NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Genetically Modified Model Report on the

Toxicology and Carcinogenicity Study of Mixtures of 3′-Azido-3′-Deoxythymidine (AZT), Lamivudine (3TC), and Nevirapine (NVP) (CASRNs 30516-87-1, 134678-17-4, 129618-40-2) in Genetically Modified C3B6.129F1-*Trp53*^{tm1Brd} N12 Haploinsufficient Mice (*In Utero* and Postnatal Gavage Study)

NTP GMM 16

OCTOBER 2013

NTP REPORT

ON THE

TOXICOLOGY AND CARCINOGENICITY STUDY OF MIXTURES OF 3′-AZIDO-3′-DEOXYTHYMIDINE (AZT), LAMIVUDINE (3TC), AND NEVIRAPINE (NVP)

(CAS Nos. 30516-87-1, 134678-17-4, 129618-40-2)

IN GENETICALLY MODIFIED C3B6.129F1-*Trp53*^{tm1Brd} N12 HAPLOINSUFFICIENT MICE

(*IN UTERO* AND POSTNATAL GAVAGE STUDY)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

October 2013

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National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Genetically Modified Model (GMM) Report series began in 2005 with studies conducted by the NTP. The studies described in the GMM Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies. Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP GMM Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP GMM Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov) or in hardcopy upon request from the NTP Central Data Management group at cdm@niehs.nih.gov or (919) 541-3419.

CONTRIBUTORS

The study on 3′-azido-3′-deoxythymidine, lamivudine, and nevirapine was conducted at the Food and Drug Administration's (FDA) National Center for Toxicological Research (NCTR) under an interagency agreement between the FDA and the National Institute of Environmental Health Sciences (NIEHS). The studies were monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA centers, NIEHS, and other *ad hoc* members from other governmental agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers with information for hazard identification and risk assessment.

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CONTENTS

SUMMARY

Background

3′ Azido-3′-deoxythymidine (AZT) is the most widely used chemotherapeutic agent for the treatment of people with acquired immune deficiency syndrome (AIDS) or positive for the human immunodeficiency virus (HIV). AZT treatment is also given to prevent transmission of HIV from pregnant mothers to children before or during birth, and current treatments involve combinations of drugs. Among the most commonly used are lamivudine (3TC) and nevirapine (NVP). We tested the effects of these three drugs, both individually and in combination, on the offspring of female mice (genetically modified to be sensitive to cancer induction) where the mothers were given the drugs during pregnancy.

Methods

We exposed groups of haploinsufficient C3B6.129F1-*Trp53*^{tm1Brd} N12 mice by depositing solutions containing AZT, 3TC, NVP, or combinations of the drugs in a methylcellulose solvent directly into the animals' stomachs through a tube twice daily for 28 days following birth; in addition, their mothers were exposed to the drug for seven days during pregnancy. Other sets of mothers and pups received only the methylcellulose solvent and served as the control groups. Following the dosing period, the animals were observed until 45 weeks of age. Tissues from 34 organs were examined for every animal.

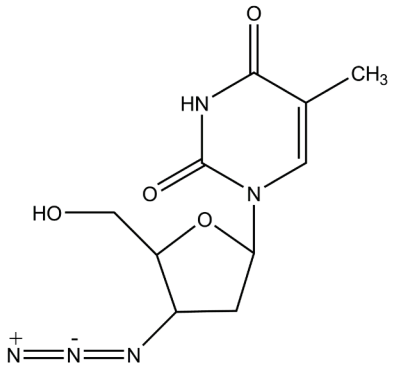
Results

Exposure to AZT caused increases in the rates of liver cancer (hepatocellular adenoma) in the male pups after 45 weeks; groups of males receiving AZT and 3TC or AZT, 3TC, and NVP also had increased rates of hepatocellular adenomas and carcinomas. In addition, there were occurrences of a few malignant lymphomas in male pups receiving AZT or AZT, 3TC, and NVP and in female pups receiving NVP, AZT plus 3TC, or the three drugs combined.

Conclusions

We conclude that AZT either alone or in combination with 3TC or with 3TC and NVP caused liver cancers in male pups exposed before and following birth. Malignant lymphomas in male and female pups may have been related to exposure to AZT alone or in combination with 3TC before and following birth.

ABSTRACT

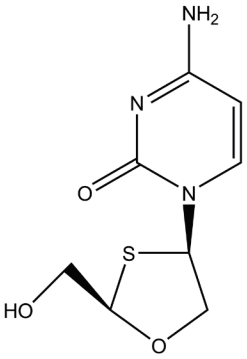


3′-AZIDO-3′-DEOXYTHYMIDINE

CAS No. 30516-87-1

Chemical Formula: $C_{10}H_{13}N_5O_4$ Molecular Weight: 267.24

Synonyms: 3′-azido-2′,3′-dideoxythymidine; azidodeoxythymidine; azidothymidine; 3′-azidothymidine; AZT; BW A509U; Compound S; 3′-deoxy-3′-azidothymidine; 3′-deoxy-(8CI) (9CI); ZDV; zidovudine.
Trade name: Retrovir® [Combivir® with 3TC]

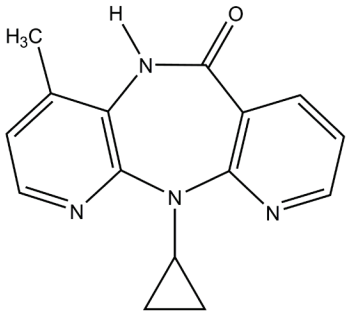


2′,3′-DIDEOXY-3′-THIACYTIDINE

CAS No. 134678-17-4

Chemical Formula: $C_8H_{11}N_3O_3S$ Molecular Weight: 229.26

Synonyms: 3TC; 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one; L-2′,3′-dideoxy-3′-thiacytidine; lamivudine
Trade name: Epivir® [Combivir® with AZT]



NEVIRAPINE

CAS No. 129618-40-2

Chemical Formula: $C_{15}H_{14}N_4O$ Molecular Weight: 266.30

Synonyms: NVP; 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*- dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one
Trade Name: Viramune®

3′-Azido-3′-deoxythymidine (AZT) is the most widely used and evaluated chemotherapeutic agent for the treatment of persons with acquired immune deficiency syndrome (AIDS). Antiviral therapy is essential for treatment and prevention of AIDS in adults and children infected with human immunodeficiency virus (HIV), and to prevent mother-to-child transmission of HIV during pregnancy and labor. The studies described in this report were designed to determine possible long-term sequelae from AZT treatment, often used in combination with other antiviral drugs, such as lamivudine (3TC) and nevirapine (NVP) in preventing mother-to-child transmission of HIV.

Male and female heterozygous F1 $p53^{+/-}$ mice were exposed to AZT, 3TC, NVP, or combinations of the chemicals *in utero* on gestation days (GD) 12 through 18, then administered the same chemical or combination of chemicals by gavage from postnatal day (PND) 1 through PND 28 and then observed until 45 weeks of age. Vehicle control mice received only an aqueous solution containing 0.2% methylcellulose and 0.1% Tween 80. Mice were dosed twice daily until PND 28. Genetic toxicology studies were conducted in mouse peripheral blood erythrocytes.

The study compared three combination doses of AZT, 3TC and NVP (AZT/3TC/NVP-L, AZT/3TC/NVP-M, and AZT/3TC/NVP-H) with the vehicle controls, and compared the individual components with each other at the highest dose (AZT-H, 3TC-H, NVP-H, AZT/3TC-H and AZT/3TC/NVP-H). Because exposure to AZT/3TC/NVP-M and AZT/3TC/NVP-H reduced pup survival, additional litters were required to provide sufficient pups for the 45-week study.

45-WEEK STUDY

In general, survival was relatively high once the pup exposure phase had been completed, with at least 75% of the mice surviving to terminal sacrifice in all groups. For males, survival was significantly greater in the AZT/3TC/NVP-L and AZT/3TC/NVP-M groups relative to the vehicle control group. There were no significant differences in survival between high dose groups of the constituent chemicals in either sex; however, survival of females in the AZT/3TC-H group was significantly less than that in the vehicle control group. Early deaths were predominantly associated with occurrences of malignant lymphoma, mammary gland tumors, and osteosarcomas.

In the combination dose comparison, males and females dosed with the AZT/3TC/NVP-H combination had significantly decreased body weights compared to the vehicle control groups from PND 11 when individual monitoring began until 20 (males) or 11 (females) weeks. In addition, mean body weights for the male and female AZT/3TC/NVP-M groups were significantly less than those of the vehicle control groups until 14 weeks. In the high dose comparison, mean body weights of the male and female AZT-H groups were significantly less than those of the vehicle control groups during some of the early weeks of dosing.

In male and female mice, absolute brain weights of the combination dose groups decreased with increasing dose and, except in low dose males, the absolute brain weights of the dosed groups were significantly less than those of the vehicle control groups. When the high doses of the constituent chemicals were compared, absolute brain weights of the male and female AZT-H and AZT/3TC/NVP-H groups were significantly less than those of the vehicle control groups. However, relative brain weights were not significantly altered. Relative liver weights of male combination dose groups followed a positive trend with dose. When the high dose groups were compared, increases in relative liver weights of male mice appeared to be associated with AZT exposure. In combination dose groups, the absolute heart weight of AZT/3TC/NVP-H females was significantly greater than that of the vehicle control group, and there was a positive trend in absolute heart weights. There was also a positive trend for relative heart weights in these combination dose groups, though no individual group relative weight was significantly greater than that of the vehicle control group. In females, absolute heart weight was also significantly increased in the AZT/3TC-H group relative to the vehicle control group.

A small but statistically significant increase in serum alanine aminotransferase activity was observed in the male AZT/3TC/NVP-H group compared to the vehicle control group.

In the combination dose comparison, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in the liver of all groups of males dosed with AZT/3TC/NVP were significantly increased compared to the vehicle control group.

In the high dose comparison, the incidences of hepatocellular adenoma in males in the AZT-H group and hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in males in the AZT/3TC-H and AZT/3TC/NVP-H groups were significantly greater than those in the vehicle control group; the incidences of these lesions in the 3TC-H and NVP-H groups were significantly less than those in the AZT/3TC/NVP-H group.

The incidences of malignant lymphoma in males administered AZT-H or AZT/3TC-H and in females administered AZT/3TC/NVP-M, AZT/3TC/NVP-H, NVP-H, or AZT/3TC-H were slightly greater than those in the vehicle control groups. The incidence of mammary gland adenoacanthoma or adenocarcinoma (combined) in females administered 3TC-H was slightly greater than that in the vehicle control group.

GENETIC TOXICOLOGY

In the peripheral blood of 1-day-old male and female mice, the percentage of total reticulocytes (RETs) was significantly decreased in groups exposed to doses that contained AZT. In addition, the percentages of micronucleated normochromatic erythrocytes (NCEs) and micronucleated RETs were generally significantly increased in groups exposed to doses containing AZT, but not in the 3TC-H or NVP-H groups. The percentages of micronucleated NCEs in the AZT/3TC/NVP-H groups were greater than in the AZT-H and the AZT/3TC-H groups. In peripheral blood of male pups evaluated at PND 28, both the percentage of micronucleated RETs and the percentage of micronucleated NCEs were significantly increased in the group where 3TC was coadministered with AZT compared to the group administered only AZT.

CONCLUSIONS

Under the conditions of this gavage study, there was *clear evidence of carcinogenic activity** of AZT alone in male heterozygous F1 $p53^{+/-}$ mice based on increased incidences of hepatocellular adenoma. There was clear evidence of carcinogenic activity of AZT in combination with 3TC, and AZT in combination with 3TC and NVP in male heterozygous F1 $p53^{+/-}$ mice based on increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). The occurrence of malignant lymphoma may have been related to treatment with AZT alone and with AZT in combination with 3TC.

There was *no evidence of carcinogenic activity* of 3TC alone in male heterozygous F1 $p53^{+/-}$ mice administered 150 mg/kg. There was *no evidence of carcinogenic activity* of NVP alone in male heterozygous F1 $p53^{+/-}$ mice administered 168 mg/kg.

There was *equivocal evidence of carcinogenic activity* of NVP alone, AZT in combination with 3TC, and AZT in combination with 3TC and NVP in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of malignant lymphoma. There was *equivocal evidence of carcinogenic activity* of 3TC alone in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of mammary gland adenoacanthoma or adenocarcinoma (combined).

There was *no evidence of carcinogenic activity* of AZT alone in female heterozygous F1 $p53^{+/-}$ mice administered 240 mg/kg.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Peer Review Panel comments and the public discussion on this Report appears on page 15.

Summary of the 45-Week Carcinogenesis and Genetic Toxicology Studies of AZT, 3TC, and NVP

	Male	Female
Doses in aqueous methylcellulose/Tween 80 by gavage		
Dams (GDs 12-18) and Pups PNDs (11-28)		
Vehicle Control	—	—
AZT-H	240 mg/kg per day	240 mg/kg per day
3TC-H	150 mg/kg per day	150 mg/kg per day
NVP-H	168 mg/kg per day	168 mg/kg per day
AZT/3TC-H	240/150 mg/kg per day	240/150 mg/kg per day
AZT/3TC/NVP-L	80/50/56 mg/kg per day	80/50/56 mg/kg per day
AZT/3TC/NVP-M	160/100/112 mg/kg per day	160/100/112 mg/kg per day
AZT/3TC/NVP-H	240/150/168 mg/kg per day	240/150/168 mg/kg per day
Pups (PNDs 1-3)		
Vehicle Control	—	—
AZT-H	120 mg/kg per day	120 mg/kg per day
3TC-H	75 mg/kg per day	75 mg/kg per day
NVP-H	21 mg/kg per day	21 mg/kg per day
AZT/3TC-H	120/75 mg/kg per day	120/75 mg/kg per day
AZT/3TC/NVP-L	40/25/7 mg/kg per day	40/25/7 mg/kg per day
AZT/3TC/NVP-M	80/50/14 mg/kg per day	80/50/14 mg/kg per day
AZT/3TC/NVP-H	120/75/21 mg/kg per day	120/75/21 mg/kg per day
Pups (PNDs 4-10)		
Vehicle Control	—	—
AZT-H	120 mg/kg per day	120 mg/kg per day
3TC-H	75 mg/kg per day	75 mg/kg per day
NVP-H	84 mg/kg per day	84 mg/kg per day
AZT/3TC-H	120/75 mg/kg per day	120/75 mg/kg per day
AZT/3TC/NVP-L	40/25/28 mg/kg per day	40/25/28 mg/kg per day
AZT/3TC/NVP-M	80/50/56 mg/kg per day	80/50/56 mg/kg per day
AZT/3TC/NVP-H	120/75/84 mg/kg per day	120/75/84 mg/kg per day
Body Weights		
Combination Dose Comparison[a]	AZT/3TC/NVP-M and AZT/3TC/NVP-H groups less than the vehicle control group from PND 11 to weeks 14 and 20, respectively	AZT/3TC/NVP-M and AZT/3TC/NVP-H groups less than the vehicle control group from PND 11 to weeks 14 and 11, respectively
High Dose Comparison[b]	AZT-H group less than the vehicle control group during the early weeks of dosing; AZT/3TC/NVP-H group less than the vehicle control group from PND 11 to week 20	AZT-H group less than the vehicle control group during the early weeks of dosing; AZT/3TC/NVP-H group less than the vehicle control group from PND 11 to week 11
Survival rates		
Combination Dose Comparison	20/25, 24/25, 22/25, 22/25	25/25, 22/25, 19/25, 21/25
High Dose Comparison	20/25, 18/25, 23/25, 22/26, 21/25, 22/25	25/25, 20/25, 20/25, 20/25, 19/25, 21/25
Nonneoplastic effects		
Combination Dose Comparison	None	None
High Dose Comparison	None	None

Summary of the 45-Week Carcinogenesis and Genetic Toxicology Studies of AZT, 3TC, and NVP

	Male	Female
Neoplastic effects		
AZT-H	Liver: hepatocellular adenoma (1/25, 8/23)	None
3TC-H	None	None
NVP-H	None	None
AZT/3TC-H	Liver: hepatocellular adenoma (1/25, 9/25); hepatocellular adenoma or carcinoma (1/25, 10/25)	None
AZT/3TC/NVP- L,M,H	Liver: hepatocellular adenoma (1/25, 7/25, 7/23, 9/23); hepatocellular adenoma or carcinoma (1/25, 9/25, 8/23, 10/23)	None
Equivocal findings		
AZT-H	All organs: malignant lymphoma (1/25, 3/24)	None
3TC-H	None	Mammary gland: adenoacanthoma or adenocarcinoma (1/25, 4/25)
NVP-H	None	All organs: malignant lymphoma (2/25, 5/25)
AZT/3TC-H	All organs: malignant lymphoma (1/25, 3/25)	All organs: malignant lymphoma (2/25, 4/24)
AZT/3TC/NVP-L,M,H	None	All organs: malignant lymphoma (2/25, 2/25, 4/22, 4/25)
Level of evidence of carcinogenic activity		
AZT	Clear evidence	No evidence
3TC	No evidence	Equivocal evidence
NVP	No evidence	Equivocal evidence
AZT/3TC	Clear evidence	Equivocal evidence
AZT/3TC/NVP	Clear evidence	Equivocal evidence
Genetic toxicology		
Total reticulocytes		
Male and female mice peripheral blood *in vivo,* PND 1:	Negative in groups exposed to AZT	
Micronucleated normochromatic erythrocytes and reticulocytes		
Male and female mice peripheral blood *in vivo,* PND 1:	Positive in AZT-H, AZT/3TC-H, AZT/3TC/NVP-M, and AZT/3TC/NVP-H groups	
Male mouse peripheral blood *in vivo,* PND 28:	Positive in AZT/3TC-H group	

[a] Vehicle control, AZT/3TC/NVP-L, AZT/3TC/NVP-M, AZT/3TC/NVP-H

[b] Vehicle control, AZT-H, 3TC-H, NVP-H, AZT/3TC-H, AZT/3TC/NVP-H

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Report on AZT, 3TC, and NVP on February 9, 2012, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW PANEL COMMENTS

On February 9, 2012, the draft Report on the study of mixtures of 3′-azido-3′-deoxythymidine (AZT), lamivudine (3TC), and nevirapine (NVP) received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.E.A. Leakey, FDA, reviewed information on the toxicity and carcinogenicity of AZT in rodents and humans, including absorption, distribution, metabolism and excretion issues and mechanisms of AZT-induced toxicity in eukaryotic cells. He presented information regarding AZT human toxicity, noting that long-term consequences of perinatal exposure to AZT are unknown. He provided background information regarding the development of the C3B6.129F1-*Trp53*^{tm1Brd} N12 haploinsufficient mouse model, which is designed to develop tumors at an increased rate and thus shorten the duration of carcinogenicity studies.

The study reported in GMM 16 was designed to test AZT in combination with 3TC and NVP. Mice were dosed twice per day with AZT, 3TC, and NVP alone or in combination from gestation day 12 until postnatal day 28. The study was designed to more closely mimic the clinical situation where human infants are dosed with drug combinations twice daily, but only prenatally and in infancy. Dr. Leakey presented the results of the study, including a significant increase in liver tumors.

The proposed conclusions for the GMM 16 study were *clear evidence of carcinogenic activity* of AZT alone in male heterozygous F1 p53$^{+/-}$ mice; *clear evidence of carcinogenic activity* of AZT in combination with 3TC, and AZT in combination with 3TC and NVP in male heterozygous F1 p53$^{+/-}$ mice; *no evidence of carcinogenic activity* of 3TC alone in male heterozygous F1 p53$^{+/-}$ mice administered 150 mg/kg; *no evidence of carcinogenic activity* of NVP alone in male heterozygous F1 p53$^{+/-}$ mice administered 168 mg/kg; *equivocal evidence of carcinogenic activity* of NVP alone, AZT in combination with 3TC, and AZT in combination with 3TC and NVP in female heterozygous F1 p53$^{+/-}$ mice; *equivocal evidence of carcinogenic activity* of 3TC alone in female heterozygous F1 p53$^{+/-}$ mice; and *no evidence of carcinogenic activity* of AZT alone in female heterozygous F1 p53$^{+/-}$ mice administered 240 mg/kg.

Dr. Olivero, the first primary reviewer, expressed concern that this mouse model may not be appropriate to evaluate the carcinogenic potential of the drugs. She acknowledged that the studies were very complex. She was also concerned that the model did not produce anemia, because that has been a signature of other studies. She suggested that, in the future, the animals' micronuclei should be examined to determine whether they have intact chromosomes. She also suggested adding protease inhibitors to future studies, because some evidence has shown that they have a protective anticarcinogenic effect.

Dr. Anderson, the second primary reviewer, called GMM 16 a remarkable study. She particularly appreciated the extra information on litter and paternal effects. She had no major scientific criticisms and agreed with the proposed conclusions.

Dr. Elwell, the third primary reviewer, agreed with the previous reviewers and concurred with the proposed conclusions. He suggested discussion in the report on the microscopic finding of centrilobular degeneration and inclusion of more information on the increased severity of vacuolization in the liver. He also noted inconsistency in the discussion of nonneoplastic findings.

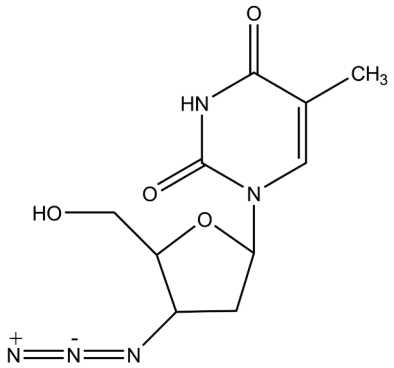
Dr. Leakey replied that tests for anemia had not been conducted to avoid adding stress to the pups, which were already in a toxic environment. He agreed to add discussion of AZT lymphoma protection and to clarify the discussion and treatment of nonneoplastic findings. Dr. G.R. Olson, Toxicologic Pathology Associates, noted that the liver tumors were quite distinct. Dr. Leakey mentioned that in the GMM series the severity scores for graded nonneoplastic lesions were not in the report, but were available on the NTP website. Dr. Elwell asked whether the other liver findings had an impact on tumor response, and if there was something unusual about this study in that most of the animals, including controls, had liver degeneration. Dr. Olson agreed that it was not a typical response. After further discussion, it was recommended that the severity score information should be incorporated into the report.

Dr. Anderson moved to approve the conclusions as written and Dr. Olivero seconded. The panel voted unanimously to accept the conclusions as written.

Dr. Bucher asked the panel for recommendations concerning future transgenic model studies. Dr. Olivero

noted that in her research program, the models, dosing, and other aspects were evolving, generating very different types of data than traditional studies. Dr. Cattley suggested formulating some type of guidance, because the studies are not full lifetime studies, the group sizes are smaller, and the genetic background is unusual. He noted what seemed to him to be a discrepancy between the intention to generate large changes in tumor incidence and the actual results, which had a more limited range. Dr. Anderson inquired whether the genetic models are in fact faster and cheaper, since they have their own special issues. Dr. Bucher said that the savings are not as great as had been anticipated, and said it remained an open question whether using the models would ultimately be cost-effective and rapid enough to actually influence clinical decision making in the use of the therapeutics and the evaluation of exposed children. Dr. Anderson suggested that there are classical models that should be considered in this context.

INTRODUCTION

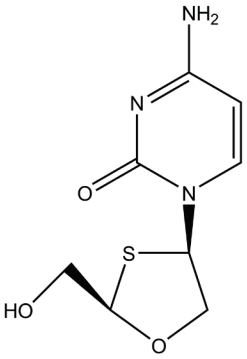


3′-AZIDO-3′-DEOXYTHYMIDINE

CAS No. 30516-87-1

Chemical Formula: $C_{10}H_{13}N_5O_4$ Molecular Weight: 267.24

Synonyms: 3′-azido-2′,3′-dideoxythymidine; azidodeoxythymidine; azidothymidine; 3′-azidothymidine; AZT; BW A509U; Compound S; 3′-deoxy-3′-azidothymidine; 3′-deoxy-(8CI) (9CI); ZDV; zidovudine.
Trade name: Retrovir® [Combivir® with 3TC]

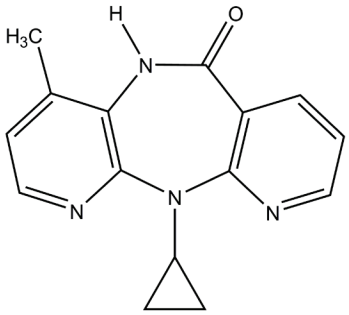


2′,3′-DIDEOXY-3′-THIACYTIDINE

CAS No. 134678-17-4

Chemical Formula: $C_8H_{11}N_3O_3S$ Molecular Weight: 229.26

Synonyms: 3TC; 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one; L-2′,3′-dideoxy-3′-thiacytidine; lamivudine
Trade name: Epivir® [Combivir® with AZT]



NEVIRAPINE

CAS No. 129618-40-2

Chemical Formula: $C_{15}H_{14}N_4O$ Molecular Weight: 266.30

Synonyms: NVP; 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*- dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one
Trade Name: Viramune®

CHEMICAL AND PHYSICAL PROPERTIES

AZT

3′-Azido-3′-deoxythymidine (AZT) is a dideoxynucleoside of thymine and a structural analogue of 2′-deoxythymidine, and it is the most widely used and evaluated chemotherapeutic agent for the treatment of persons with acquired immune deficiency syndrome (AIDS) and persons seropositive for human immunodeficiency virus (HIV). AZT is a white to off-white, odorless, crystalline solid that is moderately soluble in water (20 mg/mL) and alcohol (71 mg/mL) at 25° C. Aqueous solutions of AZT are clear to pale yellow and are mildly acidic (e.g., pH 5.5 for a 10 mg AZT/mL solution) (NTP, 2006).

3TC

Lamivudine (3TC) is an (–) enantiomer analogue of cytidine. 3TC is a white to off-white crystalline solid, with a solubility of approximately 70 mg/mL in water at 20° C (*PDR*, 2010a). It has a melting point of 160° to 162° C after recrystallization from ethanol (*Merck*, 2006a).

NVP

Neverapine (NVP) is a white to off-white crystalline powder (*PDR*, 2010b). At neutral pH, NVP has a solubility in water of approximately 100 µg/mL; it is highly soluble in water at pHs below 3. NVP has a melting point of 247° to 249° C after recrystallization from aqueous pyridine (*Merck*, 2006b) or ethyl acetate (Hargrave *et al.,* 1991).

PRODUCTION, USE, AND HUMAN EXPOSURE

AZT

AZT was first synthesized in 1964 by Horwitz *et al.* (1964), and it was subsequently reported by Mitsuya *et al.* (1985) to inhibit HIV replication *in vitro* at concentrations ranging from 50 to 500 nmol/L. Clinical activity for the treatment of AIDS was first reported by Yarchoan *et al.* (1986), and the drug was commercially developed by Burroughs Wellcome Company (Research Triangle Park, NC), under the trade name Retrovir®, which was approved by the Food and Drug Administration in March 1987, as the first nucleoside analogue reverse transcriptase inhibitor (NRTI) for the treatment of adult patients with AIDS or advanced AIDS-related complex (Anonymous, 1987; Brook, 1987). By May 2007, patent restrictions on all forms of AZT had expired allowing the United States Food and Drug Administration (FDA) to complete approval of a full range of generic alternatives for patented AZT formulations (Anonymous, 2007). Currently, AZT is available in capsules or syrup for oral administration and in formulations suitable for intravenous infusion. These include: solutions containing 10 mg AZT/mL for oral or intravenous administration, and capsules of either 100 or 300 mg AZT alone, or combination capsules containing either 300 mg AZT with 150 mg 3TC, or 300 mg AZT with 150 mg of 3TC and 300 mg of the NRTI, abacavir (DHHS, 2008a).

Antiviral therapy is essential for treatment and prevention of AIDS in adults and children infected with HIV,

and to prevent mother-to-child transmission of HIV during pregnancy and labor (DHHS, 2008a,b). AZT is a primary drug of choice for pediatric prophylactic monotherapy in infants at risk for contracting HIV and as part of triple drug combination Highly Active Antiretroviral Therapy (HAART) for infants and children infected with HIV (Cansaran *et al.*, 2006). Typical pediatric doses range from 1.5 mg AZT/kg body weight by intravenous infusion or 2 mg/kg orally every 12 hours for term neonates to 100 mg, three times daily for children weighing more than 30 kg (Cansaran *et al.*, 2006). When included as part of a therapeutic regimen to prevent mother-to-child transmission of HIV, AZT has been shown to prevent the vertical transmission of HIV by nearly 70% (7.2% in treated patients versus 21.9% in a placebo control group) (Connor *et al.*, 1994). AZT is currently a primary drug of choice for combination HAART or prophylactic treatment in pregnant women who are HIV positive, and it is the primary drug of choice for intravenous infusion during labor (PHS, 2008).

