be less sensitive to this toxicity since mice did not exhibit these clinical signs in any of the studies and they were sporadic in studies with rats. In general, gastrointestinal function did not seem to be affected since periods of increased food consumption and/or body weight gain were observed in most studies and most species. In addition, no histopathological findings were associated with any of the gastrointestinal tissues in any of the general toxicology studies or the carcinogenicity studies.

Renal toxicity was not apparent in studies conducted in monkeys, mice, rabbits, or woodchucks. It also did not produce any dose-related renal histopathology in any of the species studied except for chronic progressive nephropathy observed in rats. A marginal increase in incidence and severity of this lesion was observed in the 6-month toxicity study in this species. This is a common lesion associated with aging in rats. It affects males more than females. Normally, the small increase observed in the 6 month study would not be attributed to drug toxicity and was not considered dose limiting. However, a one-fold increase in the number of deaths attributable to chronic progressive nephropathy were observed in the mid and high dose (1000 and 2000 mg/kg/day) males and in the high dose females in the 2 year rat carcinogenicity study. Many of these deaths occurred after one year of drug administration. Dosing was stopped after 85 weeks of drug administration for the 2000 mg/kg/day dose group because of the high mortality rate in this group. The increased mortality rate attributed to this lesion suggests that chronic administration of telbivudine may potentially exacerbate pre-existing renal impairment or dysfunction in patients.

Rare cases of myopathy have been reported in several telbivudine clinical trials. Creatine kinase (CK) values were elevated in these patients. However, the degree and timing of CK elevation did not always coincide with the onset of myopathy. CK values were monitored in a 14-day intravenous study in monkeys. Skeletal as well as heart muscles were routinely evaluated histologically in the general toxicology studies. No signals suggesting muscle toxicity were observed in any of the studies performed.

Another toxicity that warrants some discussion was the spinal cord and sciatic nerve axonopathy observed in the 9-month toxicity study in monkeys. This study contained three treatment durations: 3 months, 9 months, and 9 months with a 2 month drug-free recovery period. Spinal cord hemorrhage was noted in most animals in both the control and high dose groups at all treatment durations. At three months, one more high dose male than the control males had spinal cord hemorrhage, although the mean severity score was higher in control group than that in the high dose group. However, after 9 months of daily LdT administration, all of the control animals had minimal spinal cord hemorrhage while only three out four high dose males had minimal form of this

histopathological finding. To complicate the analysis, one of these three high dose males also had mild axonal swelling. The situation in females is equally inconsistent. While the high dose groups, generally, had more incidence of spinal cord hemorrhage than the control, the severity scores were higher in the control group. The severity scores for both control and high dose groups were higher at 3-month sacrifice and the recovery groups than the 9-month sacrifice group. In addition, two out of three high dose females also had minimal to mild degeneration of sciatic nerve at the 3 month sacrifice, but only one out 4 high dose females had minimal form of this lesion. The female with sciatic nerve degeneration also had minimal hemorrhage at the spinal cord. The mechanism of axonal injury is unknown. The lesion was absent from other species and pharmacology studies did not show a signal for peripheral neuropathy. LdT is not a substrate for DNA polymerase α, β and γ. Since there was no consistent temporal pattern in the incidence and severity of this lesion and the increases were small, the axonopathy in these two tissues was probably incidental finding and not treatment related.

The genotoxic potential of telbivudine was investigated in three *in vitro* and one *in vivo* genotoxicity studies. It was found not to be mutagenic and clastogenic with and without metabolic activation. In addition, it was found not to be carcinogenic in the 2 year carcinogenicity study in rats and the 6-month carcinogenicity study in transgenic CB6F1-TgrasH2 mice. The dosing in rats was stopped for the 2000 mg/kg dose groups after 85 weeks of telbivudine administration and study terminated prematurely after 96 of drug administration because of the high mortality rate. The increased mortality rate for this study was dose-related. Many of the deaths were caused by tumors. However, except for chronic progressive nephropathy described in the previous paragraph, no statistically significant or dose related increases in the incidence of tumor and/or tumor type and deaths attributable to any tumor type were observed. Dosing in the transgenic mouse carcinogenicity study was continued to the scheduled time. No evidence of increased incidence of tumor was associated with telbivudine administration.