In 2007 there were an estimated 30 to 36 million people living with HIV infections worldwide, of which approximately half were women (UNAIDS, 2008). During 2007, 2.2 to 3.2 million new HIV infections occurred while 1.8 to 2.3 million people died from causes related to HIV. Estimates in 2007 suggested that there were 0.69 to 1.9 million people living with HIV infections in the United States, of which 140,000 to 400,000 were women (UNAIDS, 2008). Earlier estimates (WHO, 2004) have suggested that worldwide, some 2.2 million HIV-infected women give birth each year and that approximately 700,000 of their neonates become infected.

3TC

3TC was initially synthesized as a racemate in 1991 (Soudeyns *et al.*, 1991) and then in enantiomerically pure forms in 1992 (Beach *et al.*, 1992; Humber *et al.*, 1992). 3TC (as 3TC 5′-triphosphate) is thought to inhibit viral reverse transcriptase by competing with deoxycytidine 5′-triphosphate for incorporation into human immunodeficiency virus type-1 (HIV-1) DNA (Perry and Faulds, 1997). When used for the management of HIV-1 infections, 3TC is recommended to be used in combination with another NRTI (e.g., AZT) and either a protease inhibitor such as lopinavir/ritonavir, or a nonnucleoside reverse transcriptase inhibitor such as NVP (DHHS, 2011). In adults, the recommended daily dose is 300 mg, in either one or two doses (*PDR*, 2010a). Pediatric patients older than 3 months of age are given 4 mg/kg, twice daily, up to a maximum daily dose of 300 mg. Pregnant HIV-1-positive women are administered 3TC (150 mg twice daily) in combination with AZT beginning at 32 weeks of gestation; their offspring receive 2 mg 3TC, twice daily, until 6 weeks of age (DHHS, 2008b). 3TC is also administered in combination with AZT, tenofovir, stavudine, or didanosine for postexposure prophylaxis of HIV-1 infection in individuals who are exposed to HIV-1 either occupationally or nonoccupationally (DHHS, 2008a). These regimens can be expanded by the inclusion of a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor. 3TC is also used for the management of chronic hepatitis B virus; clinical trials indicate that 100 mg daily is more efficacious than 20 mg daily (*PDR*, 2010a).

NVP

NVP, a nonnucleoside reverse transcriptase inhibitor (Merluzzi *et al.*, 1990), was first synthesized in 1991 (Hargrave *et al.*, 1991). NVP inhibits HIV-1 reverse transcriptase noncompetitively by binding to an allosteric site on the enzyme (Cohen *et al.*, 1991; Wu *et al.*, 1991). This action is specific for HIV-1 reverse transcriptase (Merluzzi *et al.*, 1990; Koup *et al.*, 1991; Richman *et al.*, 1991).

NVP is usually given as part of a three-drug regimen. Typical regimens in adults and adolescents include NVP and 3TC or emtricitabine and AZT or tenofovir (DHHS 2008a,b; PHS, 2008). The recommended initial dose of NVP is 200 mg daily for the first 14 days and then 200 mg twice daily (*PDR*, 2010b). In pediatric patients, the recommended initial dose is 4 mg/kg body weight per day for the first 14 days and then 7 mg/kg twice daily for children less than 8 years of age or 4 mg/kg twice per day for children 8 years of age and older, with the total dose not to exceed 400 mg per day (*PDR*, 2010b). NVP is also given to prevent mother-to-child transmission of HIV-1. In women who have not received prior antiretroviral therapy, this typically involves a single 200 mg dose at the onset of labor followed by a single 2 mg/kg dose to the infant (PHS, 2008). NVP is also used as part of the three-part AZT regimen to prevent mother-to-child transmission of HIV-1.

PHARMACOLOGY

AZT

The antiviral activity of AZT depends on its conversion to a nucleotide triphosphate (3′-azido-2′,3′-dideoxythymidine 5′-triphosphate or AZTTP). AZT enters mammalian cells by nonfacilitated diffusion (Zimmerman *et al.*, 1987), and it is then phosphorylated

in successive reactions primarily catalyzed in proliferating cells by thymidine kinase 1, thymidylate kinase, and nucleoside diphosphokinase present in cell cytosol, resulting in AZTTP (Avramis *et al.*, 1989; Törnevik *et al.*, 1995; Bradshaw *et al.*, 2005). AZTTP is a substrate for HIV reverse transcriptase and a competitive inhibitor of deoxythymidine triphosphate. Because the 3′ position of AZT is blocked with an azido group, incorporation of AZTTP into a growing polynucleotide chain (e.g., viral DNA) terminates elongation at that position. Thus, AZT intervenes at a relatively early stage of the viral replication cycle. AZTTP is also a substrate for cellular DNA polymerases; however, the K_i and K_m of AZTTP for HIV reverse transcriptase are considerably lower than for cellular DNA polymerases. Accordingly, AZTTP inhibits viral replication at doses lower than those at which it is an efficient substrate for the cellular DNA polymerases (Furman *et al.*, 1986; Huang *et al.*, 1990; Parker *et al.*, 1991).

3TC

3TC is converted to an active antiretroviral agent by sequential 5′-phosphorylation to 3TC 5′-phosphate (catalyzed by deoxycytidine kinase) (Shewach *et al.*, 1993), 3TC 5′-diphosphate, and 3TC 5′-triphosphate (catalyzed by unspecified kinases) (Cammack *et al.*, 1992; Hart *et al.*, 1992). In a manner similar to AZT, 3TC 5′-triphosphate is thought to inhibit HIV-1 by acting as a competitive inhibitor for HIV-1 reverse transcriptase (K_i = 0.57 to 12 µM) (Hart *et al.*, 1992; Riska *et al.*, 1999a) and by causing chain termination upon incorporation into proviral DNA (Perry and Faulds, 1997). 3TC 5′-triphosphate is also a substrate for mammalian DNA polymerases α, β, γ, and ε, with K_is of 110 to 175, 10 to 25, 4 to 44, and 120 µM, respectively (Hart *et al.*, 1992; Martin *et al.*, 1994; Riska *et al.*, 1999a; Kakuda, 2000).

NVP

In contrast to AZT, 3TC, and other NRTIs, which require metabolic conversion to triphosphate derivatives in order to inhibit HIV-1 reverse transcriptase, NVP binds directly to the enzyme. This interaction is not through the reverse transcriptase catalytic site, but rather through an adjacent pocket that appears to involve two lysine residues. The interaction, which is noncompetitive in nature, does not prevent the binding of nucleoside triphosphate substrates, but rather prevents the formation of a productive complex (Cohen *et al.*, 1991; Koup *et al.*, 1991; Wu *et al.*, 1991; Smerdon *et al.*, 1994; Spence *et al.*, 1995). The K_i of NVP for HIV-1 reverse transcriptase is 200 nM, and it shows no inhibitory activity against mammalian DNA polymerases α, β, γ, or δ (Merluzzi *et al.*, 1990).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

AZT

Following oral administration, AZT is rapidly absorbed, and after oral or intravenous administration it is rapidly distributed (NTP, 2006). Elimination is also rapid, with essentially all parent drug and its metabolites being completely excreted within 24 hours (Singlas *et al.*, 1989; Taburet *et al.*, 1990; Child *et al.*, 1991; Stagg *et al.*, 1992). However, there are significant interspecies differences in the extent to which the parent compound is metabolized (NTP, 2006). In humans and monkeys, the majority of an administered dose is converted to the 5′-*O*-glucuronide and eliminated in urine along with unmetabolized parent drug and a minor metabolite, 3′-amino-2′,3′-dideoxythymidine (AMT), formed by reduction of the 3′-azido group of AZT. However, in rodents, the majority of absorbed AZT is eliminated in urine as the parent compound with relatively little conversion to the glucuronide or to AMT (Singlas *et al.*, 1989; de Miranda *et al.*, 1990; Good *et al.*, 1990; Cretton *et al.*, 1991; Mays *et al.*, 1991; Stagg *et al.*, 1992; Trang *et al.*, 1993). The reduced metabolism in rodents results in equivalent doses producing greater C_{max} values than for humans, but plasma half-life values are similar to humans (Doshi *et al.*, 1989; Patel *et al.*, 1989; Trang *et al.*, 1993). The glucuronide of AMT has been reported to be a minor urinary metabolite in monkeys and a minor biliary metabolite in humans, but it has not been identified in rats (de Miranda *et al.*, 1990).

3TC

3TC is rapidly absorbed and distributed. In mice treated orally, 3TC has a T_{max} of 30 minutes and a $t_{1/2}$ of 110 minutes (Williams *et al.*, 2003), values that are appreciably greater than those observed with AZT. Comparable values after intravenous administration are 5 minutes (T_{max}) and 96 minutes ($t_{1/2}$) (Williams *et al.*, 2003). In mouse fetuses exposed transplacentally, 3TC has a T_{max} of 60 minutes and a $t_{1/2}$ of 161 minutes, the latter being considerably greater than the $t_{1/2}$ of 44 minutes observed in the dams (Von Tungeln *et al.*, 2007). In addition, the C_{max} is substantially less in the fetuses as compared to the dams (Von Tungeln *et al.*, 2007).

3TC has a $t_{1/2}$ of 105 minutes in rats treated intravenously (Alnouti *et al.*, 2005). Rat fetuses exposed transplacentally to 3TC have C_{max} and AUC values that are appreciably less than those observed in the dams (Alnouti *et al.*, 2005). Domestic cats dosed intravenously with 3TC have a $t_{1/2}$ of 114 minutes; the comparable values after intragastric and oral dosing are 150

and 138 minutes, with T_{max} values of 30 and 66 minutes, respectively (Zhang *et al.*, 2004). The oral bioavailability of 3TC in cats is 80%. Woodchucks (*Marmota monax*) treated orally or intravenously with 3TC have a $t_{1/2}$ of 170 minutes; the oral bioavailability is 18% to 54% (Rajagopalan *et al.*, 1996). In rhesus monkeys dosed intravenously, 3TC has a $t_{1/2}$ of 84 minutes (Blaney *et al.*, 1995). The T_{max} and $t_{1/2}$ of 3TC in *Erythrocebus patas* monkeys given an oral mixture of AZT and 3TC are 50 and 136 minutes, respectively (Divi *et al.*, 2008).

In humans administered 3TC orally, the T_{max} is approximately 1 hour, the $t_{1/2}$ is 3.5 to 11.5 hours, and the bioavailability is 86% (Perry and Faulds, 1997; King *et al.*, 2002; *PDR*, 2010a). The $t_{1/2}$ for 3TC in infants and children appears to be slightly shorter than in adults (Perry and Faulds, 1997; King *et al.*, 2002).

In humans and experimental animals, the majority of 3TC is excreted unchanged, primarily in the urine. The percent excreted as 3TC varies across species, with 75% being reported in rats (Rajagopalan *et al.*, 1996), 26% in woodchucks (Rajagopalan *et al.*, 1996), 32% to 59% in rhesus monkeys (Blaney *et al.*, 1995), and 68% to 71% in humans (Dudley, 1995; *PDR*, 2010a). Other than 5′-phosphate derivatives, the only reported metabolite of 3TC is 3TC sulfoxide, which has been detected in the urine of dogs and humans (Plumb *et al.*, 1996; *PDR*, 2010a).

NVP

NVP is readily absorbed following oral dosing. In chimpanzees, more than 64% of an oral dose is bioavailable (Cheeseman *et al.*, 1993); the corresponding value in humans is greater than 90% (Lamson *et al.*, 1999; *PDR*, 2010b), with a T_{max} occurring 1.3 to 4.6 hours after dosing (Cheeseman *et al.*, 1995; Lamson *et al.*, 1999; *PDR,* 2010b). Compared to AZT and 3TC, NVP is eliminated very slowly. In chimpanzees, the $t_{1/2}$ is 11 to 24 hours (Cheeseman *et al.*, 1993), while the value in humans after a single oral dose is 40 to 51 hours (Cheeseman *et al.*, 1993; Lamson *et al.*, 1999; Riska *et al.*, 1999a). A similar $t_{1/2}$ is obtained following intravenous dosing (Lamson *et al.*, 1999). Repeated administration of NVP to humans results in a decrease in $t_{1/2}$ (Riska *et al.*, 1999a), possibly resulting from autoinduction of cytochrome P450 (CYP) enzymes. The $t_{1/2}$ in infants appears to be greater than that in adults (Luzuriaga *et al.*, 1996; Mirochnick *et al.*, 1998).

The disposition, biotransformation, and elimination of NVP have been reported in mice, rats, rabbits, dogs, monkeys (cynomolgus), chimpanzees, and humans (Riska *et al.*, 1999a,b). In mice, rabbits, monkeys, and humans, urinary excretion is approximately twice that found in feces. The distribution is approximately equal in rats, and in dogs fecal excretion predominates due to poor absorption of the drug. NVP is extensively metabolized. Among the identified metabolites are 3- and 8-hydroxy-NVP, 4-hydroxymethyl-NVP (12-hydroxy-NVP), 4-carboxy-NVP, and 2-, 3-, 8-, and 12-hydroxy-NVP glucuronide. The major urinary metabolites in dogs, monkeys, chimpanzees, and humans are glucuronides, primarily of 2-, 3-, and 12-hydroxy-NVP. In rats and mice, 12-carboxy-NVP is the predominant urinary metabolite. In dogs, unchanged NVP is the primary "metabolite" found in the feces. In the other species, the major fecal metabolite is 4-carboxy-NVP or 3-hydroxy-NVP.

In humans, the formation of 2-hydroxy-NVP is attributed to the CYP3A subfamily, 3-hydroxy-NVP to CYP2B6, 8-hydroxy-NVP to CYP3A4, CYP2B6, and CYP2D6, and 12-hydroxy-NVP to CYP3A4 and, possibly, CYP2D6 and CYP2C9 (Erickson *et al.,* 1999). Recently, an NVP-glutathione conjugate has been detected upon the incubation of NVP with human liver microsomes in the presence of glutathione (Wen *et al.,* 2009). The NVP-glutathione conjugate formation was catalyzed primarily by CYP3A4 and to a lesser extent by CYP2D6, CYP2C19, and CYP2A6. The oxidation of NVP by CYP3A4 also caused mechanism-based inactivation of the enzyme. *In vitro* studies have demonstrated that NVP is a potent inducer of both CYP2A6 and CYP3A4 isoforms in primary human hepatocytes (Faucette *et al.,* 2007). The induction is mediated by both the PXR and CAR gene activation pathways, but NVP is a preferential CAR/CYP2B6 inducer rather than a PXR/CYP3A4 inducer (Faucette *et al.,* 2007). The induction of CYP3A-related monooxygenase activity also occurs in rats exposed to NVP (Walubo *et al.,* 2006).

TOXICITY

Experimental Animals

AZT

In animals, AZT causes hematologic toxicities (Thompson *et al.,* 1991) and impaired function of the electron transport chain in cardiac and skeletal muscle mitochondria (Lamperth *et al.*, 1991; Walker *et al.*, 2004). The latter are associated with morphological damage including enlarged mitochondria with disorganized or absent cristae (Lamperth *et al.,* 1991; Lewis *et al.,* 1992). In Sprague-Dawley rats administered 1 mg AZT/mL drinking water for 35 days, decreases in mitochondrial DNA, RNA, and protein synthesis were observed in skeletal muscle mitochondria (Lewis *et al.,* 1992). AZTTP is an inhibitor and alternate substrate for mitochondrial DNA polymerase γ from both skeletal

and cardiac muscle (Simpson *et al.,* 1989; Lewis *et al.,* 1994), and therefore it is possible that AZT, via AZTTP, is acting as an inhibitor and chain terminator, disrupting mitochondrial DNA synthesis. However, more recent evidence suggests that AZT is also a potent inhibitor of thymidine phosphorylation in mitochondria resulting from its inhibition of mitochondrial thymidine kinase 2 (Johnson *et al.,* 1989; Lynx and McKee, 2006; Susan-Resiga *et al.,* 2007). This enzyme plays a secondary role in the conversion of thymidine into thymidine monophosphate in rapidly dividing cells, which express cytosolic thymidine kinase 1, but in nonmitotic cells such as mature hepatocytes or cardiac myocytes, it provides the primary source of thymidine triphosphate that is required for mitochondrial DNA replication and nuclear DNA repair (Pérez-Pérez *et al.,* 2005; Samuels, 2006). AZT inhibits thymidine kinase 2 at lower concentrations (7 to 14 μM) than AZTTP inhibits DNA polymerase γ (Lynx and McKee, 2006) and pharmacologic levels of AZT would be expected to result in accumulative mitochondrial damage and increased observed mutation frequencies through this mechanism.

Heart toxicities associated with AZT treatment have been reported in rats, mice, and monkeys. For example, rats given AZT in drinking water at approximately 29 to 102 mg/kg per day for up to 49 days developed cardiac mitochondrial swelling with fractured and disrupted cristae (Lewis *et al.,* 1991). These ultrastructural defects did not reverse after a 14-day recovery period. Ultrastructural examination of cardiomyocytes of Sprague-Dawley rats given AZT in drinking water at approximately 90 mg/kg per day showed disruption of cristae and increased size of mitochondria after 30 or 60 days of treatment; no alterations were seen in rats that received 120 days of treatment (Corcuera Pindado *et al.,* 1994). Sprague-Dawley rats given intraperitoneal injections of AZT at approximately 17 to 51 mg/kg for 3 months developed enlarged cardiomyocytic mitochondria with disorganized or absent cristae and increased serum concentrations of creatine kinase, lactate, and glucose (Lamperth *et al.*, 1991).

Transgenic mice (that express replication-incompetent HIV) or FVB mice given AZT in drinking water at doses that delivered approximately 180 to 200 mg/kg for 35 days developed cardiac toxicity characterized by mitochondrial destruction (Lewis *et al.,* 2000). Treatment-related histopathologic changes were described as numerous cardiomyocytes with granular cytoplasm in normal and transgenic mice. The lesions were generally more severe in transgenic mice. Neither interstitial inflammation nor fibrosis were found. The National Toxicology Program (NTP) did not report cardiac toxicity in B6C3F1 mice given AZT by oral gavage in corn oil at doses of 0, 50, 100, 200, 800, or 2,000 mg/kg for 14 days, or at doses of 0, 30, 60, or 120 mg/kg for 2 years (NTP, 1999) However, in an NTP study where Swiss (CD-1®) mouse pups were exposed *in utero* via lactation and by direct gavage on postnatal days (PNDs) 4 through 28 with twice-daily doses of 75/37.5 mg/kg AZT/3TC or the vehicle control mixture of 0.1% polysorbate 80 and 0.2% methylcellulose, the hearts of PND 28 pups treated with AZT/3TC showed significant increases in the mean area and decreases in the mean number of cardiomyocytic mitochondria compared to vehicle controls (Bishop *et al.*, 2004a; NTP, 2006). AZT has also been shown to cause alteration in fat metabolism in rats. Male Wistar rats exposed to AZT (0.6 mg/mL in drinking water) for 4 weeks exhibited increased serum triglyceride levels and decreased cytochrome c oxidase and fatty acid synthase activities in their inguinal fat (Deveaud *et al.,* 2007).

Studies in monkeys at the National Cancer Institute showed that daily doses of AZT during the second half of gestation at approximately 86% of the recommended human daily dose caused mitochondrial abnormalities (Gerschenson *et al.*, 2000) that were similar to those observed in human neonates exposed to antiretroviral drugs (Divi *et al.,* 2007a). In skeletal muscle, these abnormalities were characterized as abnormally shaped mitochondria with disrupted cristae. In heart muscle, small mitochondria in myocytes with myofibrillar loss and abnormal alignment of sarcomeres were observed.

3TC

Transgenic mice expressing the mitochondrial deoxynucleotide carrier do not show indication of cardiac damage when treated with 3TC under conditions where AZT causes decreases in left ventricular mass and mitochondrial ultrastructure defects (Lewis *et al.,* 2006).

NVP

NVP causes an idiosyncratic skin rash in rats (Shenton *et al.,* 2003) through a process mediated by $CD4^+$ T cells (McIntyre, 2006; Popovic *et al.*, 2006). Female Brown Norway rats are the most sensitive to this response followed by female Sprague-Dawley rats (Shenton *et al.,* 2003). Higher concentrations of the drug induce the idiosyncratic response in male Brown Norway rats and female Lewis rats (Shenton *et al.,* 2004). Male Sprague-Dawley rats and female Stevens-Johnson syndrome mice appear to be resistant to the induction of the rash (Shenton *et al.,* 2003). Both NVP and the NVP metabolite 12-hydroxy-NVP induce the rash (Popovic *et al.,* 2006; Chen *et al.,* 2008), and it has been suggested that 12-hydroxy-NVP is the metabolite responsible for the rash as a result of subsequent metabolism to a quinone methide (Chen *et al.,* 2008). Rats treated orally with NVP do not have elevated serum

levels of alanine transferase, aspartate transferase, or alkaline phosphatase, but histological examination of the livers indicates hepatocellular hypertrophy, nuclear degranulation, disintegration, and vacuolation (Walubo *et al.,* 2006).

Oral or intraperitoneal treatment of mice with NVP causes a systemic sensitization to a subsensitizing dose of trinitrophenyl-ovalbumin (Nierkens *et al.,* 2005). Mice dosed orally with NVP show decreased creatine kinase activity in the cerebellum, hippocampus, striatum, and cortex of the brain (Streck *et al.,* 2008).

Humans

AZT

Exposure to AZT has resulted in myelosuppression and anemia in some human patients like that in experimental animals. In humans, this toxicity limits the useful therapeutic dose range of AZT (Fischl, 1989; Pluda *et al.,* 1991; Balzarini, 1994). The primary target of AZT toxicity is the hematopoietic system of the bone marrow; *in vitro* coculture studies have demonstrated that AZT is cytotoxic to human and mouse hematopoietic progenitor cells (Sommadossi and Carlisle, 1987; Dainiak *et al.,* 1988; Gallicchio *et al.,* 1989). In cultures of human bone marrow cells, the extent of incorporation of AZTTP into cellular DNA and the growth inhibition of human clonal peripheral blood mononuclear cells have been correlated (Sommadossi and Carlisle, 1987; Sommadossi *et al.,* 1989). In human erythroid K-562 leukemia cells induced to differentiate by butyric acid treatment, AZT selectively reduced the steady-state level of globin mRNA (Weidner and Sommadossi, 1990). Neither the kinetics of induction nor the steady-state mRNA levels of other components of the heme biosynthetic pathway were altered, including erythroid-specific isozymes of aminolevulinate synthase and porphobilinogen deaminase (Fowler *et al.,* 1995). These results suggest a specific effect on transcription of the globin gene in erythroid cells. A few patients receiving long-term AZT therapy have been reported to have toxic mitochondrial myopathy (Dalakas *et al.,* 1990). Clinical symptoms include myalgia, muscle weakness, and elevated levels of creatinine kinase in serum. These symptoms correlate with the presence in muscle biopsies of abnormal mitochondria containing paracrystalline inclusions. Human muscle myotubes grown in tissue culture exposed to AZT for 9 days exhibited increased numbers of mitochondria as well as enlarged mitochondria with abnormal cristae and electron-dense deposits in the matrix (Lamperth *et al.,* 1991).

The United States Department of Health and Human Services updates information on current treatment regimens for HIV and observed toxicities on an ongoing basis (PHS, 2008). Common adverse effects noted from AZT use in humans include bone marrow suppression, anemia and/or neutropenia, and subjective complaints including gastrointestinal intolerance, headache, insomnia, and asthenia. In addition, lactic acidosis with hepatic steatosis has been reported as a rare side effect from the NRTI components used in HAART, including AZT.

NRTIs such as AZT have been reported to produce mitochondrial dysfunction in human patients (Dalakas *et al.,* 1990; Arnaudo *et al.,* 1991; Lamperth *et al.,* 1991; Brinkman *et al.,* 1999; DHHS, 2008a), possibly resulting from inhibition of human mitochondrial DNA polymerase γ or thymidine kinase 2. Mitochondrial DNA dysfunction may result in pancreatitis, peripheral neuropathy, myopathy, and cardiomyopathy (Lim and Copeland, 2001; Moussian, 2008). It is thought that combinations of NRTIs will act synergistically to induce mitochondrial dysfunction. Protease inhibitors may also aggravate this mechanism (DHHS, 2008a). Lipodystrophy (fat redistribution syndrome) may be seen in patients receiving NRTIs, and is related to mitochondrial toxicities (Brinkman *et al.,* 1999; Kakuda *et al.,* 1999; Mallal *et al.,* 2000; DHHS, 2008a). Metabolic complications of HAART therapies include vascular necrosis, decreased bone density, and skin rashes (DHHS, 2008a). Another study suggests that mitochondrial damage in children of mothers taking AZT may persist for up to 2 years after birth (Artandi *et al.,* 2000). However, HIV infection by itself may lead to cardiac toxicity (Raidel *et al.,* 2002).

3TC

The toxicity of 3TC in humans has been reviewed (Perry and Faulds, 1997; *PDR,* 2010a). When used as monotherapy in adults and children for the treatment of HIV-1 or chronic hepatitis B virus infection, 3TC treatment can, in some instances, result in neutropenia, thrombocytopenia, peripheral neuropathy, headaches, gastrointestinal effects, and lactic acidosis.

NVP

The toxicity of NVP in humans has been reviewed (Pollard *et al.,* 1998; Mirochnick *et al.,* 2000; Murphy, 2003; Wen *et al.,* 2009). The most severe toxicity associated with NVP is hepatotoxity, which in some instances is fatal. The most common side effect is a rash consisting of maculopapular erythematous cutaneous eruptions. This effect occurs in children and adults (including pregnant women) and at times can be life threatening, leading to discontinuation of the drug. Whether or not the rash in humans is due to 12-hydroxy-NVP is currently uncertain (Hall and MacGregor, 2007). Other reported side effects are gastrointestinal disturbances and lipodystrophy.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

AZT

AZT has been shown to cross the placenta of mice (Child *et al.,* 1991) and monkeys (Ewings *et al.,* 2000). In a series of studies in rats, mice, and rabbits, AZT has been shown to cause adverse reproductive effects but no teratogenic effects. AZT was evaluated for adverse effects on reproductive and fetal development in CD (Sprague-Dawley) rats and New Zealand White rabbits (Greene *et al.,* 1996). Male and female CD rats were given twice-daily oral AZT doses of 0, 25, 75, or 225 mg/kg, approximately 6 hours apart. Males were dosed for 85 days prior to mating, and continued on dosing throughout two mating cycles for a total of 175 dosing days. Treated males were mated to females (F_0) dosed for 26 days prior to mating and throughout gestation and lactation. Early resorptions and decreased litter size were noted following parental dosing with 75 or 225 mg/kg. In a second mating, treated males were mated to untreated females and the pups were monitored for growth, survival, and developmental characteristics. All reproductive parameters were normal. The authors concluded that the embryotoxicity of AZT noted with the first mating (treated males with treated females) was not mediated by a genotoxic effect in the males. The liveborn offspring showed no developmental abnormalities or teratogenic effects. Also in these studies, pregnant New Zealand White rabbits given an oral dose of 250 mg AZT/kg body weight per day from gestational days (GDs) 6 to 18 had reduced weight gain, anemia, and increased late fetal deaths. The liveborn offspring showed no developmental abnormalities or teratogenic effects.

When pregnant CD-1® mice were given daily intragastric AZT doses of 25 mg/kg from GDs 12 to 18, no developmental toxicity was seen in the F_1 generation (Diwan *et al.,* 2000). When these treated offspring were mated to untreated offspring, the liveborn F_2 pups showed no adverse effects on reproductive parameters. Other studies have shown that AZT can cause cytotoxic effects in preimplantation mouse embryos by inhibition of blastocyst and post-blastocyst development at doses similar to human therapeutic doses (Toltzis *et al.*, 1991; DHHS, 2008b).

3TC

Transplacental treatment of rabbits with 3TC results in some evidence of embryolethality (*PDR*, 2010a). The effect is not observed in rats treated similarly; likewise, there is no indication of teratogenicity in either species (*PDR*, 2010a).

Perinatal exposure to 3TC is associated with mitochondrial toxicity in mice as indicated by a decrease in mitochondrial DNA (Chan *et al.,* 2007). *E. patas* monkeys transplacentally exposed to 3TC show evidence of morphological damage to umbilical cord artery endothelial cell mitochondria but no evidence of skeletal muscle mitochondrial morphological damage at birth (Divi *et al.,* 2007b).

Perinatal administration of 3TC and AZT to *E. patas* monkeys, in a model that mimics a dosing regimen used with pregnant women and their infants, induces cardiac and skeletal muscle mitochondrial damage to an extent that is equal to or only slightly greater than that induced by AZT alone (Divi *et al.,* 2005, 2007b). Infant *E. patas* monkeys exposed transplacentally to 3TC and AZT have substantial depletion of mitochondrial oxidative phosphorylation in heart and skeletal muscle (Gerschenson *et al.,* 2004). CD-1® mice treated perinatally with mixtures of AZT and 3TC show significant decreases in the mean number and area of cardiomyocytic mitochondria (Bishop *et al.,* 2004a); however, it is unclear if this is due to AZT, 3TC, or the combination of the two.

NVP

Transplacental treatment of rats with NVP causes significant decreases in fetal body weight (*PDR*, 2010b). There is no indication of teratogenicity with NVP in either rats or rabbits (*PDR*, 2010b).

Humans

AZT

There have been no reported increases in congenital abnormalities in infants born to women with antepartum AZT exposure (Connor *et al.,* 1994; Sperling *et al.,* 1998). The NIH panel cautioned that definitive conclusions regarding teratogenic risk cannot be thoroughly evaluated because of limited numbers of children evaluated (Corcuera Pindado *et al.,* 1994; DHHS, 2008a; PHS, 2008). When AZT crosses the human placenta, it is incorporated into the DNA of cord blood leukocytes (Olivero *et al.,* 1999).

3TC

Infants exposed *in utero*, during the third trimester, to 3TC or a combination of 3TC and AZT have exhibited mitochondrial dysfunction in some instances (Brogly *et al.,* 2007). Perinatal treatments with 3TC, in combination with AZT, causes seizures, lactic acidosis, anemia, altered cerebral pathology (based upon magnetic resonance imaging), impaired skeletal, heart, and/or liver oxidative phosphorylation, skeletal muscle mitochondrial abnormalities, and cardiomyopathy (Blanche *et al.,* 1999; Barret *et al.,* 2003; Tardieu *et al.*, 2005).

Transplacental exposure to 3TC and AZT results in morphological damage to mitochondria of umbilical cord artery endothelium and a decrease in mitochondrial DNA copy number in cord blood mononuclear cells and in umbilical cord tissue (Divi *et al.,* 2004, 2007a).

NVP

There are no adequate studies to assess the reproductive or developmental toxicity of NVP in humans (*PDR*, 2010b).

CARCINOGENICITY

Experimental Animals

AZT

Preclinical studies in rodents were conducted by GlaxoSmithKline to determine the potential for toxicity and/or cancer from exposure to AZT. AZT was administered to CD rats by oral gavage once a day at 0, 80, 220, or 600 mg/kg body weight for up to 2 years (Dainiak *et al.,* 1988). Because of anemia, the high dose was reduced to 450 mg/kg on day 91 and further reduced to 300 mg/kg on day 278. Squamous cell carcinoma of the vagina occurred in two females receiving 300 mg/kg; no vaginal tumors/hyperplasia occurred in any other group of female rats. These investigators also administered AZT to CD-1® mice by oral gavage at 0, 30, 60, or 120 mg/kg per day. Because of anemia, the doses were reduced to 0, 20, 30, or 40 mg/kg per day on day 90, where they remained for the rest of the 22-month study. The only neoplasms associated with administration of AZT in the mice were squamous cell carcinoma of the vagina in five females receiving 40 mg/kg, squamous cell papilloma of the vagina in one female receiving 30 mg/kg and in one female receiving 40 mg/kg, and one squamous polyp of the vagina in one female receiving 40 mg/kg. Although the incidences of hyperplasia of the vaginal epithelium were not increased above that in the controls, the severities of this lesion increased with increasing doses of AZT.

In order to clarify the role of AZT in producing vaginal tumors, AZT was administered intravaginally to CD-1® mice for 22 months (Ayers *et al.,* 1996a). Higher incidences of vaginal neoplasms occurred than were seen in the AZT oral gavage study in CD-1® mice reported by Dainiak *et al.* (1988). There was a retrograde flow of urine from the discharge point at the base of the vulva into the region of the vagina where the vaginal tumors occurred. In mice, 90% of AZT is eliminated in the urine as the parent compound following oral administration. Because there is a high rate of cell turnover in the vaginal epithelium as a consequence of the short estrous cycle in mice (4 to 5 days), the investigators concluded that prolonged exposure of the vaginal epithelium to the relatively high concentrations of AZT in the urine could explain the observed vaginal neoplasms. In humans, the concentration of free AZT in the urine is low, and the authors concluded that the vaginal tumors seen in mice would not necessarily be predictive of vaginal tumors in humans (Ayers *et al.,* 1996a).

The NTP has conducted 2-year studies of AZT and AZT/interferon in B6C3F1 mice (NTP, 1999). AZT was administered to male and female mice by oral gavage at doses of 0, 30, 60, or 120 mg/kg per day in two equal doses, at least 6 hours apart, 5 days per week for 105 weeks. In the AZT/interferon studies, male and female mice received AZT by oral gavage at daily doses of 0, 30, 60, or 120 mg/kg, given in two equal doses, 5 days per week for 105 weeks, and the groups receiving AZT also received subcutaneous injections of 500 or 5,000 U α-interferon A/D three times per week for 105 weeks. Additional groups of male and female mice received subcutaneous injections of the vehicle, 500 U α-interferon A/D, 5,000 U α-interferon A/D, or 5,000 U α-interferon A (all without AZT), three times per week for 105 weeks. There was equivocal evidence of carcinogenic activity of AZT in male mice based on marginally increased incidences of renal tubule and Harderian gland neoplasms in groups receiving AZT alone. There was clear evidence of carcinogenic activity of AZT in female mice based on increased incidences of squamous cell neoplasms of the vagina in groups that received AZT alone or in combination with α-interferon A/D. Hematotoxicity occurred in all groups that received AZT. Treatment with AZT alone and AZT in combination with α-interferon A/D resulted in increased incidences of epithelial hyperplasia of the vagina in all dosed groups of females.

In a follow-up transplacental exposure study (NTP, 2006), female Swiss (CD-1®) mice were dosed with 0, 50, 100, 200, or 300 mg AZT/kg per day via oral gavage of two equal doses from 10 to 14 days prior to conception until GD 19 and up to four pups (F_1 generation) from each litter were followed for 2 years and evaluated for carcinogenicity. Under the conditions of this study, there was clear evidence of carcinogenic activity in F_1 male mice exposed transplacentally to AZT based on increased incidences of alveolar/bronchiolar neoplasms, and no evidence of carcinogenic activity in F_1 female mice.

Studies by the National Cancer Institute suggest that AZT, when given at relatively high doses, is a moderately effective perinatal carcinogen in mice, targeting several tissue types (Olivero *et al.,* 1997; Diwan *et al.,* 1999). In these studies, AZT was given to CD-1® mice at doses of 12.5 or 25 mg (equivalent to up to 1,000 mg AZT/kg nonpregnant body weight or 450 mg AZT/kg of

terminal body weight) orally from GD 12 through GD 18. AZT was incorporated into nuclear and mitochondrial DNA of the fetuses. Dose-dependent increases in tumor multiplicities in the lung, liver, and female reproductive organs occurred. In a transplacental carcinogenicity study using lower AZT doses, CD-1® mice were given 20 or 40 mg AZT/kg body weight per day in the drinking water from gestation day 10 through lactation day 21 (Ayers *et al.,* 1997). In this study, some of the pups from the litters were then continued on AZT treatment by daily gavage at 20 or 40 mg/kg per day for 24 months; AZT tumor findings were limited to the vaginal epithelium.

Walker *et al.* (2007) exposed B6C3F1 mice and Fischer 344 rats to either once daily gavage doses of 0, 80, or 240 mg AZT/kg body weight or 480 mg/kg, given in two gavage doses per day, during the last week of gestation. Tumor incidences were evaluated at 24 months of age. In male mice, there were statistically significant increases in the incidences of hemangioma or hemangiosarcoma (combined) in all three dosed groups and of hepatocellular carcinoma in the highest dose group. In female mice, there were statistically significant increases in the incidences of neoplasms of the uterus in the highest dose group. In female rats, the incidences of mononuclear cell leukemia were increased in all three dosed groups.

3TC

The carcinogenicity of 3TC has been assessed following long-term administration to mice and rats (*PDR*, 2010a). There was no evidence of carcinogenicity in mice given 10 times the recommended therapeutic dose of 3TC for treating HIV-1 infection or in rats given 58 times the recommended therapeutic dose of 3TC.

NVP

The carcinogenicity of NVP has been assessed following long-term administration to mice and rats (*PDR*, 2010b). In mice administered 0, 50, 375, or 750 mg NVP/kg body weight per day, there were increased incidences of hepatocellular adenoma or carcinoma (combined) at all doses of NVP in male mice and at the two highest doses in female mice. In rats administered 0, 3.5, 17.5, or 35 mg/kg body weight per day, there were increased incidences of hepatocellular adenoma at all doses of NVP in male rats and at the highest dose in female rats.

Humans

AZT

There have been no studies reported in the literature on any association between AZT and/or HAART and cancer. However, a recent epidemiology study reported that patients with HIV or AIDS do have increased risk of developing lung cancer (Kirk *et al.,* 2007). Since the increased risk was not significantly correlated with either HAART or low $CD4^+$ cell count it is not yet known whether AZT exposure contributes to this increased cancer risk. Cancer often takes many years to develop, and follow-up of patients is continuing. An NIH panel has recommended long-term follow-up in children receiving *in utero* exposure to AZT and other antiretroviral drugs (Highleyman, 1997).

3TC

There have been no studies reported in the literature on any association between 3TC and the development of cancer in humans.

NVP

There have been no studies reported in the literature on any association between NVP and the development of cancer in humans.

GENETIC TOXICITY

AZT

AZT is a DNA-reactive chemical that is positive in the *Salmonella typhimurium* mutation assay (NTP, 1999) and has been shown to increase mutation frequencies and induce chromosomal damage in mammalian cells *in vivo* and *in vitro*. Its genotoxic effects have been extensively reviewed in previous NTP technical reports (NTP, 1999, 2006). A brief summary of the extensive genetic toxicity literature follows.

AZT was reported to be weakly positive in the mouse lymphoma cell mutagenicity test (Ayers, 1988; Olin and Kastrup, 1995) and to induce transformation in cultured mammalian cells (Olin and Kastrup, 1995). Results of *in vitro* cytogenetic assays with mammalian cells showed that AZT induced sister chromatid exchanges, chromosomal aberrations, and micronuclei in human lymphocytes, as well as chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells (Gonzalez Cid and Larripa, 1994). In cytogenetic studies in Chinese hamster ovary cells conducted by the NTP, sister chromatid exchanges were remarkably elevated by AZT, particularly in the absence of S9 activation, but no induction of chromosomal aberrations was observed (NTP, 1999).

In vivo, AZT has been shown in several studies to be an effective inducer of micronucleated reticulocytes (markers of chromosomal damage) in rats and mice exposed through various combinations of routes and exposure durations (Phillips *et al.*, 1991; Dertinger *et al.*, 1996a; Von Tungeln *et al.*, 2002; Bishop *et al.*,

2004b). For example, significantly increased micronucleus frequencies (6 to 27 times the frequency in concurrent controls), were noted in peripheral blood and bone marrow erythrocytes of mice after multiple treatments with 100 to 2,000 mg AZT/kg per day for periods of 72 hours, 96 hours, or 90 days. However, other studies using lower doses have reported no increase in micronucleus frequency (Motimaya *et al.,* 1994). A study (Dobrovolsky *et al.,* 2007) using C3B6F1$^{+/-}$ *Trp53*^{tm1Brd} p53 haploinsufficient mice reported that transplacental, followed by neonatal, exposure to AZT increased the reticulocyte micronucleus frequencies 4.8-, 7.1- and 11.3-fold when measured on PND 1 with maternal doses of 48, 80 and 160 mg/kg per day, respectively, and 10.3-, 10.6-, and 26.5-fold when measured on PND 10 with maternal/neonatal doses of 40/20, 80/40, and 160/80 mg/kg per day, respectively.

Incorporation of AZT into the DNA of leukocytes and multiple organs of cynomolgus monkeys was demonstrated following a 30-day treatment period (40 mg/day, by nasogastric intubation) (Olivero *et al.,* 2001). Organ specific differences in the amount of AZT incorporation were noted, and the average levels of incorporation were similar to what had been reported for human leukocytes (Olivero *et al.,* 2000). Pregnant CD-1® mice and *E. patas* monkeys were treated with AZT (mice, 12.5 or 25 mg/day; monkeys, 10 mg/day) during critical periods of gestation, and AZT incorporation into both nuclear and mitochondrial DNA, along with telomere length of chromosomes, was measured in the newborns (Comstock *et al.,* 1993). The transplacentally exposed animals showed significant AZT incorporation into nuclear as well as mitochondrial DNA of several organs, and decreased telomere lengths were seen in chromosomes from liver and brain cells of mice, but not monkeys. Similarly, a human-equivalent dose of AZT (8 mg/kg) administered continuously over 4 hours to pregnant rhesus macaques just prior to hysterotomy at the end of gestation resulted in AZT incorporation into DNA extracted from cells of several fetal organs (Poirier *et al.,* 1999).

The relevance of the positive results from animal mutation studies to humans is not yet clear, but numerous investigations have yielded data supporting a potential for genetic damage in humans exposed to AZT and other nucleoside analogues (Olivero, 2007, 2008). Several studies have demonstrated increased mutation frequencies in cultured human lymphoblastoid cells following AZT exposure. For example, there are a number of studies showing incorporation of AZT into DNA of human lymphoblastoid cells, followed by loss of heterozygosity at loci for the thymidine kinase 1, hypoxanthine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes, resulting in significant increases in mutant frequencies (Sussman *et al.,* 1999; Meng *et al.,* 2000a,b,c). Analysis of the AZT-induced mutational spectra in cultured human lymphoblastoid cells showed an increase in complete gene deletions, a result consistent with DNA chain termination, and loss of heterozygosity (Meng *et al.,* 2002). *In vivo*, anti-AZT radioimmunoassays were used to demonstrate that AZT is incorporated into lymphocyte DNA of HIV-infected adults taking AZT (Olivero *et al.,* 2000).

Currently there is some evidence that AZT exposure in conjunction with HAART can result in chromosomal damage in humans. An early paper by Shafik *et al.* (1991) reported significantly increased chromosomal aberration frequencies in lymphocytes of AIDS patients treated with AZT alone, compared to a healthy control group. In a more recent study, children born to HIV-infected mothers who received treatment with AZT and other NRTIs were evaluated periodically for up to 9 years after birth (Senda *et al.,* 2007). Heterochromatin analysis of blood leukocytes showed an increased frequency ($P<0.001$) of chromatin dispersal in samples from children exposed to NRTIs (predominantly AZT) as compared to frequencies from children of HIV-infected mothers who were not exposed to NRTIs. The heterochromatin defects persisted long after the end of the exposure period and were present in leukocytes of both myeloid and lymphoid lineages, suggesting that hematopoietic stem cells were affected.

3TC

TK6 human lymphoblastoid cells were incubated for 3 days with 0, 33, 100, or 300 µM 3TC alone (Carter *et al.,* 2007; Torres *et al.,* 2007) or in the presence of an equimolar quantity of AZT (Torres *et al.,* 2007). Compared to control cultures, incubation with 300 µM 3TC caused significant increases in the *Hprt* and *TK* mutant frequencies, while all three levels of the combined 3TC and AZT exposures caused significant increases in the *Hprt* and *TK* mutant frequencies.

Neonatal B6C3F1/*TK*$^{+/-}$ mice were treated intraperitoneally on PNDs 1 to 8 with 200 mg 3TC/kg body weight per day or a mixture of 200 mg 3TC and 200 mg AZT/kg per day (Von Tungeln *et al.,* 2002). When assessed on PNDs 9 and 10, 3TC did not increase the frequency of polychromatic erythrocytes containing micronuclei. The percentage of polychromatic erythrocytes containing micronuclei was increased by the mixture of 3TC and AZT, but the response did not differ from that observed with AZT alone. Treatment with 3TC did not affect the mutant frequency at either the *TK* gene or *Hprt* gene of spleen T-lymphocytes. The combined treatment of 3TC and AZT did increase the *TK* but not the *Hprt* mutant frequency; however, the response did not differ from treatment with AZT alone.

The increase in the *TK* mutant frequency was attributed to loss of heterozygosity.

Female C57Bl/6N and female C57Bl/6N/$TK^{+/-}$ mice were bred to male C3H/HeNMTV mice and then were treated with 3TC by gavage on GDs 12 to 17 with 0 or 120 mg/kg per day or a mixture of either 40 mg 3TC and 80 mg AZT/kg per day, 80 mg 3TC and 160 mg AZT/kg, or 120 mg 3TC and 240 mg AZT/kg (Von Tungeln *et al.,* 2007). When assessed 1 day after birth, there was no increase in micronucleated reticulocytes or micronucleated normochromatic erythrocytes in mice that had been exposed to 3TC alone, whereas there was a dose-dependent increase in mice that had been exposed to the mixtures of 3TC and AZT. Treatment with 3TC alone resulted in an increase in the *TK* mutant frequency when assessed 5 weeks after treatment, whereas the mixtures of 3TC and AZT resulted in increased *TK* mutant frequencies at 3 weeks after treatment.

Pregnant CD-1® mice were given 100 mg 3TC/kg body weight per day or a mixture of 100 mg 3TC and 200 mg AZT/kg per day for the last 7 days of gestation (Torres *et al.,* 2007). When assessed on PND 13, the mixture of 3TC and AZT, but not 3TC alone, increased the mutant frequency of the *Hprt* gene in spleen T-lymphocytes. An increase in mutant frequency was not detected at PNDs 15 or 21 with either treatment.

Female C3H/HeN ($p53^{+/+}$) mice were bred to $p53^{+/+}$ or $p53^{+/-}$ male mice and the pregnant female mice were treated by gavage on GDs 12 to 18 with a mixture of 100 mg 3TC and 160 mg AZT/kg body weight per day (Dobrovolsky *et al.,* 2007). After delivery, the $p53^{+/+}$ and $p53^{+/}$ pups were treated by gavage on PNDs 1 to 10 with 50 mg 3TC and 80 mg AZT/kg body weight per day and on PNDs 11 to 28 with 100 mg 3TC and 160 mg AZT/kg per day. When assessed on PNDs 1, 10, and 28, there were increases in micronucleated reticulocytes and micronucleated normochromatic erythrocytes that were independent of genotype. Administration of the mixtures of 3TC and AZT also produced increases in the mutant frequency at the *Hprt* gene of spleen lymphocytes in $p53^{+/-}$ mice but not in $p53^{+/+}$ mice.

Umbilical cord blood was obtained from human infants whose HIV-1-positive mothers had received antiretroviral therapy during pregnancy (Witt *et al.,* 2007). Infants whose mothers had received regimens containing 3TC and AZT plus at least one additional antiretroviral drug had significant increases in micronucleated reticulocytes compared to infants whose mothers had either not been treated or had received regimens that did not contain 3TC and AZT. Likewise, venous blood from mothers given regimens containing 3TC and AZT had significant increases in micronucleated reticulocytes compared to mothers administered regimens that did not contain 3TC and AZT or compared to typical values measured in "control" adults.

DNA was isolated from mononuclear cells of umbilical cord blood obtained from 21 infants whose HIV-1-positive mothers had been treated with 3TC and AZT during pregnancy (Meng *et al.*, 2007). When assessed by radioimmunoassay, AZT incorporation was detected (mean = 51.6 AZT molecules per 10^6 nucleotides; range = 3 to 151.5 AZT molecules per 10^6 nucleotides). This level of AZT incorporation was significantly greater than that of infants exposed to AZT alone. The levels of 3TC incorporation were not measured. AZT incorporation was also detected in mononuclear cell DNA from nine maternal blood samples (mean = 52.8 AZT molecules per 10^6 nucleotides; range = 0 to 241.7 AZT molecules per 10^6 nucleotides). In further work, the presence of mutations in glycophorin A was assessed in maternal and umbilical cord blood (Escobar *et al.*, 2007; Meng *et al.*, 2007). Compared to infants whose mothers had not been treated, the frequency of glycophorin A variants was elevated in the infants whose mothers had received mixtures of 3TC and AZT.

Umbilical cord tissue DNA of infants whose mothers had been treated during pregnancy with mixtures of AZT and 3TC was examined for sequence variations in mitochondrial DNA (Torres *et al.*, 2009). Density gradient gel electrophoresis indicated the presence of a shift in the mutation spectrum.

NVP

NVP was not mutagenic or clastogenic in a variety of assays, including microbial and mammalian gene mutation tests and micronucleus tests (*PDR*, 2010b). Synthetic esters of the NVP metabolite 12-hydroxy-NVP have been shown to react with DNA to give a number of DNA adducts (Andric *et al.*, 2006). Whether or not these DNA adducts are formed *in vivo* is currently unknown.

BACKGROUND ON GENETICALLY MODIFIED MICE USED IN THE AZT, 3TC, AND NVP STUDY

The p53 tumor suppressor gene suppresses cancer in both humans and mice. The p53 protein is critical to cell cycle control, DNA repair and apoptosis, etc., and is often mutated or lost in human and rodent cancers. The

haploinsufficient *Trp53* tumor suppressor gene mouse model heterozygous for wildtype and null (+/−) *Trp53* alleles (Donehower *et al.*, 1992, 1995) was used in this study. In this model, a *Trp53* null mutation was introduced by homologous recombination in AB1 murine embryonic stem cells that were derived from a black agouti 129Sv inbred mouse. By targeted insertion of a *pol*II neo cassette, an engineered null mutation was induced as a result of the deletion of a 450-base pair gene fragment from the *Trp53* gene that included 106 nucleotides of exon 5 and approximately 350 nucleotides of intron 4 that eliminated both mRNA and p53 protein expression from this allele. This *Trp53* protein haploinsufficient mouse model has been extensively tested as a short-term cancer bioassay mouse model (Tennant *et al*., 1995; Dunnick *et al*., 1997; French *et al*., 2001a,b; Pritchard *et al*., 2003; French, 2004) based upon the observation that mice with only a single wildtype *Trp53* allele show a significant decrease in the time required for genotoxic carcinogen-induced tumors to develop. These tumors are often associated with either a mutation and/or a loss of heterozygosity of the remaining wildtype *Trp53* allele. Few to no sporadic tumors occur in concurrent or historical study control groups in this GMM model, which allows tests to be conducted with fewer animals and direct analysis of the target wildtype *Trp53* allele to test for genotoxicity *in vivo* as a mode of action.

For the current study, an outcross between C3H/HeNTac (C3) female mice homozygous for the wildtype (+) *Trp53* allele and the C57BL/6.129Sv-*Trp53*^{tm1Brd} N12 congenic (abbreviated B6.129-*Trp53*^{tm1Brd}) N12 backcross generation males homozygous for the *Trp53* null (−) allele produced C3B6.129F1/Tac-*Trp53*^{tm1Brd} N12 progeny heterozygous for a *Trp53* wildtype (+) and null allele (−) inbred mouse progeny [hereafter referred to in the abbreviated form as the heterozygous F1 p53$^{+/-}$ mouse, Taconic Farms, Inc. (Germantown, NY)]. The heterozygous F1 p53$^{+/-}$ mouse was selected for the 45-week study of AZT because the B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient male and female mice (backcrossed to C57BL/6, subline unspecified, for two generations and then to C57BL/6NTac females for an additional three generations) were not sufficiently inbred. The N5 generation of this line retained both C57BL/6 and 129Sv strain allele heterozygosity at both the *Trp53* locus and the flanking region on chromosome 11 and at unknown loci throughout the genome of this line. This residual heterozygosity in the B6.129-*Trp53*^{tm1Brd} N5 backcross generation mice was one covariate that may have been responsible for large variations in the *p*-cresidine induced urinary bladder tumors (0% to 80%, 10 of 11 studies were positive) in males, which was used as a positive control genotoxic carcinogen in the ILSI/HESI Alternatives to Carcinogenicity Testing initiative (Storer *et al*., 2001). Therefore, additional inbreeding to the N12 generation was anticipated to decrease the variance in tumor incidence and stabilize the penetrance of tumor phenotypes in NTP studies.

The majority of B6.129-*Trp53*^{tm1Brd} homozygous null females die *in utero* and only a few are born alive and most die early. Thus, the B6.129-*Trp53*^{tm1Brd} N12 line is maintained by intercross of the B6.129-*Trp53*^{tm1Brd} female heterozygote with the B6.129-*Trp53*^{tm1Brd} homozygous null male to produce a 1:2 population of homozygous null males and heterozygous null males and females. Therefore it is necessary to select the B6.129-*Trp53*^{tm1Brd} homozygous null male as the carrier of the null allele. However, the selection of the C3H/HeNTac female as the wildtype *Trp53* allele carrier provides 1) increased fecundity and maternal instincts, 2) increased hybrid vigor of an F1 outcross that increases the number of progeny, 3) the advantage of expanding the pattern of tumor susceptibility associated with this genetic background, and 4) a genetic background similar to the B6C3F1 mouse used in NTP studies (NTP, 2013a). Together, these factors provided a rational basis for selection of this GMM test model. In addition, the NTP studies reported on AZT alone and on senna, also used the C3B6.129F1/Tac-*Trp53*^{tm1Brd} N12 haploinsufficient GMM model (NTP, 2012, 2013b), and the background rates for spontaneous tumors in the control groups of C3B6.129F1-*Trp53*^{tm1Brd} haploinsufficient mice all three studies (AZT, senna, and the current study) were not statistically different from the background rates for spontaneous tumors observed in control B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice used in previous NTP GMM studies (NTP, 2005a,b, 2007a,b,c,d,e, 2008).

STUDY RATIONALE

The development of HAART to combat the AIDS pandemic was a major public health triumph of the late 20th century. For countries where HAART drugs are widely available, they have transformed HIV infection from a death sentence into a manageable chronic disease. In the United States, the FDA has made a significant contribution to this triumph by rapidly evaluating and approving new antiretroviral drugs. An estimated 7,000 infants are born to HIV-infected women in the United States every year, and due to the implementation of HAART the vast majority of these infants escape infection (UNAIDS, 2008).

In the United States, AZT was the primary drug of choice for treating pregnant women who are HIV

positive to prevent transmission of the virus to their children when the current study was initiated (PHS, 2008). It is used either alone or in combination with other antiretroviral drugs as part of HAART. Because AZT has been in use for less than 25 years, its long-term toxicological impact on children exposed *in utero* or in infancy is currently unknown. As outlined previously, AZT has been shown to be both genotoxic and a rodent carcinogen. An initial study described elsewhere (NTP, 2013b) was designed to determine possible long-term sequelae of AZT exposure when it is used to prevent mother-to-child transmission of HIV. The C3B6F1$^{+/-}$*Trp53*^{tm1Brd} p53 haploinsufficient mouse was selected for this study because previous NTP studies showed that p53 mutations were associated with AZT-induced carcinogenesis processes (NTP, 2006) and it was hypothesized that when a p53 gene change is involved in the multiple genetic steps to cancer, a mouse deficient in this gene will develop cancer in a shorter time period than in the traditional 2-year mouse cancer studies. The study determined that when AZT was administered through late gestation and then continuously until 45 weeks of age in this model, clear evidence of carcinogenic activity was observed in male mice based on increased incidences of hepatocellular adenoma, and some evidence of carcinogenic activity in female mice based on increased incidences of malignant lymphoma (NTP, 2013b). The current study was designed to test this model further. AZT was again tested, but also in combination with 3TC and NVP. The study included 3TC because it has been used extensively in combination with AZT (Combivir®) and because neonatal C3B6F1$^{+/-}$*Trp53*^{tm1Brd} p53 haploinsufficient mice pretreated with an AZT/3TC combination had exhibited a greater frequency of micronucleated reticulocytes than those treated with an equivalent dose of AZT alone (Dobrovolsky *et al.*, 2007). The study included NVP because it has been used extensively in combination with AZT, particularly in prevention of mother-to-child transmission of HIV during labor (PHS, 2008) and because NVP causes liver toxicity in humans and therefore might potentiate the hepatocarcinogenesis of AZT in C3B6F1$^{+/-}$*Trp53*^{tm1Brd} p53 haploinsufficient mice. In addition the dosing regime was changed, in relation to other studies, to dosing only up to PND 28 so as to better mimic the gestational and early postnatal HAART given to children of HIV-infected mothers. Also, the daily dose was divided into two oral gavage exposures given approximately 6 hours apart to better mimic human exposure. The current study would, therefore, be more relevant to human exposures than the initial study (NTP, 2013b).