Telbivudine can cross blood-brain, blood-testes, and placenta barrier. Both male and female fertility were not affected at doses as high as 2000 mg/kg (about 14-times human exposure) in rats. *In utero* exposure to telbivudine did not adversely affect embryo and fetal development in rats and rabbits at doses as high as 1000 mg/kg/day. It was also secreted into rat milk. And exposure to this drug *in utero* or in milk did not affect pup delivery or neonatal development in rats. The second generations exhibited normal behavior and postnatal development, growth, sexual maturity, and fertility. The No-adverse-effect level (NOAEL) for reproductive toxicity is 1000 mg/kg/day, providing 6 to 37-fold safety margins as compared to clinical dose of 600 mg/day.

The safety of telbivudine was also investigated in a variety of in vitro and local tolerance studies. It shows low toxicity in cultured human hepatoma cells, peripheral blood mononuclear cells, none marrow progenitor cells, and numerous cells lines of human and other mammalian origin. It is not toxic to mitochondria. It is also not allergenic or irritating in the mouse local lymph node assay.

In conclusion, except for the potential to exacerbate preexisting renal dysfunction and impairment and being somewhat irritating to the gastrointestinal system, the results in the nonclinical safety studies included in this NDA package suggest that telbivudine has a good safety profile and should be well tolerated at systemic exposures > 6-fold of the clinical one.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Telbivudine is safe to be approved for marketing from the nonclinical pharmacology/toxicology perspective.

Unresolved toxicology issues (if any): None

Recommendations: None

Suggested labeling:

The language included in the sponsor's labeling for the "Carcinogenesis, Mutagenesis, Impairment of Fertility", "Pregnancy Category B", "Labor and Delivery", and "Nursing Mothers" sections is acceptable except for an editorial change regarding the word "transformation" placed in front of "assay with Chinese hamster ovary cells" in the second to the last sentence in the 2nd paragraph under "Carcinogenesis, Mutagenesis, Impairment of Fertility." The word "transformation" should be deleted since this assay is not a transformation assay as stated. The sponsor has agreed with the change. Please see the executive summary for the language to be used on the proposed label.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Appendix I: Executive CAC meeting minutes for the 2 year rat carcinogenicity study
Appendix II: Executive CAC meeting minutes for the carcinogenicity study reports
Appendix III: Executive CAC meeting minutes for the 6 month transgenic mouse carcinogenicity study using rasH2 mice
Appendix VI: Detailed descriptions on passive avoidance test and M-maze test used in rat Segments II and III reproductive toxicology study.

APPENDIX I

EXECUTIVE CAC
Date of Meeting: November 6, 2001

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
David Morse, Ph.D., HFD-150, Alternate Member
Jeri El Hage, Ph.D., HFD-510, Alternate Member
Jim Farrelly, Ph.D., HFD-530, Team Leader
Ita Yuen, Ph.D., HFD-530, Presenting Reviewer

Author of Draft: Ita Yuen, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the "Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application."

IND #: 60,459
Drug Name: β-L-2'-deoxythymidine (LdT)
Sponsor: Novirio Pharmaceuticals

Background:

β-L-2'-deoxythymidine (LdT) is the l-isomer of the naturally occurring deoxythymidine (d-isomer). It is being studied in humans for the treatment of hepatitis B infection in phase I/II clinical studies. The current clinical dosage is 400 mg QD, however, the anticipated maximum clinical dosage may be 600 mg QD. Pharmacokinetic data are available for clinical dosages up to 800 mg QD in healthy human volunteers dosed for 7 days.