The current study used a standard chronic GMM study design of a control and three dosed groups; however, because a three-drug combination was being tested, additional dose groups in which each drug of the combination was administered alone were added. An additional dose group in which AZT and 3TC were coadministered without NVP was also added. The purpose for these additional dose groups was to provide information as to which of the three component drugs might be responsible for any effects observed in the combination study.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

AZT, 3TC, and NVP were obtained from Cipla Ltd., Mumbai Central (Mumbai, India) in single lots F60731, B10250, and FX1009, respectively. Identity and purity analyses were conducted by the study laboratory at the National Center for Toxicological Research (NCTR; Jefferson, AR) and Galbraith Laboratories, Inc. (Knoxville, TN) (Appendix F). To ensure stability, the bulk chemicals were stored in sealed amber jars at room temperature. Reports on analyses performed in support of the AZT, 3TC, and NVP *in utero*/postnatal gavage study are on file at the NCTR.

AZT

Lot F60731 of the chemical, a white-to-beige crystalline solid, was identified as AZT using proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe/electron ionization (DEP/EI) mass spectrometry (MS), and liquid chromatography combined with mass spectrometry (LC-MS). Purity of lot F60731 was determined by proton and carbon-13 NMR spectroscopy, and high-performance liquid chromatography (HPLC).

No impurity resonances were detected by either proton or carbon-13 NMR analyses other than those associated with the solvent. HPLC detected no impurities with an overall purity of approximately 100%. The overall purity of lot F60731 was determined to be at least 99.9%.

3TC

Lot B10250 of the chemical, a white-to-off-white crystalline solid, was identified as 3TC using proton NMR spectroscopy, DEP/EI-MS, and LC-MS. Purity of lot B10250 was determined by elemental analyses, proton NMR spectroscopy, and HPLC.

Total impurity was estimated at 0.5% by proton NMR spectroscopy. HPLC detected one impurity with a peak area of 1.1% of the total peak area and estimated a purity of approximately 98.9%. The overall purity of lot B10250 was estimated to be approximately 99%.

NVP

Lot FX1009 of the chemical, a white-to-off-white crystalline powder, was identified as NVP using proton NMR spectroscopy, DEP/EI-MS, gas chromatography/electron ionization MS, and LC-MS. Purity of lot FX1009 was determined by elemental analyses, proton NMR spectroscopy, and HPLC.

Karl Fischer titration indicated less than 0.14% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for NVP. Total impurity was estimated at 0.2% by proton NMR. HPLC detected a single peak, indicating that the test article was 100.0% pure. The overall purity of lot FX1009 was estimated to be at least 99.5%.

Dosing Vehicle

The vehicle used for dose formulations in this study was a 0.2% methylcellulose/0.1% Tween® 80 aqueous solution. This vehicle was selected based upon preliminary experiments to find a vehicle that gave homogeneous formulations of the individual test articles and the test article combinations. Methylcellulose was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in two lots (084K0065 and 125K0055) and Tween® 80 was obtained from the same source in three lots (073K00643, 064K00631 and 125K01921). Proton and carbon-13 NMR analyses of methylcellulose lot 084K0065 were performed by the study laboratory, and spectra were consistent with those obtained previously for a methylcellulose standard obtained from Fischer Scientific (Fair Lawn, NJ).

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by admixing the test chemicals with an aqueous solution of 0.2% methylcellulose/0.1% Tween® 80 to give the required concentrations (Table F1). AZT and 3TC formed true solutions at the concentrations used, whereas NVP was insoluble and formed stable suspensions. The dose formulations were stored (with constant stirring) in sealed amber glass bottles at room temperature for up to 21 days.

Homogeneity and stability studies of 0.05 and 0.20 mg/mL formulations of AZT in the methylcellulose/Tween® 80 vehicle and of high- and low-concentration mixtures of the three test chemicals in the dosing vehicle were conducted by the study laboratory using HPLC. Homogeneity was confirmed, and stability was confirmed for up to 29 days for the AZT formulations and for up to 21 days for the three-component formulations stored at room temperature in clear glass vials sealed with Teflon®-lined silicone rubber septa, that were stirred constantly.

At fifteen time points, analyses of the dose formulations of the antiretroviral drugs were conducted by the study laboratory using HPLC. Of the 161 measured concentrations of test chemical, 139 were within 10% of the target concentration; all preparations that were accepted for use were within 15% of their target concentrations (Table F2).

STUDY DESIGN

In utero exposure was selected to mimic exposures in humans and was begun at gestational day (GD) 12, a time period coinciding with administration of the test articles in the last third of pregnancy (DHHS, 2009). Starting dosing at GD 12 also allows for maximal sensitivity for carcinogenesis studies of genotoxic agents (Rice, 1973; Anderson, 2004). Dosing was discontinued after postnatal day (PND) 28 to mimic the early postnatal Highly Active Antiretroviral Therapy (HAART) given to children of HIV-infected mothers. The doses for this study were selected to overlap human exposures based on established scaling factors (Freireich *et al.*, 1966).

The study design incorporated a vehicle control and three-dose chronic study of HAART chemical mixtures of AZT, 3TC, and NVP. These constituent chemicals increased in the same proportion (1×, 2×, 3×) across the doses. The individual constituent chemicals were also tested alone at the highest dose in order to determine which was responsible for effects observed in the dose groups exposed to the mixtures.

Male and female heterozygous F1 $p53^{+/-}$ mice were exposed to AZT, 3TC, NVP, AZT/3TC, or AZT/3TC/NVP *in utero* on GDs 12 through 18, then pups were administered the same chemical or combination of chemicals by gavage from PND 1 through PND 28 and then observed until 45 weeks of age (Figure 1). The concentrations of test chemicals

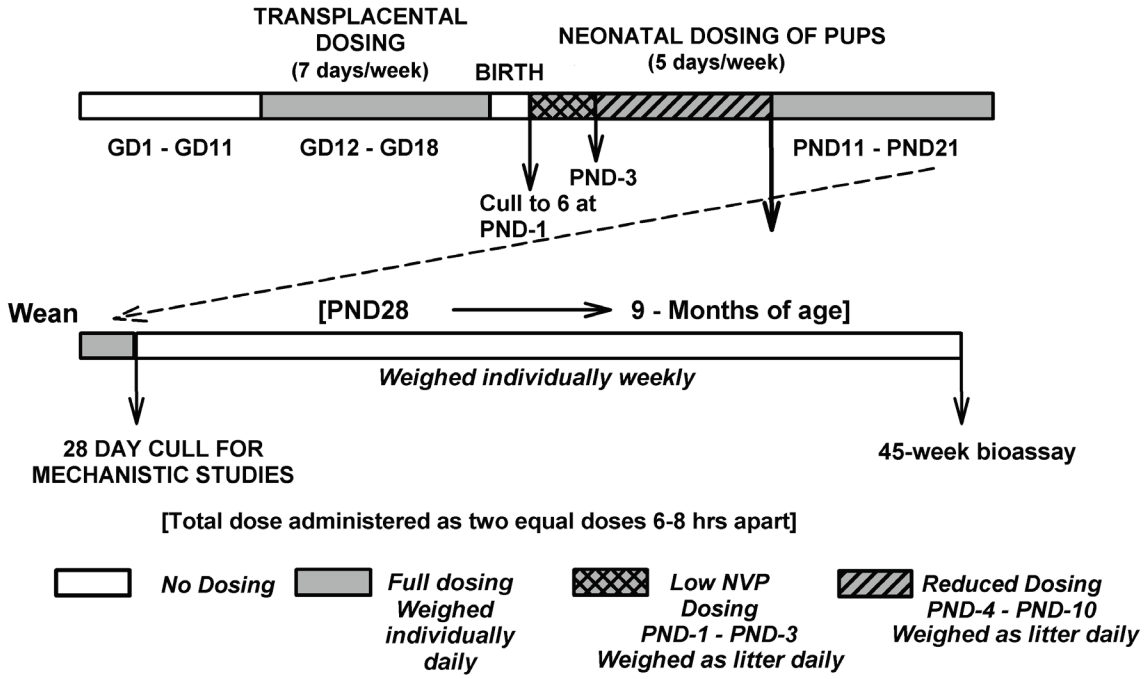


FIGURE 1
Dosing Schedules for the 45-Week Study

varied based on the age of the pups as defined in Table 1. In general, pups were administered half the maternal dose from PND 1 to PND 10, but the dose concentration of NVP was further reduced from PND 1 to PND 3 to increase pup survival. Vehicle control mice received only an aqueous solution containing 0.2% methylcellulose and 0.1% Tween 80. The dosing volume was 10 mL/kg, except on PNDs 1 through 10, when the dosing volume was 3 mL/kg. Dams were dosed twice daily, 7 days per week, and pups were dosed twice daily, 5 days per week. Full details of the dosing technique have been published by Lewis *et al.* (2010).

Because of the complexity of the dosing regimen, where dose levels changed up to three times per group during the study, describing dose groups in conventional terms of the administered dose is not practical for this report. The dose groups are therefore described in terms of their constituent chemicals and concentration type, low (L), medium (M) and high (H), and are defined in terms of constituent chemical concentration (Table 1).

TABLE 1
Dosing Regimen for the 45-Week Study[a]

Treatment	AZT (mg/kg/day)	3TC (mg/kg/day)	NVP (mg/kg/day)
Dams GDs 12 through 18 and Pups PNDs 11 to 28 (Dosing Volume = 10 mL/kg twice daily)			
Vehicle Control	0	0	0
AZT-H	240	0	0
3TC-H	0	150	0
NVP-H	0	0	168
AZT/3TC-H	240	150	0
AZT/3TC/NVP-L	80	50	56
AZT/3TC/NVP-M	160	100	112
AZT/3TC/NVP-H	240	150	168
Pups PNDs 1 through 3 (Dosing volume =3 mL/kg twice daily)			
Vehicle Control	0	0	0
AZT-H	120	0	0
3TC-H	0	75	0
NVP-H	0	0	21
AZT/3TC-H	120	75	0
AZT/3TC/NVP-L	40	25	7
AZT/3TC/NVP-M	80	50	14
AZT/3TC/NVP-H	120	75	21
Pups PNDs 4 through 10 (Dosing volume =3 mL/kg twice daily)			
Vehicle Control	0	0	0
AZT-H	120	0	0
3TC-H	0	75	0
NVP-H	0	0	84
AZT/3TC-H	120	75	0
AZT/3TC/NVP-L	40	25	28
AZT/3TC/NVP-M	80	50	56
AZT/3TC/NVP-H	120	75	84

[a] GD = gestation day, PND = postnatal day

Source and Specification of Animals

Female C3H/HeNTac wild-type mice and male C57BL/6(N12)*Trp53*$^{(-/-)}$ mice were obtained under an academic breeding license from Taconic Farms, Inc. (Germantown, NY), and were received in four groups of 10 males and 50 females (an additional 48 females were supplied to compensate for low pup survival). The animals were quarantined for 14 days prior to being assigned to the study. Each male was mated with up to nine females in succession with up to two females per breeding cage at a time. Plug-positive females were provisionally assigned to the study on the morning that the vaginal plugs were identified, which was designated GD 0 for the study. The plug-positive animals were weighed daily and those not showing signs of pregnancy were removed from the study at GD 10 and returned to the breeding pool. The heterozygous F1 p53$^{+/-}$ pups were born on GD 19 or 20 and the morning a litter was first observed was designated PND 0. On PND 1, each litter was examined, the sex of each pup was determined, and the litter was culled to six pups of equal sex ratio when possible.

Animal Maintenance

Mouse dams were housed individually with litters until PND 21. Litters were weaned and housed with littermates from PND 21 to PND 29, then housed individually. Feed and water were available *ad libitum*, except mice were fasted overnight before necropsy. In order to monitor the health of animals, blood was drawn from one sentinel mouse dam during weeks 25, 38, and 51 and from one male and one female sentinel mouse pup during weeks, 26, 38, 51, 65, and 86 after receipt of the initial breeding mice. Sera were analyzed for antibody titers to rodent viruses; all results were negative. Further details of animal maintenance are given in Table 2.

Clinical Examinations and Pathology

Animals were observed twice daily. Body weights were recorded daily for pregnant dams and for litters until PND 10; individual pups were weighed daily until PND 28 then weekly until the end of the studies. Clinical findings were recorded twice weekly.

All mice that survived to study termination were evaluated for hematology and clinical chemistry parameters. Blood was collected by cardiac puncture under CO_2 anesthesia at the time of necropsy. The samples for clinical chemistry were allowed to clot then centrifuged. The serum was removed and frozen at –60° C until analysis. Whole blood for complete blood counts was collected in EDTA, and analysis was performed the same day as collection. Complete blood counts were determined on an ABX Pentra 60 C+ analyzer (ABX, Irvine CA). Clinical chemistry analyses were conducted on an Alfa Wassermann ALERA (West Caldwell, NJ). Alfa Wassermann reagents were used for alanine aminotransferase (Henry modification), alkaline phosphatase (modified Bowers and McComb), glucose (hexokinase), and total protein (biuret) analyses. The parameters measured are listed in Table 2.

Necropsies and microscopic examinations were performed on all mice. The brain, heart, left and right kidney, liver, and lung were weighed. At necropsy, all major tissues were examined grossly for visible lesions, and all major tissues were preserved in 10% neutral buffered formalin or Davidson's solution (eyes and testes). The major tissues and gross lesions were trimmed, processed, and embedded in Formula R®, sectioned at approximately 5 µm, and stained with hematoxylin and eosin. When applicable, nonneoplastic lesions were graded for severity as 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked).

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Laboratory Data Aquisition System (LDAS) database. The slides, individual animal data records, and pathology tables were evaluated by the Toxicologic Pathology Associates (Jefferson, AR) and NCTR quality assurance units. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated slides from all tumors; there were no designated target organs. Tissues examined microscopically are listed in Table 2.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously

rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 2
Experimental Design and Materials and Methods in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

Strain and Species
Transgenic mouse model (C3H/HeNTac × B6.129SvEv)$F_1{}^{+/-}$*Trp53*^{tm1Brd} (abbr, C3B6$F_1{}^{+/-}$*Trp53*^{tm1Brd} or C3B6F_1*Trp53*$^{(+/-)}$ or heterozygous F1 p53$^{+/-}$). Dams were C3H/HeNTac; sires were C57BL/6(N12)*Trp53*$^{-/-}$

Animal Source
Dams and sires, Taconic Farms, Inc. (Germantown, NY); pups, National Center for Toxicological Research

Time Held Before Study
Male and female breeders, 6 weeks of age, were maintained 14 days in quarantine and were allocated to study.

Age When Study Began
Breeders were 8 weeks old at breeding; dosing of dams began on GD 12

Initial Conception Date
Start date for first breeders was 12/21/2006; first conception date: 12/22/2006
Start date of last breeders was 9/9/2007; last conception date: 9/21/2007

Duration of Dosing
GDs 12 through 18, then PNDs 1 through 28; observed through week 45

Date of Last Dose
11/08/2007

Date of Necropsy
07/15/2008

Age at Necropsy
45 weeks for terminal sacrifice; early deaths and moribund animals were also necropsied

Size of Study Groups
25 or 26 males and 24 or 25 females

Animals per Cage
One male and up to two females during breeding; dams housed individually with litters until PND 21; pups housed with littermates PNDs 21 to 28 then individually beginning PND 29

Method of Animal Identification
Paw tattoo (by PND 11), ear clipped at PND 21, tail tattoo with cage bar code number

Diet
NIH-31 autoclavable pellets (5022CGP3, Lab Diet, Purina Mills, Inc., St. Louis, MO) available *ad libitum* until day before necropsy

Water
Millipore-filtered water (Jefferson, AR, municipal supply) via plastic water bottles fitted with rubber stoppers and sipper tubes (bottles: Allentown Caging Equipment Co., Inc., Allentown, NJ; stoppers and tubes: Ancare Corp., Bellmore, NY), available *ad libitum*

TABLE 2
Experimental Design and Materials and Methods in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Cages
Polycarbonate single mouse cages for breeder males and females with litters. After PND 29, offspring were housed individually in polycarbonate double cages with dividers (Allentown Caging Equipment Co., Inc., Allentown, NJ, and Lab Products, Seaford, DE), changed weekly, rotated monthly

Bedding
Hardwood chip bedding (Northeastern Products Corp., Warrensburg, NY), changed weekly for animals housed individually and twice weekly for cages with litters present

Cage Filters
Micro-vent cage filtration with 0.2 micron HEPA filter (Allentown Caging Equipment Co., Inc., Allentown, NJ), changed weekly

Racks
Stainless steel (Allentown Caging Equipment Co., Inc., Allentown, NJ), changed every 3 weeks

Animal Room Environment
Temperature: 22° ± 4° C
Relative humidity: 40%-70%
Room fluorescent light: 12 hours/day
Room air changes: 10-15/hour

Doses
Dams GDs 12 through 18 and Pups PNDs 11 through 28, dosing volume 10 mL/kg twice daily.
AZT-H: 240 mg/kg AZT
3TC-H: 150 mg/kg 3TC
NVP-H: 168 mg/kg NVP
AZT/3TC-H: 240/150 mg/kg AZT/3TC
AZT/3TC/NVP-L: 80/50/56 mg/kg AZT/3TC/NVP
AZT/3TC/NVP-M: 160/100/112 mg/kg AZT/3TC/NVP
AZT/3TC/NVP-H: 240/150/168 mg/kg AZT/3TC/NVP

Pups PNDs 1 through 3, dosing volume 3 mL/kg twice daily
AZT-H: 120 mg/kg AZT
3TC-H: 75 mg/kg 3TC
NVP-H: 21 mg/kg NVP
AZT/3TC-H: 120/75 mg/kg AZT/3TC
AZT/3TC/NVP-L: 40/25/7 mg/kg AZT/3TC/NVP
AZT/3TC/NVP-M: 80/50/14 mg/kg AZT/3TC/NVP
AZT/3TC/NVP-H: 120/75/21 mg/kg AZT/3TC/NVP

Pups PNDs 4 through 10, dosing volume 3 mL/kg twice daily
AZT-H: 120 mg/kg AZT
3TC-H: 75 mg/kg 3TC
NVP-H: 84 mg/kg NVP
AZT/3TC-H: 120/75 mg/kg AZT/3TC
AZT/3TC/NVP-L: 40/25/28 mg/kg AZT/3TC/NVP
AZT/3TC/NVP-M: 80/50/56 mg/kg AZT/3TC/NVP
AZT/3TC/NVP-H: 120/75/84 mg/kg AZT/3TC/NVP

Dosing was twice daily, 7 days/week from GD 12 through GD 18 and then twice daily, 5 days/week from PND 1 through PND 28

Method of Distribution
Sires and dams were assigned to breeding cages randomly; pregnant dams were assigned to dose groups so as to maximize the distribution of an individual sire's progeny across the dose groups.

Type and Frequency of Observation
Observed twice daily; body weights recorded daily for pregnant dams, for litters until PND 10, and for individual pups from PNDs 11 through 28, then weekly for individual pups; clinical findings were recorded twice weekly

Method of Sacrifice
CO_2 asphyxiation

TABLE 2
Experimental Design and Materials and Methods in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Necropsy
All mice were necropsied. Organs weighed were brain, heart, left and right kidney, liver, and lung.

Clinical Pathology
At the end of the study, blood was collected via cardiac puncture under carbon dioxide anesthesia for hematology and clinical chemistry.
Hematology: hematocrit, erythrocyte and platelet counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and leukocyte count and differentials
Clinical chemistry: alanine aminotransferase, alkaline phosphatase, blood glucose, lactic acid, and total protein

Histopathology
Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, blood vessel, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.

STATISTICAL METHODS

Two substudies were analyzed separately. The first substudy, termed the combination dose comparison, compared the vehicle control group to the different dose level group (L, M, and H) of the AZT/3TC/NVP combination. This is a one-way substudy and the results are presented in that format with dose trend and comparisons to the vehicle control group. The second substudy, termed the high dose comparison, compared high doses of the six drug combinations to one another. These six combinations were: vehicle control, AZT-H, 3TC-H, NVP-H, AZT/3TC-H, and AZT/3TC/NVP-H. Because there is not necessarily any directionality among the comparisons with the exception of the comparisons to the vehicle control group, a single-sided test was used with "N" appended to denote negative comparisons. The alpha-level was 5% for comparisons to the vehicle control group but 2.5% for comparisons among combinations.

Survival Analyses

Survival was analyzed using the proportional hazards model (Cox, 1972). All vehicle control female pups survived to terminal sacrifice. To allow reasonable inferences, synthetic events were added to all female treatment groups. These events were uncorrelated to any litters, were given event times of 336 days, and were weighted as one-half of an actual pup.

Four covariance models were run to account for clustering. The first model assumed pup independence and used the usual Cox model-based covariance estimates. The second model assumed pup independence but used the unaggregated sandwich-estimator empirical covariance (Lin and Wei, 1989). The model was run to assess the impact of using the empirical covariance estimate alone, regardless of intralitter correlation. The third model assumed correlation within litters by aggregating the sandwich-estimator empirical covariance (Binder, 1992). The fourth model assumed correlation within sires by aggregating the sandwich-estimator empirical covariance.

Analysis of Continuous Variables

In addition to the standard repeated-measures mixed model for predicting body weight based on a fixed treatment group effect and age, this study required the addition of a random litter effect to account for correlation among littermates. Furthermore, since the correlation could be negative within litters, the mixed model specified the litter effect in the "R" matrix rather than the "Z" matrix. In this study, pups share the dam and littermates (implicitly dam-mates) are one possible correlation. However, multiple litters can share a common sire and sire-mates are another possible correlation. Therefore, three correlation models had to be conducted: unadjusted (assumes pup independence), dam-adjusted (assumes correlation among littermates but independence among litters), and sire-adjusted (assumes pups from different sires are independent). The combination of the heteroscedastic variance over time coupled with the litter correlation makes a repeated-measures model difficult. Therefore, since the time course is not really of great concern, a basic mixed model was run by time point. Dunnett's adjustment was used to compare to the vehicle control group.

The log-rank test (Kalbfleisch and Prentice, 1980) was used for the one-way subdesigns. This test makes no adjustment for litter clusters. It does allow adjustment for multiple comparisons. In this report, Kramer's extension of Tukey's method (Kramer, 1956) was used

when all comparisons were of interest while Hsu's extension of Dunnett's test (Hsu, 1992) was used where comparisons to the vehicle control group are of primary interest. Because there is not necessarily any directionality among the comparisons with the exception of the comparisons to the vehicle control group, the analyses used two-sided tests. The relative hazard ratios indicate the direction. Two substudies, as described for survival analysis, were analyzed separately.

For the organ weight analysis, the same three correlation models were run: unadjusted (assumes pup independence), dam-adjusted (assumes correlation among littermates but independence among litters), and sire-adjusted (assumes pups from different sires are independent). However, because the correlation model generally made no difference in the conclusions, the report lists only the dam-adjusted correlation structure. Dunnett's tests (Dunnett, 1955) were used to compare to the particular control group (vehicle control or AZT/3TC/NVP-H).

The organ weight endpoint was analyzed as an absolute value using no covariate and as a relative ratio to body weight. Because survival may have impacted some organ weights, the analyses were conducted on the subgroup of terminal kill animals.

Clinical pathology data and pup survival within litters to PND 10 were analyzed in SAS (version 9.1) under the SAS General Linear Models program to produce means, standard error values and evaluation of significant differences between dose groups. Combination dose comparisons were evaluated using a Dunnett test and a linear trend test. A Tukey test was used for the high dose comparison of constituent chemicals.

Calculation of Lesion Incidences and Severities

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, A4, A6, A7, A9, A10, and A12 as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For all neoplasms and nonneoplastic lesions, the Poly-3 method of Bailer and Portier (1988) as modified by Bieler and Williams (1993) and NIEHS (continuity-correction) was used to analyze age-adjusted incidence (Tables A2, A5, A8, and A11).

To adjust for intralitter correlation, the Poly-3 method was envisioned as a weighted regression with binomial weights. This allows the methods of generalized linear models to be utilized with generalized estimating equation methods to allow for the estimation of intracluster correlation and adjustment for this. The binomial event is then the simple flag variable indicating whether the subject had the tumor of interest. The binomial trials count is 1 if the animal had the tumor of interest or lived to terminal sacrifice. Otherwise, it is the Poly-3 adjusted fractional value. For this study, we did not attempt to use quasi-likelihood (McCullagh and Nelder, 1989) to implement the Bieler and Williams variance correction model. This means that a uniform dose group (that is, all successes or all failures) leads to invalid variance estimates. To fix this issue, dummy events were added, if necessary, to break the uniformity. If added, they were added to all groups equally. These augmented records were assigned to dummy litters to prevent interaction with the correlation estimates. The weights of these dummy events were set to 0.005.

The model was fit using an ANOVA-style effects model with appropriate contrasts. This differs from the Poly-3 method, which is run as a series of individual regressions. This was done primarily to prevent the intralitter correlation from wobbling too much. It seems unlikely that the dose should alter the intralitter correlation but fitting the model as a series of regressions would allow this. The model-estimated means are reported as the Poly-3, cluster-adjusted incidences for these models. The decision on whether to include a lesion or lesion pool was based on this adjusted incidence exceeding 5%.

For nonneoplastic lesions, the non-zero severity score averages were computed and, to incorporate lesion severity scores, the distribution-free (but unadjusted for age or clustering) method of Jonckheere (1954) and Terpstra (1952) was used to compute monotonic trend tests and the method of Shirley (1977) as modified by Williams (1986) was used to compute comparisons to control. These results are listed as JT/SW within the tabulated results.

QUALITY ASSURANCE METHODS

The 45-week study was conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, records from these studies including protocols and any amendments, deviations, or related information; study-related standard operating procedures (SOPs) and documentation; test article accountability and characterization; raw data generated in operational areas as defined in applicable SOPs; computer records containing INLIFE and pathology raw

data; and daily animal room logs and a copy of the laboratory study report will be submitted to the NCTR Archives.

GENETIC TOXICOLOGY

Peripheral blood was collected at sacrifice from excess heterozygous F1 $p53^{+/-}$ mice that were culled on PND 1 or PND 28, and aliquots were diluted with anticoagulant, fixed in cold (–80° C) methanol, and stored at –85° C. The fixed blood samples were shipped to Litron Laboratories (Rochester, NY) on dry ice for analysis. Micronucleated cells were identified and quantified using the MicroFlow^{PLUS} mouse kit from Litron Laboratories (Dertinger *et al.,* 1996b, 2006). Briefly, reticulocytes were identified by fluorescein isothiocyanate-labeled antibodies against the CD71 mouse surface antigen, platelets were identified by phycoerythrin-labeled antibodies against CD61 antigen, and DNA, including micronuclei, was stained with propidium iodide. Data provided by Litron was sorted in the form of sorted spreadsheets of differences in reticulocyte micronucleus frequency between dose groups, and as audited study reports which have been added to the Study Archive. The spreadsheet data were then analyzed at NCTR in SAS (version 9.1) to produce means, standard error values, and significant differences between dose groups via a Tukey test evaluation run under the SAS General Linear Models program. Only one male and/or one female pup from any one litter were evaluated so that potential litter effects were eliminated.