The toxicity potential of this drug has been studied in rats and monkeys. Twenty-eight-day toxicity studies were submitted to the Agency to support the initial introduction to humans. The highest doses studied for both species were 2000 mg/kg/day. The NOAEL for the rat study was 2000 mg/kg/day. At this dose, the steady state AUC value was approximately 20-fold above the anticipated maximum clinical dosage. Soft feces were associated with drug levels of 500 mg/kg/day in monkeys. However, this toxicity was not thought to be dose-limiting and 2000 mg/kg/day was considered the NOAEL. To support the dose selection for the 2-year carcinogenicity study in rats, the sponsor has

submitted the results of a 6-month toxicity study with a 3-month interim sacrifice. The highest dose studied was 1000 mg/kg/day which was found to be the NOAEL. LdT was found not to be genotoxic in the standard battery of tests.

The absorption, distribution, metabolism, and excretion (ADME) profile for this drug is similar to other nucleoside analogs. It is excreted mainly as unchanged compound in urine with short plasma half-life (~2 hours in monkeys, 3 hours in rats, and 7.5 hours in woodchucks). No metabolites were detected in monkeys and woodchucks following the administration of 3H-LdT. The extent of plasma protein binding for this drug is in the process of being evaluated. However, plasma protein binding of nucleoside analogs is low and estimated at less than 25%. Nucleoside analogs become activated once they are taken up into cells and phosphorylated. However, cellular uptake is low.

Mouse Carcinogenicity Protocol:

The sponsor proposed to use the limit dose criterion for dose selection and proposed to study 0, 375, 750, and 1500 mg/kg/day dose using 65 mice/sex/dose. The animals will receive the drug by daily oral gavage for 104 weeks. Histopathology will be evaluated on control and high dose animals only and also on those that die or are sacrificed at an unscheduled interval. There are no toxicological or pharmacokinetic data on this species.

Rat Carcinogenicity Protocol:

The dosages and study design for the rat carcinogenicity study is identical to that for mice. Doses of 0, 375, 750, and 1500 mg/kg/day were proposed with 65 rats/sex/group. The NOAEL at 1 month is 2000 mg/kg/day and at 6 months is 1000 mg/kg/day (the highest dose studied). At 1000 mg/kg/day, the steady state AUC value was ~ 13 fold above the AUC value for 400 mg/day clinical dose and 8.5-fold above the anticipated maximum clinical dose. At 2000 mg/kg/day, the AUC value is estimated to be about 30-fold and 20-fold above the clinical doses of 400 and 600 mg/day, respectively.

Executive CAC Recommendations and Conclusions:

Mouse:

The Committee could not concur with doses without data from any dose range-finding study. Given the short plasma half-life of the drug and the lack of toxicity seen by gavage, the Committee strongly recommended using the dietary route of drug administration in the mouse for the carcinogenicity study. The supporting dose range-finding study in mice should be 3 months in duration giving drug in the diet if that is the route of administration chosen for the carcinogenicity study. If the maximum tolerated dose cannot be reached in the study, a maximum feasible dose of five percent of diet can be used in the carcinogenicity study. Once the data become available, a new protocol can be submitted to the Committee for review.

Rat:

It is appropriate for the sponsor to use "limit dose" as the criterion for selection the dose. LdT is not genotoxic. The ICH guidance S1C(R) "Addendum to Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes" indicated that 1500 mg/kg/day can be used as the high dose if (1) the human dose does not exceed 500 mg/day and (2) the rodent systemic exposure at 1500 mg/kg would be at least 10-fold of the human exposure. The guidance also stated that "if human dose exceeds 500 mg/day, the high dose may be increased up to the maximum feasible dose." Since the anticipated maximum clinical dosage is 600 mg/day, the high dose for the rat carcinogenicity study should be set at 2000 mg/kg/day. At this dose, the AUC value is estimated to be 20-fold of that at clinical dose of 600 mg/day and 13-fold of that at clinical dose of 800 mg/day. Even if the sponsor decide to market 800 mg/day at the time of NDA submission, the criteria for using "limit dose" would still apply. Thus, doses of 0, 500, 1000, and 2000 mg/kg/day are recommended for the 2-year carcinogenicity study in rats.