RESULTS

LITTER SUCCESS AND PUP SURVIVAL

As shown in Table 3, the chemical combinations were relatively nontoxic to the pregnant dams at the doses used, because most produced viable litters. However, there were apparent treatment-related effects on both litter success and pup survival to postnatal day (PND) 28. The average number of pups born per litter was lower in the AZT/3TC/NVP-H group compared to the vehicle control group. Pup survival was not affected by treatment between PNDs 1 and 10, which suggested that the reduction of the concentration of the NVP component of the doses between PNDs 1 and 3 had protected the neonatal mice from NVP toxicity. However, survival was significantly decreased between PNDs 11 and 28 in the AZT/3TC/NVP-M and AZT/3TC/NVP-H groups relative to the vehicle control group. The majority of pup deaths occurred between PNDs 13 and 20. This effect on pup mortality was likely due to a combination of multiple components of the dose, because, while survival was significantly reduced in the AZT-H group relative to the vehicle control group (Table 4), the observed decrease was significantly less than that of the AZT/3TC/NVP-H group. Overall, pup survival across all the dose groups was significantly decreased relative to that observed in previous studies that used heterozygous F1 $p53^{+/-}$ mice (NTP, 2013b), and because of this reduction in pup survival, more dams (and litters) had to be supplied to the study than the 25 dams per dose group anticipated in the experimental design. There were also treatment effects on rates of pup growth between PND 1 and PND 10 (Figures H3 and H4), which potentially contributed to the treatment effects on survival between PNDs 11 and 28 (Tables H1 through H4 and Figures H1 and H2).

TABLE 3
Litter Parameters and Pup Survival to 28 Days in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
Total dams pregnant[b]	30	26	24	37
Dams that did not litter	2	2	1	2
Total litters	28	24	23	35
Total pups born	204	180	156	208
Average per litter	7.29	7.5	6.78	5.94
Number of males born	105	85	71	93
Sex ratio of live pups (female:male)	1:1.18	1:1.10	1:0.90	1:0.89
Pups born dead (%)	10 (4.9)	18 (10.0)	6 (3.8)	11 (5.3)
Percent survival PND 1 - PND 10[c]	82.74%	78.70%	90.58%	81.03%
Survival analysis[d]	NS	NS	NS	NS
Percent survival PND 11 - PND 28[e]	96.54%	98.02%	81.52%	48.82%
Survival analysis[f]	P<0.001	NS	P=0.006	P<0.001
Pups assigned to 45-week study	50	50	50	50
Litters used for 45-week study	24	16	16	24

[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

[b] Plug positive dams were randomly assigned to dose groups on gestational day (GD) 0 (day plug was observed), and their weight gain was monitored to confirm pregnancy. Dams that did not gain sufficient weight to indicate pregnancy by GD 11 were returned to the breeding pool and are not included in this table.

[c] Excludes pups that were culled on postnatal day (PND) 1, and includes both males and females

[d] Overall survival within litters to PND 10. Beneath the vehicle control group is the P value associated with a linear trend test. Beneath the dosed groups are the P values corresponding to pairwise comparisons by Dunnett's test. NS=not significant

[e] Excludes pups that died or were culled before PND 11, and includes both males and females

[f] Beneath the vehicle control group is the P value associated with a linear trend test. Beneath the dosed groups are the P values corresponding to pairwise comparisons by Dunnett-Hsu adjusted comparisons among groups in log-rank analysis between the vehicle controls and that dosed group.

TABLE 4
Litter Parameters and Pup Survival to 28 Days in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Total dams pregnant[b]	30	29	28	26	28	37
Dams that did not litter	2	0	3	3	1	2
Total litters	28	29	25	23	27	35
Total pups born	204	206	147	155	184	208
Average per litter	7.29	7.10	5.88	6.74	6.81	5.94
Number of males born	105	100	64	74	91	93
Sex ratio of live pups (female:male)	1:1.18	1:0.99	1:0.84	1:0.94	1:1.12	1:0.89
Pups born dead (%)	10 (4.9)	5 (2.4)	7 (4.8)	2 (1.3)	12 (6.5)	11 (5.3)
Percent survival PND 1 - PND 10[c]	82.74%	83.10%	86.11%	86.23%	94.00%	81.03%
Survival analysis [vs. vehicle control][d]		NS	NS	NS	NS	NS
Survival analysis [vs. AZT/3TC/NVP-H][d]	NS	NS	NS	NS	NS	
Percent survival PND 11 - PND 28[e]	96.54%	80.60%	99.09%	99.24%	71.47%	48.82%
Survival analysis [vs. vehicle control][f]		P=0.037	NS	NS	P<0.001	P<0.001
Survival analysis [vs. AZT/3TC/NVP-H][f]	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	
Pups assigned to 45-week study	50	50	50	51	50	50
Litters used for study	24	22	17	19	20	24

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Plug positive dams were randomly assigned to dose groups on gestational day (GD) 0 (day plug was observed), and their weight gain was monitored to confirm pregnancy. Dams that did not gain sufficient weight to indicate pregnancy by GD 11 were returned to the breeding pool and are not included in this table.

[c] Excludes pups that were culled on postnatal day (PND) 1, and includes both males and females

[d] Overall survival within litters to PND 10. P values represent pairwise comparisons with a Tukey's test. NS=not significant

[e] Excludes pups that died or were culled before PND 11, and includes both males and females

[f] P values represent Tukey-Kramer adjusted comparisons among groups in log-ranked analysis. NS=not significant

45-WEEK STUDY

Survival

Estimates of survival probabilities for male and female mice are shown in Tables 5 and 6 and Figures 2 and 3. In general, survival was relatively high with at least 75% surviving to terminal sacrifice in all groups. No obvious dose-dependent effects were observed in either males or females when the combination doses were compared or when the individual high dose constituent chemicals were compared. For males, survival was significantly greater in the AZT/3TC/NVP-L and AZT/3TC/NVP-M groups relative to the vehicle control group. This result was not considered to be treatment related. The natural deaths and morbidity in the control group were not associated with any single pathological lesion, but were the result of osteosarcomas and other malignant sarcomas, or malignant lymphoma. For females, there was no significant treatment effect for any of the three combination doses relative to the vehicle controls. There were no significant differences in survival between high dose groups of the constituent chemicals in either sex (statistical data not presented); however, survival of females in the AZT/3TC-H group was significantly less than that of the vehicle control group.

Additional statistical analyses were performed to determine possible litter and common sire effects on survival (Appendix G). This required different analytical methods than used for the standard Cox Proportional Hazards Model (Cox, 1972) and resulted in significant increases in hazard for all dosed female groups relative to the vehicle controls. However, these effects were are attributed to the 100% survival in the vehicle control group rather than a genuine significant dose effect. There were no significant differences in survival in either male or female mice due to clustering of litter or sire effects.

TABLE 5
Survival of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Male				
Mice initially in study	25	25	25	25
Removals[b]	0	0	2	1
Moribund	2	0	1	1
Natural deaths	3	1	0	1
Mice surviving to study termination	20	24	22	22
Probability of survival to end of study[c]	80.0%	96.0%	95.7%	91.7%
Mean survival (days)	321.0	332.6	334.0	324.8
Survival analysis[d]	P=0.206**N**	P=0.043**N**	P=0.047**N**	P=0.157**N**
Female				
Mice initially in study	25	25	25	25
Removals[b]	0	0	3	0
Moribund	0	2	2	2
Natural deaths	0	1	1	2
Mice surviving to study termination	25	22	19	21
Probability of survival to end of study	100%	88.0%	86.4%	84.0%
Mean survival (days)	332.8	324.4	313.9	328.7
Survival analysis	P=0.078	P=0.104	P=0.088	P=0.079

[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Censored from survival analysis
[c] Kaplan-Meier determinations
[d] Cox Proportional Hazards Regression Model results. Tests formed by suitable contrasts in the Cox Estimates. All tests are single degree of freedom and one sided. The P value in the vehicle control column is the trend test; other P values are pairwise comparisons to the vehicle control group. A negative trend or a lower mortality in a dose group is indicated by **N**.

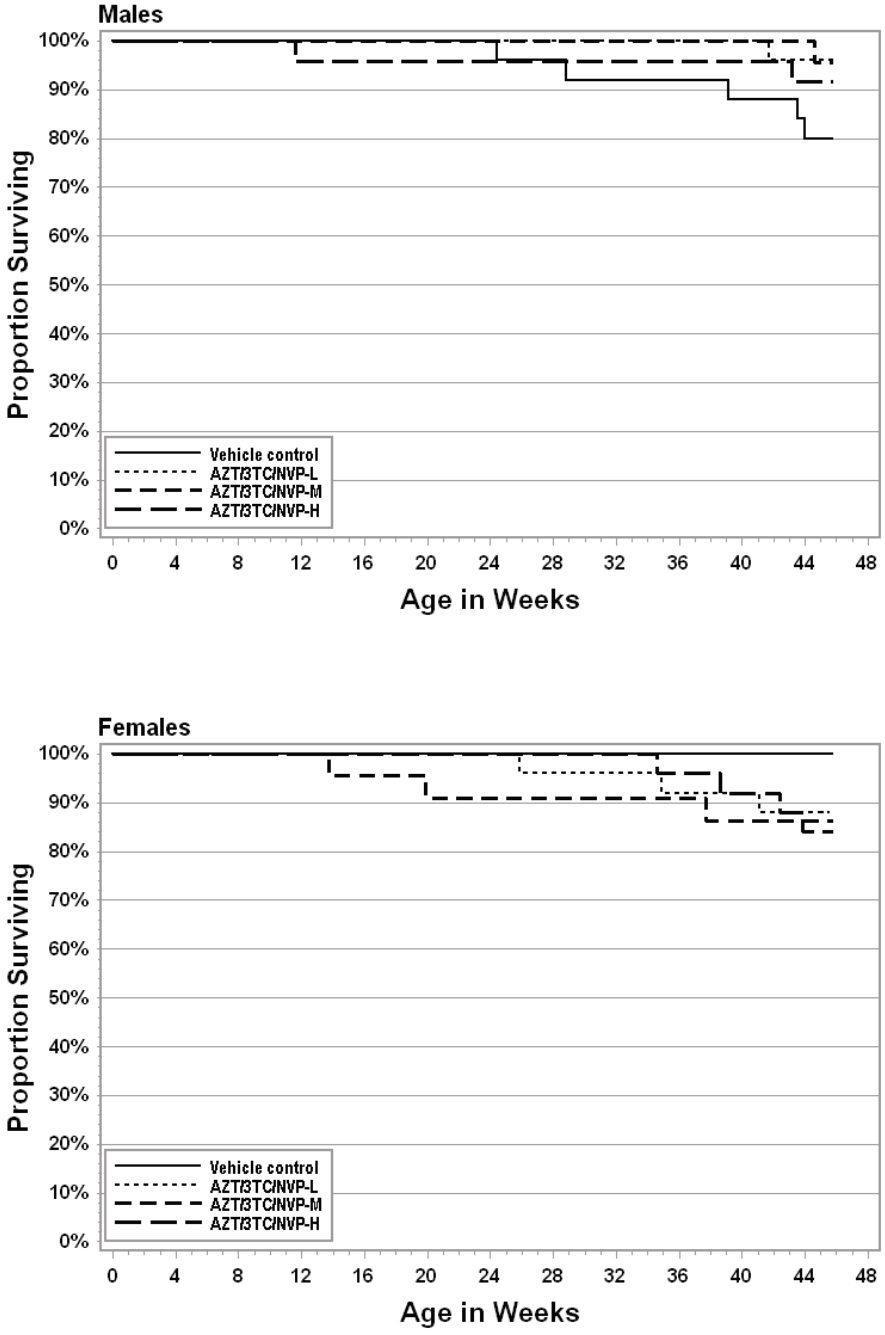


FIGURE 2
Kaplan-Meier Survival Curves for Heterozygous F1 p53$^{+/-}$ Mice
in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison
AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg

TABLE 6
Survival of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Male						
Mice initially in study	25	25	25	26	25	25
Removals[b]	0	1	0	0	0	1
Moribund	2	4	0	0	3	1
Natural deaths	3	2	2	4	1	1
Mice surviving to study termination	20	18	23	22	21	22
Probability of survival to end of study[c]	80.0%	75.0%	92.0%	84.6%	84.0%	91.7%
Mean survival (days)	321.0	302.7	325.3	310.7	312.8	324.8
Survival analysis[d]		P=0.418	P=0.085**N**	P=0.263**N**	P=0.283**N**	P=0.171**N**
Female						
Mice initially in study	25	25	25	25	25	25
Missexed[b]	0	0	0	0	1	0
Moribund	0	3	3	2	4	2
Natural deaths	0	2	2	3	1	2
Mice surviving to study termination	25	20	20	20	19	21
Probability of survival to end of study	100%	80.0%	80.0%	80.0%	79.2%	84.0%
Mean survival (days)	332.8	328.2	326.2	325.4	309.6	328.7
Survival analysis		P=0.051	P=0.054	P=0.051	P=0.037	P=0.075

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Censored from survival analysis

[c] Kaplan-Meier determinations

[d] Cox Proportional Hazards Regression Model results. Tests formed by suitable contrasts in the Cox Estimates. All tests are single degree of freedom, one sided, pairwise comparisons to the vehicle control group. A lower mortality in a dose group is indicated by **N**.

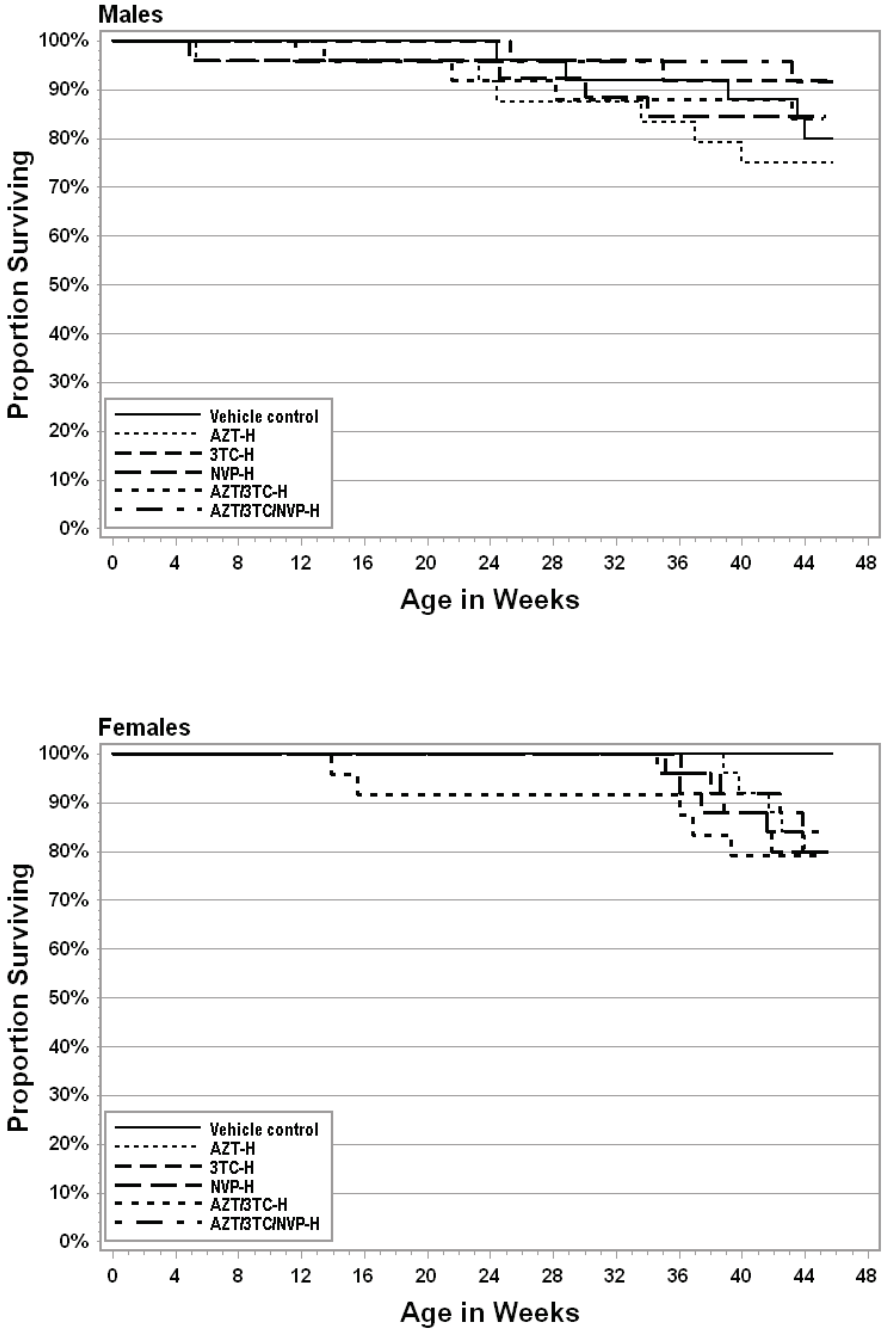


FIGURE 3
Kaplan-Meier Survival Curves for Heterozygous F1 p53$^{+/-}$ Mice
in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison
AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP;
AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

Body Weights, Organ Weights, and Clinical Pathology

Growth curves for males and females are shown in Figures 4 and 5. Body weight data are also presented in Appendix D. In the combination dose comparison, males and females dosed with the AZT/3TC/NVP-H combination had significantly decreased body weights compared to the vehicle control groups for several weeks after PND 11 when individual monitoring began (until 11 weeks for females and 20 weeks for males) (Tables D1 and D3). There were also significant linear trends across the dose groups during this period. In addition, mean body weights for the male and female AZT/3TC/NVP-M groups were significantly less than those of the vehicle control groups until 14 weeks. The high dose comparison suggested that AZT was the major contributor to the observed body weight decreases in the combination chemical groups. Mean body weights for the male and female AZT-H groups were significantly less than those of the vehicle control groups during some of the early weeks of dosing, whereas those of the 3TC-H and NVP-H groups tended to be greater than those of the vehicle control groups (Tables D2 and D4).

In male and female mice, absolute brain weights in the combination dose groups decreased with increasing dose and, except in low dose males, the absolute brain weights of the dosed groups were significantly less than those of the vehicle control groups (Table E1). Because body weights also decreased with increasing dose, there were no significant decreases in relative brain weights compared to the vehicle controls. When the high dose constituent chemicals were compared, absolute brain weights of the male and female AZT-H and AZT/3TC/NVP-H groups were significantly less than those of the vehicle control groups (Table E2). When the high dose groups were compared for females, the decrease appeared to be dependent on AZT exposure; the absolute brain weights of the AZT-H and AZT/3TC/NVP-H groups were statistically similar to one another and the remaining dose groups had absolute brain weights that were significantly greater than that of the AZT/3TC/NVP-H group. Compared to the vehicle control group, there were no significant differences in relative brain weights among the high dose groups of male and female mice.

Relative liver weights of male combination dose groups followed a positive trend with dose (Table E1). When the high dose groups were compared, increases in relative liver weights of male mice appeared to be associated with AZT exposure (Table E2). This is shown in that the relative liver weight of the AZT/3TC/NVP-H group was significantly greater than that of the vehicle control group but similar to the AZT-H and AZT/3TC-H group, while the relative liver weights of the 3TC-H and NVP-H groups were significantly less than that of the AZT/3TC/NVP-H group. In female mice, neither absolute or relative liver weights were significantly affected by any treatment (Tables E1 and E2).

In combination dose groups, the absolute heart weight of AZT/3TC/NVP-H females was significantly greater than that of the vehicle control group, and there was a positive trend in absolute heart weights (Table E1). There was also a positive trend for relative heart weights in these combination dose groups, though no individual group relative weight was significantly greater than that of the vehicle control group. In females, absolute heart weight was also significantly increased in the AZT/3TC-H group relative to the vehicle control group (Table E2). Neither absolute or relative heart weights of males were affected in any group (Tables E1 and E2).

Serum alanine aminotransferase activity was increased in the male AZT/3TC/NVP-H group compared to the vehicle control group (Tables C1 and C2) by a small but statistically significant amount with a significant dose trend across the combination doses. There were no other significant effects on clinical pathology parameters in treated groups of mice.

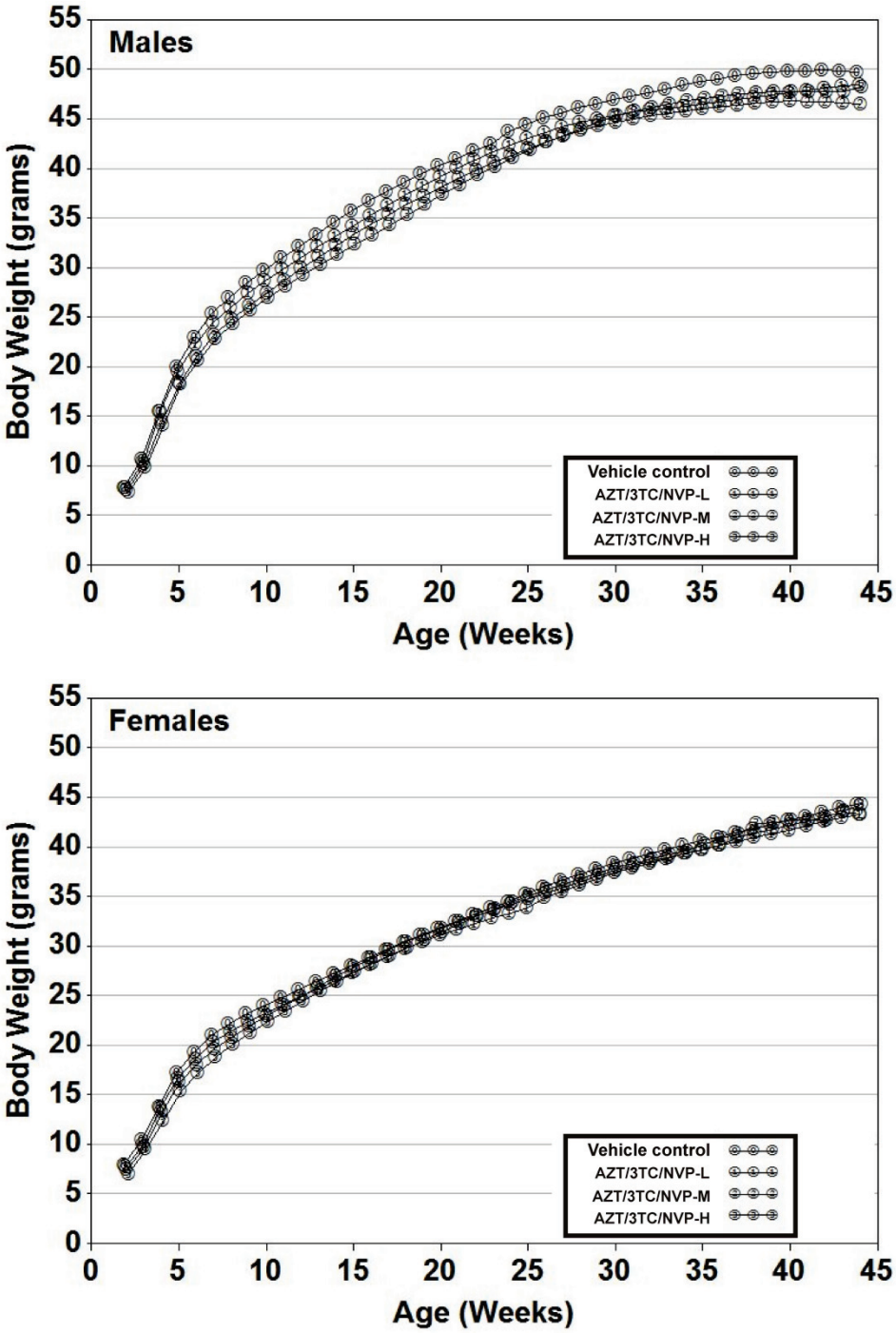


FIGURE 4
Growth Curves for Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison
AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg;
H = 240/150/168 mg/kg

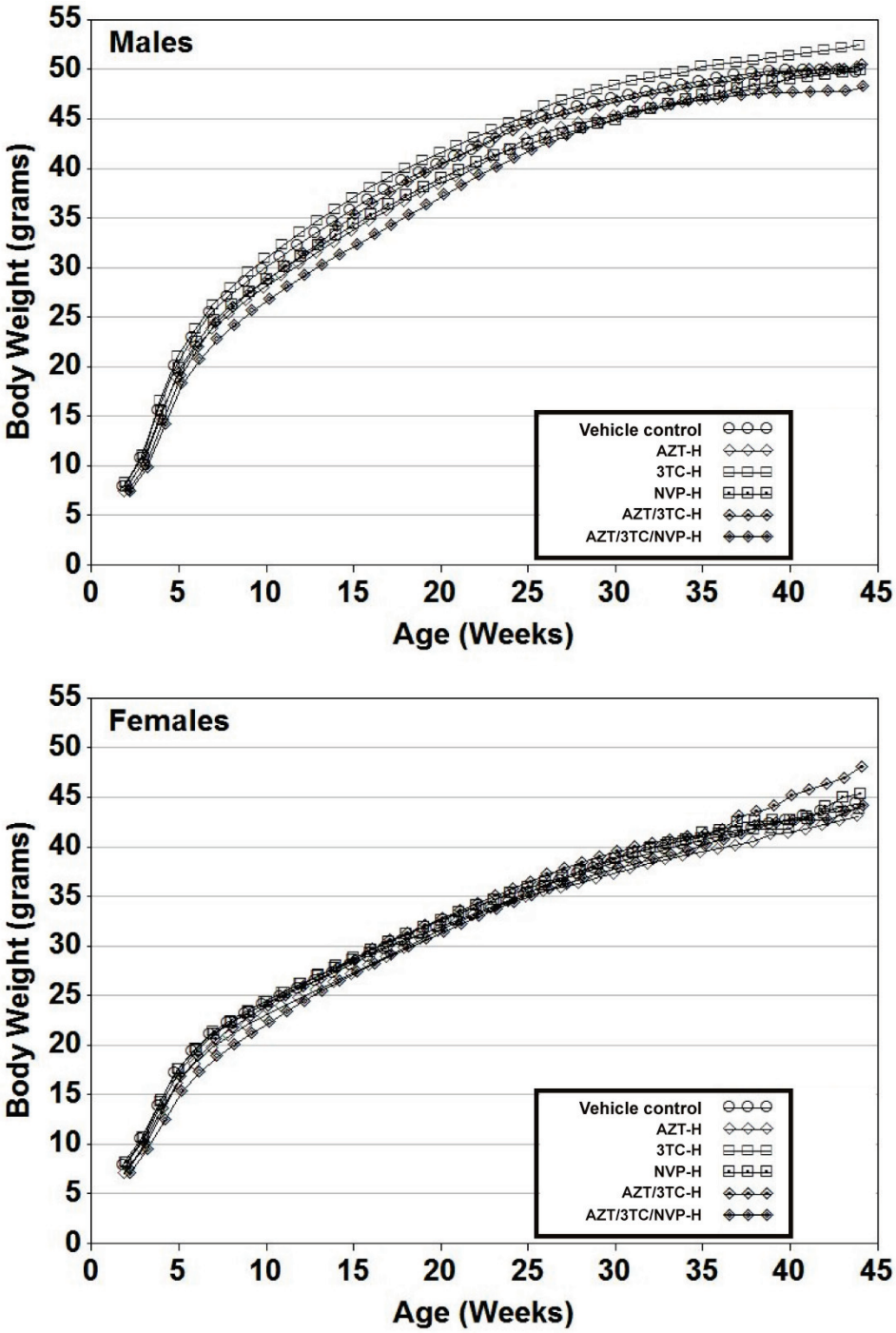


FIGURE 5
Growth Curves for Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison
AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP;
AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms and/or nonneoplastic lesions of the liver, mammary gland, kidney, and spleen. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analysis of selected primary neoplasms are presented in Appendix A.

Liver: In the combination dose comparison, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in all groups of males dosed with AZT/3TC/NVP were significantly increased compared to the vehicle control group, and the incidences increased across the dose range with significant trends (Tables 7, A1, and A2).

The severity of cytoplasmic vacuolization in males in the AZT/3TC/NVP groups was greater than that in the vehicle control group, and the severities increased with a significant ($P \leq 0.029$) trend (Tables A3 and G8). The background incidences of cytoplasmic vacuolization were relatively high: 19/25 and 14/25 for male and female mice, respectively (Tables A3 and A9). High background incidences of this lesion have also been reported in other studies that used the methylcellulose/Tween® 80 dosing vehicle used in the current study (NTP, 2013a,b). The incidences of basophilic focus were slightly increased in AZT/3TC/NVP-M and AZT/3TC/NVP-H males (Table 7).

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Number Examined Microscopically	25	25	23	23
Basophilic Focus[b]	0	0	2	2
Hepatocellular Adenoma, Multiple	0	3	2	2
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	1/25 (4.0%)	7/25 (28.0%)	7/23 (30.4%)	9/23 (39.1%)
Adjusted rate[e]	1/22.9 (4.4%)	7/24.8 (28.3%)	7/22.9 (30.5%)	9/22.8 (39.4%)
Terminal rate[f]	0/20 (0.0%)	7/24 (29.2%)	7/22 (31.8%)	9/22 (40.9%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test[g]	P=0.006	P=0.030	P=0.021	P=0.003
Hepatocellular Carcinoma, Multiple	0	0	0	1
Hepatocellular Carcinoma (includes multiple)	0	2	2	1
Hepatocellular Adenoma or Carcinoma				
Overall rate	1/25 (4.0%)	9/25 (36.0%)	8/23 (34.8%)	10/23 (43.5%)
Adjusted rate	1/22.9 (4.4%)	9/24.8 (36.4%)	8/22.9 (34.9%)	10/22.8 (43.8%)
Terminal rate	0/20 (0.0%)	9/24 (37.5%)	8/22 (36.4%)	10/22 (45.5%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	P=0.004	P=0.006	P=0.009	P=0.001

(T) Terminal kill

[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg

[b] Number of animals with lesion

[c] Historical incidence for control groups in 40- and 45-week heterozygous F1 p53$^{+/-}$ mouse studies: 8/100, range 4.0%-12.5%

[d] Number of animals with neoplasm per number of animals with liver examined microscopically

[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[f] Observed incidence at terminal kill

[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

In the high dose comparison, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in males in the AZT-H, AZT/3TC-H, and AZT/3TC/NVP-H groups were significantly greater than those in the vehicle control group (Tables 8, A4, and A5). The incidences of these lesions in the 3TC-H and NVP-H groups were significantly less than those in the AZT/3TC/NVP-H group suggesting that the AZT component of the remaining dosed groups was the main factor contributing to the increased incidences of liver neoplasms.

TABLE 8
Incidences of Neoplasms of the Liver in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Number Examined Microscopically	25	23	25	25	25	23
Hepatocellular Adenoma, Multiple[b]	0	4	1	0	2	2
Hepatocellular Adenoma (includes multiple)[c]						
Overall rate[d]	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	9/25 (36.0%)	9/23 (39.1%)
Adjusted rate[e]	1/22.9 (4.4%)	8/19.9 (40.2%)	3/23.6 (12.7%)	2/22.4 (8.9%)	9/22.2 (40.5%)	9/22.8 (39.4%)
Terminal rate[f]	0/20 (0.0%)	8/18 (44.4%)	3/23 (13.0%)	2/22 (9.1%)	9/21 (42.9%)	9/22 (40.9%)
First incidence (days)	313	316 (T)	318 (T)	322 (T)	316 (T)	316 (T)
Poly-3 test vs. Vehicle Control[g]		P=0.004	P=0.313	P=0.493	P=0.003	P=0.003
Poly-3 test vs. AZT/3TC/NVP-H[h]	P=0.003	P=0.601	P=0.036**N**	P=0.016**N**	P=0.589	
Hepatocellular Carcinoma, Multiple	0	0	0	0	1	1
Hepatocellular Adenoma or Carcinoma						
Overall rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	10/25 (40.0%)	10/23 (43.5%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	3/23.6 (12.7%)	2/22.4 (8.9%)	10/22.2 (45.0%)	10/22.8 (43.8%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	3/23 (13.0%)	2/22 (9.1%)	10/21 (47.6%)	10/22 (45.5%)
First incidence (days)	313	316 (T)	318 (T)	322 (T)	316 (T)	316 (T)
Poly-3 test vs. Vehicle Control		P=0.004	P=0.313	P=0.493	P<0.001	P=0.001
Poly-3 test vs. AZT/3TC/NVP-H	P=0.001	P=0.529**N**	P=0.017**N**	P=0.007**N**	P=0.585	

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of animals with neoplasm

[c] Historical incidence for control groups in 40- and 45-week heterozygous F1 p53$^{+/-}$ mouse studies: 8/100, range 4.0%-12.5%

[d] Number of animals with neoplasm per number of animals with liver examined microscopically

[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[f] Observed incidence at terminal kill

[g] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

[h] Beneath the dose group incidence are the P values corresponding to pairwise comparisons between the AZT/3TC/NVP-H group and that dose group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

Malignant Lymphoma: The incidences of malignant lymphoma in females administered AZT/3TC/NVP-M or AZT/3TC/NVP-H were slightly greater than that in the vehicle control group and the time to first tumor was earlier in all AZT/3TC/NVP groups than in the vehicle controls (Tables 9, A7 and A8). When the high dose constituent chemicals were compared, the highest incidence occurred in the NVP-H group suggesting that the NVP contributed to the increase (Tables 10, A10, and A11). However, the incidence in the AZT/3TC-H group was also higher than that in the vehicle control group. Historical control data from other NTP and NCTR studies using heterozygous F1 p53$^{+/-}$ mice, which includes this study, give an overall incidence of 3/102 (2.9%) for malignant lymphoma in females (Tables 9, 10, and J2).

In the high dose comparison, the incidences of malignant lymphoma were slightly greater in males administered AZT-H (3/24) or AZT/3TC-H (3/25) than in the vehicle controls (1/25) (Tables A4 and A5). The historical incidence of malignant lymphoma in male control groups of F1 p53$^{+/-}$ mice in 40- and 45-week studies is 3/101 (0.0%-8.0%) (Table J1).

Mammary Gland: The incidence of mammary gland adenoacanthoma or adenocarcinoma (combined) was sightly increased in the 3TC-H group of females relative to the vehicle control group and the AZT/3TC/NVP-H groups (Tables 10, A10, and A11). Mammary gland neoplasms did not occur in the AZT/3TC/NVP-L or AZT/3TC/NVP-M groups of females (Table A7). Historical control data from other NTP and NCTR studies using heterozygous F1 p53$^{+/-}$ mice, which includes the current study, give overall incidences of 0/101 for mammary gland adenoacanthoma and 1/101 (1.0%) for mammary gland adenocarcinoma in females (Table J2). While the increases in the incidences of malignant mammary gland neoplasms in female mice were small and did not reach statistical significance, the possibility that they were treatment related cannot be ruled out under the experimental conditions of this study.

TABLE 9
Incidences of Malignant Lymphoma in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Malignant Lymphoma[b]				
Overall rate[c]	2/25 (8.0%)	2/25 (8.0%)	4/22 (18.2%)	4/25 (16.0%)
Adjusted rate[d]	2/25.0 (8.0%)	2/24.4 (8.2%)	4/22.0 (18.2%)	4/24.5 (16.3%)
Terminal rate	2/25 (8.0%)	0/22 (0.0%)	1/19 (5.3%)	2/21 (9.5%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test[f]	P=0.156	P=0.687	P=0.274	P=0.324

(T) Terminal kill
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Historical incidence for control groups in 40- and 45-week heterozygous F1 p53$^{+/-}$ mouse studies: 3/102, range 0.0%-8.0%
[c] Number of animals with malignant lymphoma per number of animals examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

TABLE 10
Incidences of Selected Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs						
Malignant Lymphoma[b]						
Overall rate[c]	2/25 (8.0%)	2/25 (8.0%)	1/25 (4.0%)	5/25 (20.0%)	4/24 (16.7%)	4/25 (16.0%)
Adjusted rate[d]	2/25.0 (8.0%)	2/24.4 (8.2%)	1/23.7 (4.2%)	5/23.7 (21.1%)	4/23.6 (16.9%)	4/24.5 (16.3%)
Terminal rate[e]	2/25 (8.0%)	0/20 (0.0%)	0/20 (0.0%)	4/20 (20.0%)	0/19 (0.0%)	2/21 (9.5%)
First incidence (days)	316 (T)	277	258	251	102	247
Poly-3 test vs.						
Vehicle Control[f]		P=0.686	P=0.518**N**	P=0.186	P=0.307	P=0.324
Poly-3 test vs.						
AZT/3TC/NVP-H[g]	P=0.324	P=0.336	P=0.182	P=0.479**N**	P=0.628**N**	
Mammary Gland						
Adenocarcinoma[h]						
Overall rate	1/25 (4.0%)	0/25 (0.0%)	3/25 (12.0%)	0/23 (0.0%)	2/23 (8.7%)	0/25 (0.0%)
Adjusted rate	1/25.0 (4.0%)	0/23.8 (0.0%)	3/23.6 (12.7%)	0/21.8 (0.0%)	2/21.1 (9.5%)	0/23.7 (0.0%)
Terminal rate	1/25 (4.0%)	0/20 (0.0%)	2/20 (10.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	319 (T)	—	277	—	280	—
Poly-3 test vs.						
Vehicle Control		P=0.510**N**	P=0.282	P=0.527**N**	P=0.440	P=0.510**N**
Poly-3 test vs.						
AZT/3TC/NVP-H	P=0.510**N**	—	P=0.112**N**	—	P=0.208**N**	
Adenoacanthoma or Adenocarcinoma						
Overall rate	1/25 (4.0%)	1/25 (4.0%)	4/25 (16.0%)	0/23 (0.0%)	2/23 (8.7%)	1/25 (4.0%)
Adjusted rate	1/25.0 (4.0%)	1/23.9 (4.2%)	4/24.0 (16.6%)	0/21.8 (0.0%)	2/21.1 (9.5%)	1/23.9 (4.2%)
Terminal rate	1/25 (4.0%)	0/20 (0.0%)	2/20 (10.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	319 (T)	313	271	—	280	302
Poly-3 test vs.						
Vehicle Control		P=0.750	P=0.161	P=0.527**N**	P=0.440	P=0.751
Poly-3 test vs.						
AZT/3TC/NVP-H	P=0.751	P=0.760**N**	P=0.173**N**	P=0.519	P=0.455**N**	

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Historical incidence for control groups in 40- and 45-week heterozygous F1 p53$^{+/-}$ mouse studies: 3/102, range 0.0%-8.0%

[c] Number of animals with neoplasm per number of animals with liver examined microscopically

[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[e] Observed incidence at terminal kill

[f] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

[g] Beneath the dose group incidence are the P values corresponding to pairwise comparisons between the AZT/3TC/NVP-H group and that dose group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

[h] Historical incidence: 1/101, range 0.0%-4.0%

Kidney: In males, the incidence of protein casts in the AZT/3TC/NVP-H group and the incidences of hydronephrosis in the AZT/3TC/NVP-L and AZT/3TC/NVP-H groups were significantly greater than those in the vehicle control group. The incidences of these lesions also exhibited positive trends (P=0.019 and P=0.006, respectively) (Tables 11 and A3).

Spleen: The incidence of hematopoietic cell proliferation in female mice in the AZT/3TC/NVP-M group was significantly increased compared to that in the vehicle control group, and the incidences of this lesion exhibited a positive trend (P=0.007) (Tables 11 and A9).

Litter Correlation Analysis: Because up to two littermates per sex were used to populate the 45-week study, statistical analysis incorporating correlation of influence among littermates was also used to compute incidences of neoplasms (Appendix G). Statistical significance generally increased suggesting that there was a negative rather than positive correlation between lesion incidence and litter. However, these changes were insufficient to decrease the P values for neoplasm incidences below the 5% definition of significance.

GENETIC TOXICITY

In 1-day-old mice, the percentages of total reticulocytes (RETs) were significantly decreased in groups exposed to doses that contained AZT, but not in the groups exposed to doses containing 3TC or NVP alone (Table B1). In addition, both the percentages of micronucleated normochromatic erythrocytes (NCEs) and the percentages of micronucleated RETs (except percent micronucleated RETs in AZT/3TC-H females) were significantly increased in groups exposed to doses containing AZT, but not in the 3TC-H or NVP-H groups. The percentages of micronucleated NCEs in the AZT/3TC/NVP-H groups were greater than in the AZT-H and the AZT/3TC-H groups. This suggests that the three-drug combination might potentiate the effect of AZT alone (Table B1); however, in blood from male pups evaluated at PND 28, the percentage of micronucleated RETs and the percentage of micronucleated NCEs were significantly increased in the group where 3TC was coadministered with AZT compared to the group administered only AZT (Table B2)

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Male				
Kidney[b]	25	25	23	23
Protein Casts[c]	3 (1.0)[d]	7 (1.0)	7 (1.3)	10* (1.0)
Hydronephrosis	1 (2.0)	13** (1.2)	5 (1.2)	12** (2.0)
Female				
Spleen	25	25	22	25
Hematopoietic Cell Proliferation	2 (2.5)	2 (2.5)	8* (2.0)	7 (2.6)

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

DISCUSSION AND CONCLUSIONS

While the advent of Highly Active Antiretroviral Therapy (HAART) has dramatically improved rates of morbidity and mortality in people infected with HIV, the long-term toxicological consequences of such therapy are unknown (PHS, 2008). AZT is a key component of HAART and although it has previously been studied in cancer bioassays, it has not been extensively studied in combination with other antiretroviral drugs. A major objective of this study was to develop and evaluate the heterozygous F1 $p53^{+/-}$ mouse model as a tool for investigating the potential carcinogenicity of AIDS drug combinations.

The genetically modified mouse in this study was designed to produce a p53 haploinsufficiency on a genetic background that was similar to the B6C3F1 mouse. However, the parental strains were reversed relative to the B6C3F1 mouse to take advantage of the readily available B6.129-*Trp53*$(N12)^{tm1Brd(-/-)}$ mouse to supply p53 haploinsufficiency from the paternal side so that healthy wild type female mice would be exposed to AZT during pregnancy. Unlike many other strains, C3H female mice show excellent tolerance to the dosing of their pups. The observed incidences of hepatocellular adenoma in the male heterozygous F1 $p53^{+/-}$ mice used in this study suggest that these genetically modified mice do express a tumor profile that is similar to the B6C3F1 mouse.

The B6.129-*Trp53*$(N12)^{tm1Brd(+/-)}$ p53 haploinsufficient mouse model, which was used in previous NTP genetically modified model (GMM) studies (NTP, 2012, 2013b) exhibited very low incidences of liver tumors and was unresponsive to exposure to dichloroacetate (Brook, 1987), a chemical that has been shown to be hepatocarcinogenic in both B6C3F1 mice and Fischer 344 rats (Bull *et al.*, 1990; DeAngelo *et al.*, 1996).

The preceding study in this series of experiments (NTP, 2013b), which utilized AZT alone given continuously until 45 weeks of age, demonstrated increased incidences of hepatocellular adenoma with a positive dose trend in male heterozygous F1 $p53^{+/-}$ mice continuously exposed to either 160/80/160 or 240/120/240 mg/kg AZT. In the current study, where heterozygous F1 $p53^{+/-}$ mice were dosed only from postnatal day (PND) 1 though PND 28, significantly increased incidences of hepatocellular adenoma or hepatocellular adenoma or carcinoma (combined), were also observed in male mice evaluated at 45 weeks of age that were exposed to either AZT alone or AZT in combination with either 3TC or 3TC and NVP, but not in male mice exposed to either 3TC or NVP alone.

While 3TC has not been reported to be carcinogenic in rodents, it has been reported to enhance the genotoxicity of AZT in mice simultaneously exposed to both drugs (Von Tungeln *et al.*, 2002; Dobrovolsky *et al.*, 2007). Nevirapine is reported in be a constitutive androstane receptor (CAR)-type cytochrome P450 inducer and to be hepatocarcinogenic in both rats and mice following 2-year exposures (*PDR*, 2010b). CAR agonists generally increase mouse liver tumor incidences in cancer bioassays (McClain, 1995) via promotional mechanisms that require continuous exposure and the relevance of such tumors to human risk assessment is questionable (McClain, 1990). However, the presence of such tumors could potentially mask other hepatocarcinogenic mechanisms such as genotoxicity. NVP metabolites have been reported to form adducts with DNA (Antunes *et al.*, 2008). The heterozygous F1 $p53^{+/-}$ mouse model as used in this study eliminated the potential for CAR-related tumor promotion by dosing only up to PND 28. Clearly, this eliminated NVP-dependent hepatocarcinogenicity while still enabling the manifestation of AZT-dependent hepatocarcinogenicity. Thus, the mouse model, as used in this study, provides a mechanism to investigate hepatocarcinogenic effects of CAR-dependent tumor promotors that are additional and unrelated to CAR-related signal transduction.

Although NVP exposure as used in this study did not significantly increase liver tumor incidences in either male or female mice, there were greater than twofold increases in the incidences of malignant lymphoma in female heterozygous F1 $p53^{+/-}$ mice to over six times the mean historical incidence for all NTP studies that have used this mouse model (Table J2). However, the increases were not statistically significant and so may not be indicative of a treatment-related carcinogenic response. While incidences of malignant lymphoma were increased approximately twofold over vehicle control values in the AZT/3TC/NVP-M and AZT/3TC/NVP-H groups, they were also increased

more than twofold in the AZT/3TC-H group which was not exposed to NVP. The incidence of malignant lymphoma was not increased by AZT alone; the overall incidences in the vehicle control and AZT-H groups were both 2/25. In addition, incidences of mammary gland adenoacanthoma and adenocarcinoma (combined) were increased in female heterozygous F1 $p53^{+/-}$ mice exposed to 3TC, from an overall rate of 4% in the vehicle control group to 16% in the 3TC-H group and 8.7% in the AZT/3TC-H group. While these increases were not statistically significant the incidences were greater than the historical incidence of mammary gland adenoacanthoma and adenocarcinoma for all NTP studies that have used this mouse model (1%; Table J2). The mouse model used in this study was not designed to detect small changes in the incidences of neoplasms with low background rates of occurrence. Increased experimental group size is required to detect effects on rarer neoplasm endpoints. Evidence of carcinogenicity for these increases in neoplasm incidence in female heterozygous F1 $p53^{+/-}$ mice should therefore be considered equivocal because, although the increases are not statistically significant, the experimental design does not eliminate the possibility that they are treatment related.

The heterozygous F1 $p53^{+/-}$ mouse model used in this study has demonstrated clear evidence of hepatocarcinogenicity of AZT in male heterozygous F1 $p53^{+/-}$ mice. The model not only provides a bioassay system that is of comparable sensitivity to the male B6C3F1 mouse in detecting hepatocarcinogens, it also has the ability to differentiate between promotional mouse hepatocarcinogens such as CAR and peroxisome proliferator-activated receptor agonists and more dangerous genotoxic and epigenetic agents.

Early rodent cancer bioassays of AZT using low doses (20 or 40 mg/kg per day) did not report clinically relevant carcinogenic activity (Ayers, 1988), but NTP studies have reported carcinogenic activity when higher doses have been used. For example, B6C3F1 mice exposed to 60 or 120 mg/kg AZT by gavage for 2 years exhibited increased incidences of vaginal squamous cell carcinoma (females) and Harderian gland adenoma (males) (NTP, 1999). While no increase in the incidence of hepatocellular tumors were reported in this study, data interpretation was complicated by a high background liver tumor incidence associated with active infections of *Helicobacter hepaticus.* In a follow-up study using Swiss CD-1® mice exposed to AZT throughout gestation via maternal gavage (NTP, 2006), male F_1 mice exposed to 200 or 300 mg/kg exhibited increased incidences of lung tumors [alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined)] at 2 years of age. Walker *et al.* (2007) reported increased incidences of hepatocellular carcinoma in 2-year-old male B6C3F1 mice exposed during gestation to either 240 or 480 mg/kg AZT. However, a more recent study in B6C3F1 mice that were exposed to AZT transplacentally from gestational day (GD) 12 to GD 18 at doses up to only 240 mg/kg per day, did not report increased rates of hepatocarcinogenicity when the mice were evaluated at 2 years of age (NTP, 2013a).

The mechanisms for the carcinogenic effects of AZT in rodents may also be related to how AZT is metabolized. AZT is metabolized by three pathways: glucuronidation, which accounts for up to 75% of the human urinary product; mixed-function oxidase-mediated reactions, giving 3′-amino-3′-deoxythymidine (AMT), a minor urinary metabolite; and phosphorylation, which occurs throughout the tissues. Phosphorylation is fundamental to the antiviral activity of AZT but accounts for only about 1% of its total disposition. Unchanged AZT constitutes about 20% of the human urinary products; in contrast, the unchanged drug in rats and mice accounts for up to 90% of the drug recovered in the urine (Doshi *et al.*, 1989; Patel *et al.*, 1989; de Miranda *et al.*, 1990). It has been argued that increased incidences of tumors of the vaginal epithelium observed in rodent bioassays may be due to chronic local exposure to unconjugated AZT via urine which would not occur in humans (Ayers *et al.*, 1996b). While lack of conjugation could also influence AZT concentrations in liver and lung tissues following oral AZT exposure, tissue concentrations have not been directly compared between humans and rodents. Despite low rates of glucuronidation, oral doses of AZT are rapidly eliminated in mice; for example, the serum half-life of AZT in adult female C57BL/6N mice treated orally with 400 mg/kg AZT was reported to be 44 minutes (Williams *et al.*, 2003). Toxicokinetic studies associated with the current study are presented in Appendix I. Both the C_{max} and AUC values for AZT in mice receiving 160 or 240 mg/kg were considerably greater than those of human patients receiving therapeutic doses of AZT. For example, AZT therapy is designed to maintain serum AZT concentrations above 1 μM and typically produces C_{max} values of between 5 and 10 μM (Klecker *et al.*, 1987; Donnerer *et al.*, 2008), whereas when measured in heterozygous F1 $p53^{+/-}$ mouse pups on PND 28, oral gavage of AZT at 160 or 240 mg/kg resulted in apparent C_{max} concentrations of 152 and 718 μM, respectively (Table I4). However, the current study demonstrated a significant increase in the incidence of hepatocellular adenomas in male heterozygous F1 $p53^{+/-}$ mice in the AZT/3TC/NVP-L dose group (Table 7), which received 20 mg AZT/kg twice daily by gavage from PND 1 through PND 10, 40 mg/kg twice daily by maternal gavage from GD 12

through GD 18, and 40 mg/kg twice daily by gavage from PND 11 through PND 28. The simulation in Appendix I predicts that administration of 40 mg AZT/kg body weight twice daily to 28-day-old mice would result in apparent C_{max} and AUC values of 20.6 µM and 60.3 µM•hour, respectively (Table I7). These values are 212% and 136%, respectively, of those predicted for human patients receiving therapeutic doses of AZT (Figure I3 and Table I7).

AZT has a relatively high rate of incorporation into liver and lung nuclear and mitochondrial DNA after transplacental exposure (Olivero *et al.*, 1997), and this may explain why these organs are targets for transplacental and neonatal carcinogenicity of the drug. AZT has been reported to evoke a wide range of genotoxic effects in both *in vitro* and *in vivo* test systems, and specifically increases the incidences of micronucleated erythrocytes and reticulocytes in blood from exposed mice (NTP, 1999, 2006, 2013a,b; Bishop *et al.*, 2004b; Witt *et al.*, 2004; Dobrovolsky *et al.*, 2007). In the current genotoxicity studies, erythrocyte and reticulocyte micronucleus frequencies were increased by AZT exposure in heterozygous F1 $p53^{+/-}$ mice evaluated on PND 1 and PND 28, but not after exposure to 3TC or NVP in the absence of AZT; there was some evidence that 3TC and/or NVP enhanced the effect of AZT (Appendix B).

In vitro studies have reported that AZT accumulates in mitochondria as AZT-monophosphate and inhibits mitochondrial synthesis of thymidine triphosphate (TTP) (Lynx *et al.*, 2006). Lack of mitochondrial TTP synthesis may explain at least some of the genotoxic activity of AZT, because in nonproliferating cells, mitochondria are the sole source of nuclear TTP that is required as a substrate for mitochondrial DNA replication and nuclear DNA repair processes (Song *et al.*, 2003; McKee *et al.*, 2004).

While there have been no reports of AZT causing cancer in humans, a recent epidemiology study reported that patients with HIV infection or AIDS have increased risk of developing lung cancer (Kirk *et al.*, 2007). Since the increased risk was not significantly correlated with either HAART or low $CD4^+$ cell count it is not yet known whether AZT exposure contributes to this increased cancer risk. Another recent study from California has reported that homosexual men have a higher incidence of cancer diagnosis than heterosexual men, but the explanation for this has not been investigated (Boehmer *et al.*, 2012). Cancer takes time to develop, and further follow-up of patients is required to determine any association between AZT (and other antiretroviral drugs) and long-term adverse effects, including cancer. While the benefits of HAART clearly outweigh the potential short-term risks of AZT and other AIDS therapeutics for individuals exposed to HIV, more information is needed on the long-term consequences of HAART so that the therapeutic regimes can be optimized for long-term health. The heterozygous F1 $p53^{+/-}$ mouse model provides a useful tool for further evaluating the hepatocarcinogenic potential of AZT when used in combination with other therapeutic agents. It has the advantage of potentially providing data within 12 months of study initiation, and if sufficient animal numbers are used to provide statistical power, it promises high sensitivity for detecting hepatocarcinogenicity and possibly other neoplasias.

The previous study in this series (NTP, 2013b) exposed heterozygous F1 $p53^{+/-}$ mice to AZT alone through the same dosing period used in the current study, but continued the dosing 5 days per week from PND 20 until evaluation at 45 weeks of age. The Poly-3 adjusted incidence rates for hepatocellular adenoma or carcinoma (combined) in the 80/40/80, 160/80/160, and 240/120/240 mg/kg AZT dose groups of males in this study were 13.2%, 22.2%, and 36.5%, respectively. This corresponds to Poly-3-adjusted rates of 36.4%, 34.9%, and 43.8% for the AZT/3TC/NVP-L, AZT/3TC/NVP-M, and AZT/3TC/NVP-H dose groups of males in the current study. In females, the incidences of malignant lymphoma in the vehicle control, 80/40/80, 160/80/160, and 240/120/240 mg/kg AZT dose groups were 0/26, 0/27, 1/27 and 3/27, respectively, whereas in the current study they were 2/25, 2/25, 4/22, 4/25 and 2/25 for the vehicle control, AZT/3TC/NVP-L, AZT/3TC/NVP-M, AZT/3TC/NVP-H, and AZT-H dose groups, respectively. The mice in the corresponding dose groups in both studies were exposed to identical total daily doses of AZT between GD 12 and PND 28. The studies differed in that (1) dosing continued until terminal evaluation in the previous study (NTP, 2013b) but ceased at PND 28 in the current study, (2) the dose was administered as a single gavage dose in the previous study but was split into two gavage administrations 6 hours apart in the current study, and (3) AZT was administered alone in the previous study but was part of a three-drug combination in the current study.

Despite the shorter exposure time, there is clearly a greater carcinogenic response in the male mice of the current study than in the previous study (NTP, 2013b),

particularly with the low and mid doses. The Poly-3 adjusted rates for the incidences of hepatocellular adenoma or carcinoma (combined) for the AZT-H and AZT/3TC-H dose groups in the current study are 40.2% and 45.0%, respectively, and as such are not able to significantly demonstrate that 3TC and/or NVP exposure enhances AZT-induced hepatocarcinogenicity. Likewise, the small difference between the Poly-3 adjusted liver neoplasm incidence rates in the AZT-H group in the current study and that of the 240/120/240 AZT dose group in the previous study (NTP, 2013b) is insufficient to significantly demonstrate that splitting the dose enhances carcinogenicity. Unfortunately, the current study did not include an AZT-L dose group, which might have been able to resolve this. In contrast, there was no evidence from the current study that AZT alone increased incidence of malignant lymphoma in female heterozygous F1 $p53^{+/-}$ mice, despite equivocal evidence for this effect from the previous study. Diwan *et al.,* (1999) also observed no increase in the incidence of malignant lymphoma in CD-1 mice exposed to AZT *in utero*. Thus, it is possible that adult exposure to AZT is responsible for the increased incidence of malignant lymphoma observed in the previous study. Nevertheless, it is apparent from comparing the two studies that a combination of twice-daily dosing and coadministration of 3TC and NVP lowers the threshold AZT dose that produces hepatocellular adenomas and carcinomas in male heterozygous F1 $p53^{+/-}$ mice. This observation has some relevance to the potential long-term human risk of HAART therapy, because AZT is used therapeutically in multidrug combinations, and AZT is administered two or three times daily to achieve constant blood levels.