The Committee noted that the sponsor could select doses based on the AUC ratio. To reach 25-fold of human AUC, the Committee would recommend doses of 4000, 1000, and 500 mg/kg/day in anticipation that 800 mg/day might be selected for market approval.

Since the sponsor plans to conduct histological evaluation of tissues from only control and high dose treatment groups, histopathologic examination of other dose groups will be needed under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, tissue for all of the dose groups will need to be examined;
(b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, tissue at the next lower dose group will need to be evaluated;
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986), all relevant tissues for that dose level and the next lower dose level will need to be examined;
(d) for an excessive decrease in body weight or survival in the examined dose group, all tissues and organs from lower dose groups need to be evaluated.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\
/ Division File, HFD-530

/JFarrelly, HFD-530
/IYuen, HFD-530
/GCarmouze, HFD-530
/ASeifried, HFD-024

APPENDIX II

Executive CAC
Date of Meeting: October 17, 2006

Committee: Joseph Contrera, Ph.D., OPS, Acting Chair
David Morse, Ph.D., DODP, Alternate Member
Dan Mellon, Ph.D., DAARP, Alternate Member
Jim Farrelly, Ph.D., DAVP, Team Leader
Ita Yuen, Ph.D., DAVP, Presenting Reviewer

Author of Draft: Ita Yuen, Ph.D., DAVP

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 22,011
Drug Name: β-L-2'-deoxythymidine; LdT; telbivudine; Tyzeka™
Sponsor: Idenix Pharmaceutical Inc.

Background:

β-L-2'-deoxythymidine (LdT) is the l-isomer of the naturally occurring deoxythymidine (d-isomer). It is under review for marketing approval for the treatment of human hepatitis B virus infection. The proposed dosage is 600 mg QD. The absorption, distribution, metabolism, and excretion (ADME) profile of LdT has been demonstrated to be similar across the species studied (rats, mice, rabbits, monkeys, woodchucks, and humans). LdT is eliminated mainly through renal excretion as unchanged drug. Rat was the only species that showed minor metabolism of LdT.

The toxicological potential of LdT has been extensively characterized in several species. The only effects associated with LdT treatment are gastrointestinal (GI) irritation and possible exacerbation of existing renal dysfunction. In monkeys, LdT caused occasional but dose-related increase in the incidence of abnormal feces sometimes accompanied by emesis. Body weight gain was occasionally reduced at the 1000 mg/kg/day dose (AUC value 8-fold above that of humans at 600 mg/day) but was not affected at the end of 9-month treatment. This GI toxicity is not dose-limiting in monkeys. In pregnant rabbits, 1000 mg/kg/day dose (AUC values 34-fold above that of the clinical dose) caused one death, one abortion, and two premature deliveries as well as reduced body weight and food consumption as well as abnormal feces. This was clearly the maximum tolerated dose for rabbits. In the clinical trials, GI system-related adverse events for LdT was observed at comparable occurrences as that for the comparator arm treated with 3TC. One discontinuation of drug was associated with LdT treatment where the patients had unresolved nausea and loose stool after at least one year of LdT treatment. The symptom resolved after LdT administration was stopped.

The other LdT associated toxicity was the exacerbation of chronic progressive nephropathy in rats. This is a common lesion in older rats and is more prominent in males than females. Dosing in the 2 year carcinogenicity study in rats at 2000 mg/kg/day was stopped at 85 weeks of treatment because of the high mortality rate. Deaths attributable to chronic nephropathy were also increased at this dose as compare to the concurrent control groups. These results suggest that chronic treatment with LdT has the potential to exacerbate pre-existing renal impairment/dysfunction.

Rat Carcinogenicity Study:

The doses used in the 2 year study are 0, 500, 1000, and 2000 mg/kg/day. LdT is not genotoxic. However, the criterion where 25-fold over clinical AUC value could not be used to set the high dose because of the high exposure required in the clinical dosage. Instead, limit dose of 2000 mg/kg/day was determined by the Executive CAC to be the appropriate high dose.

Dosing at 2000 mg/kg/day had to be stopped after 85 weeks of treatment because of the high mortality rate (41/65 deaths each in males and females). The study was terminated after 96 weeks of treatment. The survival rates at week 85 were 55%, 42%, 46%, and 37% in control, low, mid, and high dose males, respectively, and 49%, 45%, 51%, and 37% for females. Even though most of the deaths were attributed to neoplasms, no single type of tumor contributed significantly to the cause of death. Chronic progressive nephropathy may have been exacerbated by the LdT treatment since deaths attributable to this lesion were 4, 2, 8, and 9 in control, low, mid, and high dose males, respectively, and 0, 0, 0, 3 in females. Clearly, 2000 mg/kg/day exceeded the maximum tolerated dose.

Statistically significant increases were seen in the incidences of pancreas acinar cell adenoma in the high dose males ($p=0.214$, two-tailed) and adrenal medulla pheochromocytomas ($p= 0.438$, two-tailed) in the high dose females. However, these increases were observed in the high dose groups only. When the tumors from the high dose groups are excluded from statistical analysis, no significance in any tumor types was found for the study. Therefore, it is concluded that LdT is not carcinogenic in the rat model under the conditions of the assay.

TgRasH2 Mouse Carcinogenicity Study:

The sponsor had originally submitted a protocol proposal for a 2 year mouse carcinogenicity study. The proposed dose selection criterion was limit dose for the high dose of 1500 mg/kg/day without any supporting study in this species. The Exec CAC did not give concurrence for the dose selection and asked the sponsor to conduct a 3 month toxicology study in order to provide rationale for the proposed dose. Instead, the sponsor inquired about the possibility of a carcinogenicity study using transgenic mouse model. Since LdT id not genotoxic, the appropriate transgenic mouse model would have been the

mouse. However, LdT is high soluble in aqueous solution, it was difficult to formulate it in a solution appropriate for dermal application. Therefore, it was suggested that TgrasH2 mouse model, which allows oral administration of test drug, could be used. The sponsor performed a one month toxicology study in CB6F1 mice, the parent strain of TgrasH2 mice. It was found that the 2000 mg/kg/day dose was well tolerated and was below the maximum tolerated dose. Therefore, the limit dose criterion was used to set the high dose at 2000 mg/kg/day.

Doses of 0, 500, 1000, and 2000 mg/kg/day LdT were administered to TgrasH2 transgenic mice for 6 months. The positive control used was 75 mg/kg/day N-methyl-N-nitrosourea. While the positive control induced tumors of various types at multiple organs, high number of deaths caused by tumors, and many adverse effects resulting from the tumors, LdT did not affect any parameters monitored. The toxicokinetic results verified that the animals were exposed to LdT systemically. The exposure at 2000 mg/kg/day was about 14 fold over that in the humans taking 600 mg LdT once a day. The study results indicate that LdT is not carcinogenic in the TgrasH2 transgenic mouse model under the conditions of the assay.

Executive CAC Recommendations and Conclusions:

Rat:

The Committee agreed that the study was adequate. The selected doses had prior concurrence from the Executive CAC. After reviewing the results from the 2 year carcinogenicity study, the Committee found that the study was negative for drug-related neoplasms.