CONCLUSIONS

Under the conditions of this gavage study, there was *clear evidence of carcinogenic activity** of AZT alone in male heterozygous F1 $p53^{+/-}$ mice based on increased incidences of hepatocellular adenoma. There was clear evidence of carcinogenic activity of AZT in combination with 3TC, and AZT in combination with 3TC and NVP in male heterozygous F1 $p53^{+/-}$ mice based on increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). The occurrence of malignant lymphoma may have been related to treatment with AZT alone and with AZT in combination with 3TC.

There was *no evidence of carcinogenic activity* of 3TC alone in male heterozygous F1 $p53^{+/-}$ mice administered 150 mg/kg. There was *no evidence of carcinogenic activity* of NVP alone in male heterozygous F1 $p53^{+/-}$ mice administered 168 mg/kg.

There was *equivocal evidence of carcinogenic activity* of NVP alone, AZT in combination with 3TC, and AZT in combination with 3TC and NVP in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of malignant lymphoma. There was *equivocal evidence of carcinogenic activity* of 3TC alone in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of mammary gland adenoacanthoma or adenocarcinoma (combined).

There was *no evidence of carcinogenic activity* of AZT alone in female heterozygous F1 $p53^{+/-}$ mice administered 240 mg/kg.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Peer Review Panel comments and the public discussion on this Report appears on page 15.

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APPENDIX A
SUMMARY OF LESIONS IN HETEROZYGOUS F1 p53$^{+/-}$ MICE IN THE *IN UTERO*/POSTNATAL GAVAGE STUDY OF AZT, 3TC, AND NVP

TABLE A1
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Disposition Summary				
Animals initially in study	25	25	25	25
Early deaths				
Moribund	2			
Natural deaths	1	1		1
Survivors				
Died/moribund after day 320	2		1	1
Terminal kill	20	24	22	22
Removals			2	1
Animals examined microscopically	25	25	23	24
Alimentary System				
Esophagus	(25)	(25)	(23)	(24)
Gallbladder	(22)	(24)	(23)	(23)
Intestine large, cecum	(22)	(24)	(23)	(23)
Intestine large, colon	(22)	(24)	(23)	(23)
Intestine large, rectum	(22)	(24)	(23)	(23)
Intestine small, duodenum	(23)	(24)	(23)	(23)
Leiomyosarcoma	1 (4%)			
Intestine small, ileum	(22)	(24)	(23)	(23)
Intestine small, jejunum	(23)	(24)	(23)	(23)
Adenocarcinoma	1 (4%)			
Liver	(25)	(25)	(23)	(23)
Hepatocellular adenoma	1 (4%)	4 (16%)	5 (22%)	7 (30%)
Hepatocellular adenoma, multiple		3 (12%)	2 (9%)	2 (9%)
Hepatocellular carcinoma		2 (8%)	2 (9%)	
Hepatocellular carcinoma, multiple				1 (4%)
Mesentery	(3)	(1)	(2)	(0)
Hemangiosarcoma		1 (100%)		
Leiomyosarcoma, metastatic, intestine small, duodenum	1 (33%)			
Sarcoma	1 (33%)			
Pancreas	(24)	(25)	(23)	(23)
Leiomyosarcoma, metastatic, intestine small, duodenum	1 (4%)			
Sarcoma, metastatic, tissue NOS	1 (4%)			
Salivary glands	(25)	(25)	(23)	(23)
Stomach, forestomach	(25)	(25)	(23)	(23)
Stomach, glandular	(23)	(24)	(23)	(23)
Cardiovascular System				
Blood vessel	(25)	(25)	(23)	(24)
Heart	(25)	(25)	(23)	(24)
Endocrine System				
Adrenal cortex	(25)	(25)	(23)	(23)
Adrenal medulla	(25)	(25)	(23)	(22)
Pheochromocytoma benign			1 (4%)	
Islets, pancreatic	(24)	(25)	(23)	(23)
Parathyroid gland	(23)	(23)	(23)	(23)
Pituitary gland	(24)	(25)	(23)	(23)
Thyroid gland	(25)	(25)	(23)	(23)

TABLE A1
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Abdominal, sarcoma	1 (100%)			
Genital System				
Epididymis	(25)	(25)	(23)	(23)
Preputial gland	(25)	(25)	(23)	(23)
Squamous cell carcinoma			1 (4%)	
Squamous cell papilloma				1 (4%)
Prostate	(25)	(25)	(23)	(23)
Seminal vesicle	(25)	(25)	(23)	(23)
Testes	(25)	(25)	(23)	(23)
Hematopoietic System				
Bone marrow	(23)	(25)	(23)	(23)
Lymph node	(2)	(3)	(1)	(1)
Mediastinal, sarcoma, metastatic, tissue NOS	1 (50%)			
Lymph node, mandibular	(25)	(25)	(23)	(23)
Lymph node, mesenteric	(24)	(25)	(23)	(23)
Sarcoma, metastatic, tissue NOS	1 (4%)			
Spleen	(25)	(25)	(23)	(23)
Thymus	(24)	(25)	(20)	(20)
Integumentary System				
Mammary gland	(1)	(1)	(0)	(0)
Skin	(25)	(25)	(23)	(24)
Osteosarcoma, metastatic, bone				1 (4%)
Squamous cell papilloma			1 (4%)	
Musculoskeletal System				
Bone	(3)	(0)	(1)	(1)
Osteosarcoma	1 (33%)			
Cranium, neuroblastoma, metastatic, nose			1 (100%)	
Humerus, osteosarcoma	1 (33%)			
Rib, osteosarcoma				1 (100%)
Tibia, osteosarcoma	1 (33%)			
Bone, femur	(25)	(25)	(23)	(24)
Skeletal muscle	(3)	(0)	(0)	(0)
Leiomyosarcoma, metastatic, intestine small, duodenum	1 (33%)			
Diaphragm, sarcoma, metastatic, tissue NOS	1 (33%)			
Nervous System				
Brain, brain stem	(25)	(24)	(23)	(23)
Brain, cerebellum	(25)	(24)	(23)	(23)
Brain, cerebrum	(25)	(25)	(23)	(23)
Olfactory lobe, neuroblastoma, metastatic, nose			1 (4%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Nervous System (continued)				
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord, cervical	(1)	(0)	(0)	(0)
Spinal cord, lumbar	(1)	(0)	(0)	(0)
Spinal cord, thoracic	(1)	(0)	(0)	(0)
Respiratory System				
Lung	(25)	(25)	(23)	(23)
Alveolar/bronchiolar adenoma			2 (9%)	
Osteosarcoma, metastatic, bone	1 (4%)			
Nose	(25)	(25)	(23)	(24)
Neuroblastoma			1 (4%)	
Trachea	(25)	(25)	(23)	(23)
Special Senses System				
Eye	(22)	(24)	(23)	(23)
Harderian gland	(25)	(25)	(23)	(23)
Adenoma		1 (4%)		
Neuroblastoma, metastatic, nose			1 (4%)	
Urinary System				
Kidney	(25)	(25)	(23)	(23)
Urinary bladder	(25)	(25)	(23)	(23)
Systemic Lesions				
Multiple organs[b]	(25)	(25)	(23)	(24)
Leukemia granulocytic		1 (4%)		
Lymphoma malignant	1 (4%)		1 (4%)	2 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	7	11	13	14
Total primary neoplasms	9	12	16	14
Total animals with benign neoplasms	1	7	11	10
Total benign neoplasms	1	8	11	10
Total animals with malignant neoplasms	7	4	5	4
Total malignant neoplasms	8	4	5	4
Total animals with metastatic neoplasms	3		1	1
Total metastatic neoplasms	8		3	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg

[b] Number of animals with any tissue examined microscopically

[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
Bone: Osteosarcoma				
Overall rate[b]	3/25 (12.0%)	0/25 (0.0%)	0/23 (0.0%)	1/24 (4.2%)
Adjusted rate[c]	3/23.9 (12.6%)	0/24.8 (0.0%)	0/22.9 (0.0%)	1/22.9 (4.4%)
Terminal rate[d]	1/20 (5.0%)	0/24 (0.0%)	0/22 (0.0%)	1/22 (4.5%)
First incidence (days)	207	—[f]	—	326 (T)
Poly-3 test[e]	P=0.137**N**	P=0.107**N**	P=0.121**N**	P=0.319**N**
Liver: Hepatocellular Adenoma				
Overall rate	1/25 (4.0%)	7/25 (28.0%)	7/23 (30.4%)	9/23 (39.1%)
Adjusted rate	1/22.9 (4.4%)	7/24.8 (28.3%)	7/22.9 (30.5%)	9/22.8 (39.4%)
Terminal rate	0/20 (0.0%)	7/24 (29.2%)	7/22 (31.8%)	9/22 (40.9%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	P=0.006	P=0.030	P=0.021	P=0.003
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	1/25 (4.0%)	9/25 (36.0%)	8/23 (34.8%)	10/23 (43.5%)
Adjusted rate	1/22.9 (4.4%)	9/24.8 (36.4%)	8/22.9 (34.9%)	10/22.8 (43.8%)
Terminal rate	0/20 (0.0%)	9/24 (37.5%)	8/22 (36.4%)	10/22 (45.5%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	P=0.004	P=0.006	P=0.009	P=0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/25 (0.0%)	0/25 (0.0%)	2/23 (8.7%)	0/23 (0.0%)
Adjusted rate	0/22.8 (0.0%)	0/24.8 (0.0%)	2/22.9 (8.7%)	0/22.8 (0.0%)
Terminal rate	0/20 (0.0%)	0/24 (0.0%)	2/22 (9.1%)	0/22 (0.0%)
First incidence (days)	—	—	318 (T)	—
Poly-3 test	P=0.379	—[g]	P=0.236	—
All Organs: Malignant Lymphoma				
Overall rate	1/25 (4.0%)	0/25 (0.0%)	1/23 (4.3%)	2/24 (8.3%)
Adjusted rate	1/23.6 (4.2%)	0/24.8 (0.0%)	1/22.9 (4.4%)	2/23.0 (8.7%)
Terminal rate	0/20 (0.0%)	0/24 (0.0%)	1/22 (4.5%)	1/22 (4.5%)
First incidence (days)	176	—	325 (T)	307
Poly-3 test	P=0.238	P=0.491**N**	P=0.754	P=0.491
All Organs: Osteosarcoma				
Overall rate	3/25 (12.0%)	0/25 (0.0%)	0/23 (0.0%)	1/24 (4.2%)
Adjusted rate	3/23.9 (12.6%)	0/24.8 (0.0%)	0/22.9 (0.0%)	1/22.9 (4.4%)
Terminal rate	1/20 (5.0%)	0/24 (0.0%)	0/22 (0.0%)	1/22 (4.5%)
First incidence (days)	207	—	—	326 (T)
Poly-3 test	P=0.137**N**	P=0.107**N**	P=0.121**N**	P=0.319**N**
All Organs: Malignant Neoplasms				
Overall rate	7/25 (28.0%)	4/25 (16.0%)	5/23 (21.7%)	4/24 (16.7%)
Adjusted rate	7/25.0 (28.0%)	4/25.0 (16.0%)	5/23.0 (21.7%)	4/23.0 (17.4%)
Terminal rate	2/20 (10.0%)	3/24 (12.5%)	4/22 (18.2%)	3/22 (13.6%)
First incidence (days)	176	297	318 (T)	307
Poly-3 test	P=0.278**N**	P=0.249**N**	P=0.435**N**	P=0.300**N**

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
All Organs: Benign Neoplasms				
Overall rate	1/25 (4.0%)	7/25 (28.0%)	11/23 (47.8%)	10/24 (41.7%)
Adjusted rate	1/22.9 (4.4%)	7/24.8 (28.3%)	11/23.0 (47.8%)	10/22.9 (43.8%)
Terminal rate	0/20 (0.0%)	7/24 (29.2%)	10/22 (45.5%)	10/22 (45.5%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	P<0.001	P=0.030	P<0.001	P=0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	7/25 (28.0%)	11/25 (44.0%)	13/23 (56.5%)	14/24 (58.3%)
Adjusted rate	7/25.0 (28.0%)	11/25.0 (44.0%)	13/23.0 (56.5%)	14/23.0 (60.8%)
Terminal rate	2/20 (10.0%)	10/24 (41.7%)	12/22 (54.5%)	13/22 (59.1%)
First incidence (days)	176	297	318 (T)	307
Poly-3 test	P=0.009	P=0.190	P=0.040	P=0.019

(T) Terminal kill
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Disposition Summary				
Animals initially in study	25	25	25	25
Early deaths				
Moribund	2			
Natural deaths	1	1		1
Survivors				
Died/moribund after day 320	2		1	1
Terminal kill	20	24	22	22
Removals			2	1
Animals examined microscopically	25	25	23	24
Alimentary System				
Esophagus	(25)	(25)	(23)	(24)
Gallbladder	(22)	(24)	(23)	(23)
Intestine large, cecum	(22)	(24)	(23)	(23)
Intestine large, colon	(22)	(24)	(23)	(23)
Intestine large, rectum	(22)	(24)	(23)	(23)
Intestine small, duodenum	(23)	(24)	(23)	(23)
Ectopic pancreas				1 (4%)
Intestine small, ileum	(22)	(24)	(23)	(23)
Intestine small, jejunum	(23)	(24)	(23)	(23)
Liver	(25)	(25)	(23)	(23)
Basophilic focus			2 (9%)	2 (9%)
Hematopoietic cell proliferation	1 (4%)	1 (4%)		
Infiltration cellular, lymphocyte		1 (4%)	1 (4%)	
Inflammation, suppurative			1 (4%)	
Inflammation, chronic active		1 (4%)	1 (4%)	1 (4%)
Necrosis	1 (4%)	1 (4%)	1 (4%)	2 (9%)
Tension lipidosis		2 (8%)	2 (9%)	1 (4%)
Vacuolization cytoplasmic	19 (76%)	21 (84%)	19 (83%)	20 (87%)
Centrilobular, degeneration	15 (60%)	20 (80%)	18 (78%)	17 (74%)
Mesentery	(3)	(1)	(2)	(0)
Fat, necrosis	1 (33%)		2 (100%)	
Pancreas	(24)	(25)	(23)	(23)
Cyst	1 (4%)			
Infiltration cellular, lymphocyte		2 (8%)		
Acinus, degeneration			1 (4%)	
Salivary glands	(25)	(25)	(23)	(23)
Infiltration cellular, lymphocyte	18 (72%)	14 (56%)	16 (70%)	16 (70%)
Stomach, forestomach	(25)	(25)	(23)	(23)
Hyperkeratosis			1 (4%)	
Epithelium, hyperplasia	1 (4%)			
Stomach, glandular	(23)	(24)	(23)	(23)
Cardiovascular System				
Blood vessel	(25)	(25)	(23)	(24)
Heart	(25)	(25)	(23)	(24)
Mineralization		1 (4%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Endocrine System				
Adrenal cortex	(25)	(25)	(23)	(23)
Accessory adrenal cortical nodule	1 (4%)			1 (4%)
Hypertrophy			1 (4%)	2 (9%)
Subcapsular, hyperplasia	13 (52%)	14 (56%)	11 (48%)	12 (52%)
Adrenal medulla	(25)	(25)	(23)	(22)
Islets, pancreatic	(24)	(25)	(23)	(23)
Hyperplasia	13 (54%)	9 (36%)	13 (57%)	6 (26%)
Parathyroid gland	(23)	(23)	(23)	(23)
Pituitary gland	(24)	(25)	(23)	(23)
Thyroid gland	(25)	(25)	(23)	(23)
Ectopic thymus	2 (8%)			
Follicle, degeneration				1 (4%)
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Genital System				
Epididymis	(25)	(25)	(23)	(23)
Exfoliated germ cell				1 (4%)
Hypospermia				1 (4%)
Inflammation, chronic active			1 (4%)	
Preputial gland	(25)	(25)	(23)	(23)
Cyst		1 (4%)		
Infiltration cellular, lymphocyte		1 (4%)		
Inflammation, suppurative			1 (4%)	
Inflammation, chronic active	1 (4%)		1 (4%)	
Keratin cyst		1 (4%)		
Acinus, degeneration	3 (12%)	4 (16%)	7 (30%)	5 (22%)
Duct, ectasia	3 (12%)	3 (12%)	5 (22%)	5 (22%)
Prostate	(25)	(25)	(23)	(23)
Inflammation, suppurative			1 (4%)	
Seminal vesicle	(25)	(25)	(23)	(23)
Testes	(25)	(25)	(23)	(23)
Seminiferous tubule, degeneration			1 (4%)	1 (4%)
Hematopoietic System				
Bone marrow	(23)	(25)	(23)	(23)
Hyperplasia	3 (13%)	2 (8%)	4 (17%)	
Lymph node	(2)	(3)	(1)	(1)
Axillary, hyperplasia, lymphoid		1 (33%)		
Lumbar, hyperplasia, lymphoid		1 (33%)		
Mediastinal, hyperplasia, lymphoid	1 (50%)	2 (67%)		
Mediastinal, infiltration cellular, plasma cell		1 (33%)		
Renal, hyperplasia, lymphoid		1 (33%)		
Lymph node, mandibular	(25)	(25)	(23)	(23)
Hyperplasia, lymphoid	2 (8%)	2 (8%)	4 (17%)	5 (22%)
Infiltration cellular, plasma cell			1 (4%)	

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Hematopoietic System (continued)				
Lymph node, mesenteric	(24)	(25)	(23)	(23)
Hyperplasia, lymphoid	9 (38%)	10 (40%)	5 (22%)	9 (39%)
Infiltration cellular, plasma cell		1 (4%)	1 (4%)	
Infiltration cellular, polymorphonuclear			1 (4%)	
Spleen	(25)	(25)	(23)	(23)
Hematopoietic cell proliferation	7 (28%)	9 (36%)	6 (26%)	6 (26%)
Hyperplasia, lymphoid	12 (48%)	15 (60%)	13 (57%)	12 (52%)
Thymus	(24)	(25)	(20)	(20)
Atrophy	2 (8%)	1 (4%)		1 (5%)
Hyperplasia, lymphoid	2 (8%)	3 (12%)	1 (5%)	1 (5%)
Integumentary System				
Mammary gland	(1)	(1)	(0)	(0)
Skin	(25)	(25)	(23)	(24)
Inflammation, suppurative				1 (4%)
Epithelium, hyperplasia				1 (4%)
Musculoskeletal System				
Bone	(3)	(0)	(1)	(1)
Bone, femur	(25)	(25)	(23)	(24)
Fibro-osseous lesion	1 (4%)		1 (4%)	
Skeletal muscle	(3)	(0)	(0)	(0)
Nervous System				
Brain, brain stem	(25)	(24)	(23)	(23)
Brain, cerebellum	(25)	(24)	(23)	(23)
Brain, cerebrum	(25)	(25)	(23)	(23)
Mineralization	2 (8%)	1 (4%)	3 (13%)	1 (4%)
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord, cervical	(1)	(0)	(0)	(0)
Spinal cord, lumbar	(1)	(0)	(0)	(0)
Spinal cord, thoracic	(1)	(0)	(0)	(0)
Compression	1 (100%)			
Respiratory System				
Lung	(25)	(25)	(23)	(23)
Hemorrhage		1 (4%)		
Alveolar epithelium, hyperplasia		1 (4%)		1 (4%)
Nose	(25)	(25)	(23)	(24)
Hyaline droplet		3 (12%)	2 (9%)	
Trachea	(25)	(25)	(23)	(23)
Special Senses System				
Eye	(22)	(24)	(23)	(23)
Harderian gland	(25)	(25)	(23)	(23)
Infiltration cellular, lymphocyte	1 (4%)	1 (4%)	1 (4%)	

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Urinary System				
Kidney	(25)	(25)	(23)	(23)
Casts protein	3 (12%)	7 (28%)	7 (30%)	10 (43%)
Cyst			1 (4%)	
Hydronephrosis	1 (4%)	13 (52%)	5 (22%)	12 (52%)
Infarct			1 (4%)	
Infiltration cellular, lymphocyte	8 (32%)	9 (36%)	9 (39%)	7 (30%)
Nephropathy	1 (4%)			1 (4%)
Urinary bladder	(25)	(25)	(23)	(23)
Infiltration cellular, lymphocyte	2 (8%)	1 (4%)	1 (4%)	1 (4%)
Lumen, dilatation	1 (4%)			

TABLE A4
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Disposition Summary						
Animals initially in study	25	25	25	26	25	25
Early deaths						
Moribund	2	4			3	
Natural deaths	1	2	2	4		1
Survivors						
Died/moribund after day 320	2				1	1
Terminal kill	20	18	23	22	21	22
Removals		1				1
Animals examined microscopically	25	24	25	26	25	24
Alimentary System						
Esophagus	(25)	(23)	(25)	(25)	(24)	(24)
Gallbladder	(22)	(21)	(25)	(22)	(24)	(23)
Intestine large, cecum	(22)	(22)	(24)	(22)	(24)	(23)
Adenoma				1 (5%)		
Intestine large, colon	(22)	(22)	(25)	(23)	(24)	(23)
Intestine large, rectum	(22)	(22)	(23)	(22)	(24)	(23)
Intestine small, duodenum	(23)	(22)	(23)	(22)	(24)	(23)
Leiomyosarcoma	1 (4%)					
Intestine small, ileum	(22)	(22)	(24)	(22)	(24)	(23)
Intestine small, jejunum	(23)	(22)	(23)	(22)	(24)	(23)
Adenocarcinoma	1 (4%)					
Liver	(25)	(23)	(25)	(25)	(25)	(23)
Hemangiosarcoma					1 (4%)	
Hepatocellular adenoma	1 (4%)	4 (17%)	2 (8%)	2 (8%)	7 (28%)	7 (30%)
Hepatocellular adenoma, multiple		4 (17%)	1 (4%)		2 (8%)	2 (9%)
Hepatocellular carcinoma, multiple					1 (4%)	1 (4%)
Sarcoma, metastatic, uncertain primary site			1 (4%)			
Capsule, alveolar/bronchiolar carcinoma, metastatic, lung			1 (4%)			
Mesentery	(3)	(0)	(0)	(1)	(2)	(0)
Fibrosarcoma					1 (50%)	
Leiomyosarcoma, metastatic, intestine small, duodenum	1 (33%)					
Sarcoma	1 (33%)			1 (100%)		
Pancreas	(24)	(23)	(25)	(25)	(24)	(23)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (4%)			
Leiomyosarcoma, metastatic, intestine small, duodenum	1 (4%)					
Sarcoma, metastatic, tissue NOS	1 (4%)					
Sarcoma, metastatic, uncertain primary site			1 (4%)			
Salivary glands	(25)	(23)	(25)	(25)	(24)	(23)
Stomach, forestomach	(25)	(23)	(25)	(23)	(24)	(23)
Stomach, glandular	(23)	(22)	(24)	(22)	(24)	(23)

TABLE A4
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Cardiovascular System						
Blood vessel	(25)	(23)	(25)	(25)	(24)	(24)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (4%)			
Heart	(25)	(24)	(25)	(25)	(24)	(24)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (4%)			
Endocrine System						
Adrenal cortex	(25)	(23)	(25)	(25)	(23)	(23)
Adrenal medulla	(25)	(23)	(25)	(23)	(21)	(22)
Islets, pancreatic	(24)	(23)	(25)	(25)	(24)	(23)
Parathyroid gland	(23)	(20)	(24)	(18)	(19)	(23)
Pituitary gland	(24)	(21)	(25)	(25)	(24)	(23)
Thyroid gland	(25)	(23)	(25)	(23)	(23)	(23)
General Body System						
Tissue NOS	(1)	(0)	(1)	(3)	(0)	(0)
Abdominal, sarcoma	1 (100%)		1 (100%)	1 (33%)		
Genital System						
Epididymis	(25)	(23)	(25)	(25)	(24)	(23)
Sarcoma, metastatic, uncertain primary site			1 (4%)			
Preputial gland	(25)	(24)	(25)	(25)	(24)	(23)
Squamous cell papilloma						1 (4%)
Prostate	(25)	(23)	(25)	(25)	(24)	(23)
Seminal vesicle	(25)	(23)	(24)	(25)	(24)	(23)
Sarcoma, metastatic, uncertain primary site			1 (4%)			
Testes	(25)	(24)	(24)	(25)	(24)	(23)
Hematopoietic System						
Bone marrow	(23)	(24)	(24)	(25)	(24)	(23)
Lymph node	(2)	(2)	(1)	(1)	(2)	(1)
Sarcoma, metastatic, uncertain primary site			1 (100%)			
Mediastinal, sarcoma, metastatic, tissue NOS	1 (50%)					
Mediastinal, sarcoma, metastatic, uncertain primary site			1 (100%)			
Lymph node, mandibular	(25)	(23)	(25)	(25)	(24)	(23)
Lymph node, mesenteric	(24)	(23)	(25)	(25)	(24)	(23)
Sarcoma, metastatic, tissue NOS	1 (4%)					
Sarcoma, metastatic, uncertain primary site			1 (4%)			
Spleen	(25)	(23)	(24)	(25)	(24)	(23)
Hemangiosarcoma					1 (4%)	
Thymus	(24)	(22)	(24)	(23)	(22)	(20)
Sarcoma, metastatic, uncertain primary site			1 (4%)			

TABLE A4
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Integumentary System						
Mammary gland	(1)	(0)	(0)	(1)	(1)	(0)
Skin	(25)	(24)	(25)	(25)	(24)	(24)
Osteosarcoma, metastatic, bone						1 (4%)
Musculoskeletal System						
Bone	(3)	(0)	(1)	(1)	(0)	(1)
Osteosarcoma	1 (33%)					
Cranium, osteosarcoma			1 (100%)			
Humerus, osteosarcoma	1 (33%)					
Pelvis, osteosarcoma				1 (100%)		
Rib, osteosarcoma						1 (100%)
Tibia, osteosarcoma	1 (33%)					
Bone, femur	(25)	(24)	(25)	(25)	(25)	(24)
Osteosarcoma			1 (4%)			
Skeletal muscle	(3)	(2)	(1)	(0)	(0)	(0)
Leiomyosarcoma, metastatic, intestine small, duodenum	1 (33%)					
Rhabdomyosarcoma		1 (50%)				
Diaphragm, sarcoma, metastatic, tissue NOS	1 (33%)					
Diaphragm, sarcoma, metastatic, uncertain primary site			1 (100%)			
Nervous System						
Brain, brain stem	(25)	(23)	(25)	(25)	(24)	(23)
Brain, cerebellum	(25)	(23)	(25)	(25)	(24)	(23)
Brain, cerebrum	(25)	(23)	(25)	(25)	(24)	(23)
Peripheral nerve	(1)	(0)	(0)	(0)	(0)	(0)
Spinal cord, cervical	(1)	(0)	(0)	(0)	(0)	(0)
Spinal cord, lumbar	(1)	(0)	(0)	(0)	(0)	(0)
Spinal cord, thoracic	(1)	(0)	(0)	(0)	(0)	(0)
Respiratory System						
Lung	(25)	(24)	(25)	(25)	(24)	(23)
Alveolar/bronchiolar adenoma			1 (4%)	1 (4%)		
Alveolar/bronchiolar carcinoma		1 (4%)	1 (4%)			
Osteosarcoma, metastatic, bone	1 (4%)					
Nose	(25)	(24)	(25)	(25)	(25)	(24)
Osteosarcoma		1 (4%)				
Trachea	(25)	(23)	(25)	(25)	(24)	(23)
Special Senses System						
Eye	(22)	(22)	(23)	(23)	(24)	(23)
Harderian gland	(25)	(22)	(25)	(25)	(24)	(23)
Adenoma			1 (4%)	1 (4%)		
Carcinoma			1 (4%)			

TABLE A4
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Urinary System						
Kidney	(25)	(23)	(25)	(25)	(24)	(23)
Capsule, alveolar/bronchiolar carcinoma, metastatic, lung			1 (4%)			
Urinary bladder	(25)	(23)	(25)	(25)	(24)	(23)
Sarcoma				1 (4%)		
Systemic Lesions						
Multiple organs[b]	(25)	(24)	(25)	(26)	(25)	(24)
Histiocytic sarcoma				1 (4%)	1 (4%)	
Lymphoma malignant	1 (4%)	3 (13%)			3 (12%)	2 (8%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	7	12	9	9	17	14
Total primary neoplasms	9	14	10	10	17	14
Total animals with benign neoplasms	1	8	5	5	9	10
Total benign neoplasms	1	8	5	5	9	10
Total animals with malignant neoplasms	7	6	5	5	8	4
Total malignant neoplasms	8	6	5	5	8	4
Total animals with metastatic neoplasms	3		2			1
Total metastatic neoplasms	8		14			1
Total animals with malignant neoplasms, uncertain primary site			1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of animals with any tissue examined microscopically