TgRasH2 mouse:

The Committee agreed that the study was adequate. The selected doses had prior concurrence from the Executive CAC. After reviewing the results from the 6-month transgenic mouse carcinogenicity study, the Committee found that the study was negative for drug-related neoplasms.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DAVP
/JFarrelly, DAVP
/IYuen, DAVP
/KShade, DAVP
/ASeifried, OND IO

APPENDIX III

EXECUTIVE CAC
Date of Meeting: August 12, 2003

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joe Contrera, Ph.D., HFD-901, Permanent Member
Abby Jacobs, Ph.D., HFD-540, Permanent Member
Lois Freed, Ph.D., HFD-120, Rotating Member
Jim Farrelly, Ph.D., HFD-530, Team Leader
Ita Yuen, Ph.D., HFD-530, Presenting Reviewer

Author of Draft: Ita Yuen, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the "Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application."

IND #: 60,459
Drug Name: β-L-2'-deoxythymidine (LdT)
Sponsor: Idenix Pharmaceuticals

Background:

β-L-2'-deoxythymidine (LdT) is the l-isomer of the naturally occurring deoxythymidine (d-isomer). It is being studied in humans for the treatment of hepatitis B infection in phase II/III clinical studies. The proposed clinical dosage in phase III clinical trials is 600 mg QD. Pharmacokinetic data are available for clinical dosages up to 800 mg QD in healthy human volunteers dosed for 7 days.

The toxicity potential of this drug has been studied in mice, rats and monkeys. The highest dose used in rat and monkeys were 1000 mg/kg/day in the 6 and 9 month general toxicology studies. The NOAEL was 1000 mg/kg/day. A 2-year carcinogenicity study in rats is ongoing and the in-life phase of which will end on November 2003. The "limit dose" criterion was used to set the high dose at 2000 mg/kg/day for that study. At the end of last month (July 2003), there were only 21 out of 65 animals left in the high dose female group. Thus, 2000 mg/kg/day is likely the maximum tolerated dose for that study.

The sponsor has approached the Division regarding the acceptability of using a transgenic mouse model to study the carcinogenic potential of L-dT. Since the compound is found not to be genotoxic in the standard genotoxicity testing battery, the logical choice would be the Tg.AC transgenic mouse model. However, since this compound has poor solubility in the solvents available for dermal formulation, L-dT can only be easily administered orally. After consultation with Dr. Frank Sistare, the rasH2 transgenic mouse model was suggested and accepted by the sponsor. It was also suggested that the sponsor should perform a dose range-finding study in the parental strain of rasH2 transgenic mice as a basis for the dose selection for the definitive study.

The results from the dose range finding study suggest that the maximum tolerated dose has not been achieved even at 2000 mg/kg/day. At this dose, the AUC value was at least 30-fold above that at the clinical dosage of 600 mg QD. The absorption, distribution, metabolism, and excretion (ADME) profile for this drug is similar across all species studied. It is excreted as unchanged compound in urine and feces equally with short plasma half-life. No metabolite was detected following the oral administration of 3H-L-dT except for the phosphorylated L-dT. The plasma protein binding is low. The sponsor has used the AUC ratio criterion as a justification for setting the high dose at 2000 mg/kg/day. However, this criterion has not been used in the transgenic mouse models. It is felt that the use of the "limit dose" criterion as outlined in the ICH Guidance S1C(R) "Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes," is more appropriate in the present situation.

Mouse Carcinogenicity Protocol:

The sponsor proposed to study the carcinogenic potential of L-dT in rasH2 transgenic mice. The proposed doses are 0 (0.5% CMC-Na solution), 500, 1000, and 2000 mg/kg/day dose using 25 mice/sex/dose. Positive control animals (25 mice/sex) will receive a single intraperitoneal dose of 75 mg/kg N-methyl-N-nitrosourea. The animals will receive the drug by daily oral gavage for 6 month. Histopathology will be performed in vehicle control, positive control and high dose and early deaths/sacrifice animals at necropsy. There are no toxicological or pharmacokinetic data on this species.

Executive CAC Recommendations and Conclusions:

Mouse:

The Committee concurs with doses proposed for the carcinogenicity study in rasH2 transgenic mice. However, because the AUC ratio criterion has not been used in transgenic mouse models and does not have any data supporting the use of this criterion, it is felt that the use "limit dose" as the criterion for selection the dose is more appropriate in the present case. LdT is not genotoxic. The ICH guidance S1C(R) "Addendum to Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes" indicated that 1500 mg/kg/day can be used as the high dose if (1) the human dose does not exceed 500 mg/day and (2) the rodent systemic exposure at 1500 mg/kg would be at least 10-fold of the human exposure. The guidance also stated that "if

human dose exceeds 500 mg/day, the high dose may be increased up to the maximum feasible dose." Becasue the anticipated maximum clinical dosage is 600 mg/day, the high dose of 2000 mg/kg/day is acceptable.

Since the sponsor plans to conduct histological evaluation of tissues from only control and high dose treatment groups, histopathologic examination of other dose groups will be needed under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, tissue for all of the dose groups will need to be examined;
(b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, tissue at the next lower dose group will need to be evaluated;
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986), all relevant tissues for that dose level and the next lower dose level will need to be examined;
(d) for an excessive decrease in body weight or survival in the examined dose group, all tissues and organs from lower dose groups need to be evaluated.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/ Division File, HFD-530
/JFarrelly, HFD-530
/IYuen, HFD-530
/JO'Neil, HFD-530
/ASeifried, HFD-024

Appendix IV

Passive avoidance test:

The passive avoidance apparatus consisted of a two-compartment chamber with hinged Plexiglas® lids. One compartment was fitted with a bright light and Plexiglas□ floor. The other compartment was fitted with a grid floor to which a brief (1 sec) pulse of mild electric current (1 mA) was delivered. The two compartments were separated by a sliding door. On each test trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore the apparatus until it entered the "dark" compartment. The sliding door was the immediately closed, the light turned off and the brief pulse of current was delivered to the grid floor. The rat was then removed from the appratus and placed into a holding cage for 30 seconds before the start of the next trial. Trials were repeated until the rat remained in the "bright" compartment for 60 seconds on two consecutive trials (the criterion for learning) or until 15 trials has been completed. The latency to enter the dark compartment or the maximum 60-second interval was recorded for each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval, and the criterion was the same for both days of testing. Dosage groups were compared for the following dependent measures: the number of trials to the criterion in the first session (this measure was used to compare groups for overall learning performance), the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first test session (this measure was used to compare groups for activity levels and exploratory tendencies in novel environment), the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session (this measure was used to compare groups for short-term retention), the number of trials to the criterion in the second test session (this measure was used to compare groups for long-term retention) and the latency (in seconds) to enter the "dark" compartment form the "bright" compartment on trial 1 in the second session (this value was another indication of long-term retention).

M-maze test:

A watertight 16-gauge stainless steel modified M-maze was filled with water to a depth of approximately 9 inches; the water was monitored for temperature (range of 21 ± 1°C). On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal and then remove from the water. A 15-second interval separated each trial. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured

in seconds) to choose the correct goal or the maximum 60-second intervals was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval; the correct goal and the criterion were the same for both test sessions. Dosage groups were compared for the following dependent measures: the number of trials to criterion on the first day of testing (this measure was used to compare groups for overall learning performance), the latency 9in seconds) to reach the correct goal on trial 2 of the first day of testing (this measure was used to compare groups for short-term retention), the number of trials to criterion on the second day of testing (this measure was used to compare groups for long-term retention), the average number of errors for each trial on the second day of testing (this measure was also used to compare groups for long-term retention) and the latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing (this was another indication of long-term retention).

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

/s/

Ita Yuen
10/24/2006 01:22:06 PM
PHARMACOLOGIST

James Farrelly
10/24/2006 01:29:36 PM
PHARMACOLOGIST