[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A5
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Bone: Osteosarcoma						
Overall rate[b]	3/25 (12.0%)	0/24 (0.0%)	1/25 (4.0%)	1/26 (3.8%)	0/25 (0.0%)	1/24 (4.2%)
Adjusted rate[c]	3/23.9 (12.6%)	0/19.9 (0.0%)	1/23.6 (4.2%)	1/22.9 (4.4%)	0/22.2 (0.0%)	1/22.9 (4.4%)
Terminal rate[d]	1/20 (5.0%)	0/18 (0.0%)	1/23 (4.3%)	1/22 (4.5%)	0/21 (0.0%)	1/22 (4.5%)
First incidence (days)	207	—[f]	318 (T)	322 (T)	—	326 (T)
	Vehicle Control	P=0.150**N**	P=0.307**N**	P=0.319**N**	P=0.127**N**	P=0.319**N**
		AZT-H	P=0.534	P=0.528	—[g]	P=0.528
Poly-3 test[e]			**3TC-H**	P=0.753	P=0.512**N**	P=0.753
				NVP-H	P=0.506**N**	P=0.761
					AZT/3TC-H	P=0.506
Liver: Hepatocellular Adenoma						
Overall rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	9/25 (36.0%)	9/23 (39.1%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	3/23.6 (12.7%)	2/22.4 (8.9%)	9/22.2 (40.5%)	9/22.8 (39.4%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	3/23 (13.0%)	2/22 (9.1%)	9/21 (42.9%)	9/22 (40.9%)
First incidence (days)	313	316 (T)	318 (T)	322 (T)	316 (T)	316 (T)
	Vehicle Control	**P=0.004**	P=0.313	P=0.493	**P=0.003**	**P=0.003**
		AZT-H	**P=0.038N**	**P=0.017N**	P=0.615	P=0.601**N**
Poly-3 test			**3TC-H**	P=0.524**N**	**P=0.031**	**P=0.036**
				NVP-H	**P=0.014**	**P=0.016**
					AZT/3TC-H	P=0.589**N**
Liver: Hepatocellular Adenoma or Carcinoma						
Overall rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	10/25 (40.0%)	10/23 (43.5%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	3/23.6 (12.7%)	2/22.4 (8.9%)	10/22.2 (45.0%)	10/22.8 (43.8%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	3/23 (13.0%)	2/22 (9.1%)	10/21 (47.6%)	10/22 (45.5%)
First incidence (days)	313	316 (T)	318 (T)	322 (T)	316 (T)	316 (T)
	Vehicle Control	**P=0.004**	P=0.313	P=0.493	**P<0.001**	**P=0.001**
		AZT-H	**P=0.038N**	**P=0.017N**	P=0.499	P=0.529
Poly-3 test			**3TC-H**	P=0.524**N**	**P=0.014**	**P=0.017**
				NVP-H	**P=0.006**	**P=0.007**
					AZT/3TC-H	0.585**N**
Lung: Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	0/25 (0.0%)	1/24 (4.2%)	2/25 (8.0%)	1/25 (4.0%)	0/24 (0.0%)	0/23 (0.0%)
Adjusted rate	0/22.8 (0.0%)	1/19.9 (5.0%)	2/24.2 (8.3%)	1/22.4 (4.5%)	0/21.4 (0.0%)	0/22.8 (0.0%)
Terminal rate	0/20 (0.0%)	1/18 (5.6%)	1/23 (4.3%)	1/22 (4.5%)	0/21 (0.0%)	0/22 (0.0%)
First incidence (days)	—	319 (T)	250	320 (T)	—	—
	Vehicle Control	P=0.473	P=0.248	P=0.497	—	—
		AZT-H	P=0.568	P=0.734**N**	P=0.486**N**	P=0.473**N**
Poly-3 test			**3TC-H**	P=0.526**N**	P=0.263**N**	P=0.248**N**
				NVP-H	P=0.510**N**	P=0.497**N**
					AZT/3TC-H	—

TABLE A5
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Malignant Lymphoma						
Overall rate	1/25 (4.0%)	3/24 (12.5%)	0/25 (0.0%)	0/26 (0.0%)	3/25 (12.0%)	2/24 (8.3%)
Adjusted rate	1/23.6 (4.2%)	3/22.1 (13.6%)	0/23.6 (0.0%)	0/22.9 (0.0%)	3/24.8 (12.1%)	2/23.0 (8.7%)
Terminal rate	0/20 (0.0%)	0/18 (0.0%)	0/23 (0.0%)	0/22 (0.0%)	0/21 (0.0%)	1/22 (4.5%)
First incidence (days)	176	168	—	—	99	307
	Vehicle Control	P=0.277	P=0.500**N**	P=0.507**N**	P=0.321	P=0.491
		AZT-H	P=0.101**N**	P=0.106**N**	P=0.609**N**	P=0.480**N**
Poly-3 test			**3TC-H**	—	P=0.123	P=0.229
				NVP-H	P=0.130	P=0.236
					AZT/3TC-H	P=0.535**N**
All Organs: Osteosarcoma						
Overall rate	3/25 (12.0%)	1/24 (4.2%)	2/25 (8.0%)	1/26 (3.8%)	0/25 (0.0%)	1/24 (4.2%)
Adjusted rate	3/23.9 (12.6%)	1/19.9 (5.0%)	2/23.6 (8.5%)	1/22.9 (4.4%)	0/22.2 (0.0%)	1/22.9 (4.4%)
Terminal rate	1/20 (5.0%)	1/18 (5.6%)	2/23 (8.7%)	1/22 (4.5%)	0/21 (0.0%)	1/22 (4.5%)
First incidence (days)	207	318 (T)	318 (T)	322 (T)	—	326 (T)
	Vehicle Control	P=0.371**N**	P=0.505**N**	P=0.319**N**	P=0.127**N**	P=0.319**N**
		AZT-H	P=0.560	P=0.730**N**	P=0.478**N**	P=0.729**N**
Poly-3 test			**3TC-H**	P=0.512**N**	P=0.249**N**	P=0.512**N**
				NVP-H	P=0.506**N**	P=0.761
					AZT/3TC-H	P=0.506
All Organs: Benign Neoplasms						
Overall rate	1/25 (4.0%)	8/24 (33.3%)	5/25 (20.0%)	5/26 (19.2%)	9/25 (36.0%)	10/24 (41.7%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	5/23.6 (21.2%)	5/22.9 (21.9%)	9/22.2 (40.5%)	10/22.9 (43.8%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	5/23 (21.7%)	5/22 (22.7%)	9/21 (42.9%)	10/22 (45.5%)
First incidence (days)	313	316 (T)	318 (T)	320 (T)	316 (T)	316 (T)
	Vehicle Control	**P=0.004**	P=0.099	P=0.091	**P=0.003**	**P=0.001**
		AZT-H	P=0.150**N**	P=0.166**N**	P=0.615	P=0.530
Poly-3 test			**3TC-H**	P=0.615	P=0.135	P=0.089
				NVP-H	P=0.150	P=0.102
					AZT/3TC-H	P=0.532
All Organs: Malignant Neoplasms						
Overall rate	7/25 (28.0%)	6/24 (25.0%)	5/25 (20.0%)	5/26 (19.2%)	8/25 (32.0%)	4/24 (16.7%)
Adjusted rate	7/25.0 (28.0%)	6/22.4 (26.8%)	5/24.2 (20.7%)	5/25.0 (20.0%)	8/25.0 (32.0%)	4/23.0 (17.4%)
Terminal rate	2/20 (10.0%)	2/18 (11.1%)	4/23 (17.4%)	2/22 (9.1%)	4/21 (19.0%)	3/22 (13.6%)
First incidence (days)	176	168	250	177	99	307
	Vehicle Control	P=0.590**N**	P=0.397**N**	P=0.372**N**	P=0.500	P=0.300**N**
		AZT-H	P=0.444**N**	P=0.419**N**	P=0.471	P=0.344**N**
Poly-3 test			**3TC-H**	P=0.614**N**	P=0.285	P=0.532**N**
				NVP-H	P=0.262	P=0.554**N**
					AZT/3TC-H	P=0.203**N**

TABLE A5
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Benign or Malignant Neoplasms						
Overall rate	7/25 (28.0%)	12/24 (50.0%)	9/25 (36.0%)	9/26 (34.6%)	17/25 (68.0%)	14/24 (58.3%)
Adjusted rate	7/25.0 (28.0%)	12/22.4 (53.6%)	9/24.2 (37.2%)	9/25.0 (36.0%)	17/25.0 (68.0%)	14/23.0 (60.8%)
Terminal rate	2/20 (10.0%)	8/18 (44.4%)	8/23 (34.8%)	6/22 (27.3%)	13/21 (61.9%)	13/22 (59.1%)
First incidence (days)	176	168	250	177	99	307
	Vehicle Control	P=0.065	P=0.352	P=0.383	**P=0.003**	**P=0.019**
		AZT-H	P=0.206**N**	P=0.179**N**	P=0.238	P=0.424
Poly-3 test			**3TC-H**	P=0.580**N**	**P=0.027**	P=0.090
				NVP-H	**P=0.020**	P=0.074
					AZT/3TC-H	P=0.415**N**

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the group stated to the left on the same row. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence is indicated by **N**. Significant P values are bolded.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Disposition Summary						
Animals initially in study	25	25	25	26	25	25
Early deaths						
Moribund	2	4			3	
Natural deaths	1	2	2	4		1
Survivors						
Died/moribund after day 320	2				1	1
Terminal kill	20	18	23	22	21	22
Removals		1				1
Animals examined microscopically	25	24	25	26	25	24
Alimentary System						
Esophagus	(25)	(23)	(25)	(25)	(24)	(24)
Gallbladder	(22)	(21)	(25)	(22)	(24)	(23)
Intestine large, cecum	(22)	(22)	(24)	(22)	(24)	(23)
Intestine large, colon	(22)	(22)	(25)	(23)	(24)	(23)
Intestine large, rectum	(22)	(22)	(23)	(22)	(24)	(23)
Intestine small, duodenum	(23)	(22)	(23)	(22)	(24)	(23)
Ectopic pancreas						1 (4%)
Intestine small, ileum	(22)	(22)	(24)	(22)	(24)	(23)
Intestine small, jejunum	(23)	(22)	(23)	(22)	(24)	(23)
Liver	(25)	(23)	(25)	(25)	(25)	(23)
Basophilic focus			1 (4%)	1 (4%)	1 (4%)	2 (9%)
Hematopoietic cell proliferation	1 (4%)					
Infiltration cellular, lymphocyte				1 (4%)		
Inflammation, chronic active			1 (4%)		1 (4%)	1 (4%)
Mixed cell focus			1 (4%)		1 (4%)	
Necrosis	1 (4%)		1 (4%)			2 (9%)
Tension lipidosis		1 (4%)		1 (4%)	1 (4%)	1 (4%)
Vacuolization cytoplasmic	19 (76%)	18 (78%)	20 (80%)	20 (80%)	21 (84%)	20 (87%)
Centrilobular, degeneration	15 (60%)	16 (70%)	18 (72%)	17 (68%)	17 (68%)	17 (74%)
Mesentery	(3)	(0)	(0)	(1)	(2)	(0)
Inflammation, chronic active					1 (50%)	
Fat, necrosis	1 (33%)				1 (50%)	
Pancreas	(24)	(23)	(25)	(25)	(24)	(23)
Cyst	1 (4%)	1 (4%)				
Hemorrhage					1 (4%)	
Infiltration cellular, lymphocyte					1 (4%)	
Acinus, degeneration		1 (4%)	1 (4%)			
Salivary glands	(25)	(23)	(25)	(25)	(24)	(23)
Infiltration cellular, lymphocyte	18 (72%)	12 (52%)	13 (52%)	18 (72%)	11 (46%)	16 (70%)
Stomach, forestomach	(25)	(23)	(25)	(23)	(24)	(23)
Hyperkeratosis		1 (4%)				
Ulcer					1 (4%)	
Epithelium, hyperplasia	1 (4%)				1 (4%)	
Stomach, glandular	(23)	(22)	(24)	(22)	(24)	(23)

[a] Number of animals examined microscopically at the site and the number of animals with lesion. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Cardiovascular System						
Blood vessel	(25)	(23)	(25)	(25)	(24)	(24)
Heart	(25)	(24)	(25)	(25)	(24)	(24)
Endocrine System						
Adrenal cortex	(25)	(23)	(25)	(25)	(23)	(23)
Accessory adrenal cortical nodule	1 (4%)					1 (4%)
Cyst					1 (4%)	
Hypertrophy		1 (4%)				2 (9%)
Subcapsular, hyperplasia	13 (52%)	11 (48%)	13 (52%)	7 (28%)	11 (48%)	12 (52%)
Adrenal medulla	(25)	(23)	(25)	(23)	(21)	(22)
Islets, pancreatic	(24)	(23)	(25)	(25)	(24)	(23)
Hyperplasia	13 (54%)	9 (39%)	15 (60%)	10 (40%)	14 (58%)	6 (26%)
Parathyroid gland	(23)	(20)	(24)	(18)	(19)	(23)
Cyst			1 (4%)			
Pituitary gland	(24)	(21)	(25)	(25)	(24)	(23)
Thyroid gland	(25)	(23)	(25)	(23)	(23)	(23)
Ectopic thymus	2 (8%)		1 (4%)			
Follicle, degeneration						1 (4%)
General Body System						
Tissue NOS	(1)	(0)	(1)	(3)	(0)	(0)
Abscess				1 (33%)		
Abdominal, fat, necrosis			1 (100%)			
Genital System						
Epididymis	(25)	(23)	(25)	(25)	(24)	(23)
Exfoliated germ cell		1 (4%)				1 (4%)
Hypospermia						1 (4%)
Preputial gland	(25)	(24)	(25)	(25)	(24)	(23)
Infiltration cellular, lymphocyte			1 (4%)			
Infiltration cellular, plasma cell		1 (4%)				
Inflammation, suppurative		1 (4%)				
Inflammation, chronic active	1 (4%)	1 (4%)				
Acinus, degeneration	3 (12%)	2 (8%)	3 (12%)	3 (12%)	1 (4%)	5 (22%)
Duct, ectasia	3 (12%)	2 (8%)	2 (8%)	4 (16%)	1 (4%)	5 (22%)
Prostate	(25)	(23)	(25)	(25)	(24)	(23)
Infiltration cellular, lymphocyte			1 (4%)			
Inflammation, suppurative			1 (4%)			
Seminal vesicle	(25)	(23)	(24)	(25)	(24)	(23)
Testes	(25)	(24)	(24)	(25)	(24)	(23)
Seminiferous tubule, degeneration		1 (4%)	1 (4%)	2 (8%)		1 (4%)
Hematopoietic System						
Bone marrow	(23)	(24)	(24)	(25)	(24)	(23)
Hyperplasia	3 (13%)	3 (13%)	1 (4%)	2 (8%)	2 (8%)	
Lymph node	(2)	(2)	(1)	(1)	(2)	(1)
Mediastinal, hyperplasia, lymphoid	1 (50%)			1 (100%)		
Lymph node, mandibular	(25)	(23)	(25)	(25)	(24)	(23)
Hyperplasia, lymphoid	2 (8%)	1 (4%)	2 (8%)	2 (8%)	2 (8%)	5 (22%)

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Hematopoietic System (continued)						
Lymph node, mesenteric	(24)	(23)	(25)	(25)	(24)	(23)
Congestion		1 (4%)				
Hyperplasia, lymphoid	9 (38%)	5 (22%)	12 (48%)	11 (44%)	11 (46%)	9 (39%)
Infiltration cellular, plasma cell			1 (4%)	2 (8%)	1 (4%)	
Spleen	(25)	(23)	(24)	(25)	(24)	(23)
Congestion					1 (4%)	
Hematopoietic cell proliferation	7 (28%)	11 (48%)	4 (17%)	5 (20%)	11 (46%)	6 (26%)
Hyperplasia, lymphoid	12 (48%)	14 (61%)	16 (67%)	18 (72%)	14 (58%)	12 (52%)
Thymus	(24)	(22)	(24)	(23)	(22)	(20)
Atrophy	2 (8%)	1 (5%)		1 (4%)	1 (5%)	1 (5%)
Cyst		1 (5%)				
Hyperplasia, lymphoid	2 (8%)			1 (4%)	3 (14%)	1 (5%)
Integumentary System						
Mammary gland	(1)	(0)	(0)	(1)	(1)	(0)
Skin	(25)	(24)	(25)	(25)	(24)	(24)
Cyst		1 (4%)				
Granuloma		1 (4%)				
Inflammation, suppurative						1 (4%)
Epithelium, hyperplasia						1 (4%)
Musculoskeletal System						
Bone	(3)	(0)	(1)	(1)	(0)	(1)
Bone, femur	(25)	(24)	(25)	(25)	(25)	(24)
Fibro-osseous lesion	1 (4%)					
Skeletal muscle	(3)	(2)	(1)	(0)	(0)	(0)
Nervous System						
Brain, brain stem	(25)	(23)	(25)	(25)	(24)	(23)
Brain, cerebellum	(25)	(23)	(25)	(25)	(24)	(23)
Compression			1 (4%)			
Hemorrhage		1 (4%)				
Brain, cerebrum	(25)	(23)	(25)	(25)	(24)	(23)
Mineralization	2 (8%)	3 (13%)	4 (16%)	4 (16%)	2 (8%)	1 (4%)
Peripheral nerve	(1)	(0)	(0)	(0)	(0)	(0)
Spinal cord, cervical	(1)	(0)	(0)	(0)	(0)	(0)
Spinal cord, lumbar	(1)	(0)	(0)	(0)	(0)	(0)
Spinal cord, thoracic	(1)	(0)	(0)	(0)	(0)	(0)
Compression	1 (100%)					

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Respiratory System						
Lung	(25)	(24)	(25)	(25)	(24)	(23)
Hemorrhage		1 (4%)			1 (4%)	
Infiltration cellular, histiocyte		1 (4%)	1 (4%)			
Inflammation, chronic				1 (4%)		
Mineralization		1 (4%)		1 (4%)		
Alveolar epithelium, hyperplasia				1 (4%)		1 (4%)
Nose	(25)	(24)	(25)	(25)	(25)	(24)
Hyaline droplet			1 (4%)		2 (8%)	
Trachea	(25)	(23)	(25)	(25)	(24)	(23)
Special Senses System						
Eye	(22)	(22)	(23)	(23)	(24)	(23)
Inflammation, chronic active		1 (5%)				
Cornea, ulcer		1 (5%)				
Harderian gland	(25)	(22)	(25)	(25)	(24)	(23)
Infiltration cellular, lymphocyte	1 (4%)					
Urinary System						
Kidney	(25)	(23)	(25)	(25)	(24)	(23)
Casts protein	3 (12%)	4 (17%)	6 (24%)	7 (28%)	8 (33%)	10 (43%)
Hemorrhage				1 (4%)		
Hydronephrosis	1 (4%)	8 (35%)	7 (28%)	5 (20%)	9 (38%)	12 (52%)
Infiltration cellular, lymphocyte	8 (32%)	2 (9%)	10 (40%)	6 (24%)	2 (8%)	7 (30%)
Metaplasia, osseous			1 (4%)		1 (4%)	
Nephropathy	1 (4%)	1 (4%)	2 (8%)			1 (4%)
Renal tubule, regeneration			1 (4%)			
Transitional epithelium, hyperplasia			1 (4%)			
Urinary bladder	(25)	(23)	(25)	(25)	(24)	(23)
Hemorrhage					1 (4%)	
Infiltration cellular, histiocyte				1 (4%)		
Infiltration cellular, lymphocyte	2 (8%)			2 (8%)	1 (4%)	1 (4%)
Lumen, dilatation	1 (4%)		1 (4%)	1 (4%)		

TABLE A7
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Disposition Summary				
Animals initially in study	25	25	25	25
Early deaths				
Moribund		2	2	2
Natural deaths		1	1	1
Survivors				
Died/moribund after day 320				1
Terminal kill	25	22	19	21
Removals			3	
Animals examined microscopically	25	25	22	25
Alimentary System				
Esophagus	(25)	(25)	(22)	(25)
Gallbladder	(25)	(23)	(21)	(24)
Intestine large, cecum	(25)	(24)	(21)	(23)
Intestine large, colon	(25)	(25)	(21)	(24)
Intestine large, rectum	(25)	(24)	(21)	(23)
Intestine small, duodenum	(25)	(24)	(21)	(24)
Sarcoma				1 (4%)
Intestine small, ileum	(25)	(24)	(21)	(23)
Intestine small, jejunum	(25)	(24)	(21)	(23)
Liver	(25)	(25)	(22)	(25)
Mesentery	(0)	(0)	(0)	(2)
Pancreas	(25)	(25)	(21)	(24)
Salivary glands	(25)	(25)	(22)	(25)
Stomach, forestomach	(25)	(25)	(21)	(24)
Stomach, glandular	(25)	(25)	(21)	(23)
Cardiovascular System				
Blood vessel	(25)	(25)	(22)	(25)
Heart	(25)	(25)	(22)	(25)
Endocrine System				
Adrenal cortex	(25)	(25)	(22)	(25)
Adrenal medulla	(23)	(25)	(21)	(24)
Islets, pancreatic	(25)	(25)	(22)	(25)
Parathyroid gland	(20)	(21)	(21)	(23)
Pituitary gland	(25)	(24)	(22)	(23)
Pars intermedia, adenoma				1 (4%)
Thyroid gland	(23)	(25)	(22)	(25)
General Body System				
None				
Genital System				
Clitoral gland	(24)	(24)	(22)	(22)
Ovary	(25)	(25)	(22)	(25)
Uterus	(25)	(25)	(22)	(25)
Polyp stromal			1 (5%)	1 (4%)
Vagina	(25)	(25)	(22)	(23)

TABLE A7
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Hematopoietic System				
Bone marrow	(25)	(25)	(22)	(24)
Lymph node	(1)	(3)	(1)	(4)
Lymph node, mandibular	(25)	(25)	(22)	(25)
Lymph node, mesenteric	(25)	(25)	(21)	(24)
Spleen	(25)	(25)	(22)	(25)
Thymus	(25)	(25)	(21)	(24)
Integumentary System				
Mammary gland	(25)	(25)	(22)	(25)
Adenoacanthoma				1 (4%)
Adenocarcinoma	1 (4%)			
Skin	(25)	(25)	(22)	(25)
Musculoskeletal System				
Bone	(0)	(2)	(1)	(0)
Cranium, osteosarcoma			1 (100%)	
Vertebra, osteosarcoma		1 (50%)		
Bone, femur	(25)	(25)	(22)	(25)
Nervous System				
Brain, brain stem	(25)	(25)	(21)	(25)
Brain, cerebellum	(25)	(25)	(21)	(25)
Brain, cerebrum	(25)	(25)	(21)	(25)
Respiratory System				
Lung	(25)	(25)	(22)	(25)
Adenocarcinoma, metastatic, mammary gland	1 (4%)			
Alveolar/bronchiolar adenoma	1 (4%)		1 (5%)	1 (4%)
Nose	(25)	(25)	(22)	(25)
Trachea	(25)	(25)	(21)	(25)
Special Senses System				
Eye	(25)	(24)	(21)	(23)
Harderian gland	(25)	(25)	(22)	(24)
Adenoma				1 (4%)
Urinary System				
Kidney	(25)	(25)	(22)	(25)
Urinary bladder	(25)	(25)	(22)	(25)
Systemic Lesions				
Multiple organs[b]	(25)	(25)	(22)	(25)
Histiocytic sarcoma			1 (5%)	1 (4%)
Lymphoma malignant	2 (8%)	2 (8%)	4 (18%)	4 (16%)

TABLE A7
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Neoplasm Summary				
Total animals with primary neoplasms[c]	4	3	7	9
Total primary neoplasms	4	3	8	11
Total animals with benign neoplasms	1		2	4
Total benign neoplasms	1		2	4
Total animals with malignant neoplasms	3	3	6	6
Total malignant neoplasms	3	3	6	7
Total animals with metastatic neoplasms	1			
Total metastatic neoplasms	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A8
Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
All Organs: Malignant Lymphoma				
Overall rate[b]	2/25 (8.0%)	2/25 (8.0%)	4/22 (18.2%)	4/25 (16.0%)
Adjusted rate[c]	2/25.0 (8.0%)	2/24.4 (8.2%)	4/22.0 (18.2%)	4/24.5 (16.3%)
Terminal rate[d]	2/25 (8.0%)	0/22 (0.0%)	1/19 (5.3%)	2/21 (9.5%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test[e]	P=0.156	P=0.687	P=0.274	P=0.324
All Organs: Benign Neoplasms				
Overall rate	1/25 (4.0%)	0/25 (0.0%)	2/22 (9.1%)	4/25 (16.0%)
Adjusted rate	1/25.0 (4.0%)	0/23.4 (0.0%)	2/19.7 (10.2%)	4/24.1 (16.6%)
Terminal rate	1/25 (4.0%)	0/22 (0.0%)	2/19 (10.5%)	3/21 (14.3%)
First incidence (days)	316 (T)	—[f]	318 (T)	275
Poly-3 test	P=0.036	P=0.513**N**	P=0.417	P=0.162
All Organs: Malignant Neoplasms				
Overall rate	3/25 (12.0%)	3/25 (12.0%)	6/22 (27.3%)	6/25 (24.0%)
Adjusted rate	3/25.0 (12.0%)	3/25.0 (12.0%)	6/22.0 (27.3%)	6/25.0 (24.0%)
Terminal rate	3/25 (12.0%)	0/22 (0.0%)	3/19 (15.8%)	2/21 (9.5%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test	P=0.092	P=0.665	P=0.170	P=0.233
All Organs: Benign or Malignant Neoplasms				
Overall rate	4/25 (16.0%)	3/25 (12.0%)	7/22 (31.8%)	9/25 (36.0%)
Adjusted rate	4/25.0 (16.0%)	3/25.0 (12.0%)	7/22.0 (31.8%)	9/25.0 (36.0%)
Terminal rate	4/25 (16.0%)	0/22 (0.0%)	4/19 (21.1%)	5/21 (23.8%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test	P=0.023	P=0.500**N**	P=0.176	P=0.097

(T) Terminal kill

[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg

[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group.

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Disposition Summary				
Animals initially in study	25	25	25	25
Early deaths				
Moribund		2	2	2
Natural deaths		1	1	1
Survivors				
Died/moribund after day 320				1
Terminal kill	25	22	19	21
Removals			3	
Animals examined microscopically	25	25	22	25
Alimentary System				
Esophagus	(25)	(25)	(22)	(25)
Gallbladder	(25)	(23)	(21)	(24)
Intestine large, cecum	(25)	(24)	(21)	(23)
Intestine large, colon	(25)	(25)	(21)	(24)
Intestine large, rectum	(25)	(24)	(21)	(23)
Intestine small, duodenum	(25)	(24)	(21)	(24)
Intestine small, ileum	(25)	(24)	(21)	(23)
Intestine small, jejunum	(25)	(24)	(21)	(23)
Liver	(25)	(25)	(22)	(25)
Basophilic focus			1 (5%)	
Clear cell focus			1 (5%)	
Infiltration cellular, lymphocyte	1 (4%)	2 (8%)	4 (18%)	4 (16%)
Inflammation, chronic active	1 (4%)	1 (4%)		3 (12%)
Necrosis		1 (4%)		
Tension lipidosis	2 (8%)		4 (18%)	3 (12%)
Vacuolization cytoplasmic	14 (56%)	17 (68%)	13 (59%)	15 (60%)
Centrilobular, degeneration				3 (12%)
Mesentery	(0)	(0)	(0)	(2)
Fat, necrosis				2 (100%)
Pancreas	(25)	(25)	(21)	(24)
Infiltration cellular, lymphocyte	2 (8%)	3 (12%)	5 (24%)	2 (8%)
Necrosis	1 (4%)			
Salivary glands	(25)	(25)	(22)	(25)
Infiltration cellular, lymphocyte	22 (88%)	18 (72%)	12 (55%)	20 (80%)
Stomach, forestomach	(25)	(25)	(21)	(24)
Ulcer	1 (4%)			
Epithelium, hyperplasia	2 (8%)			1 (4%)
Stomach, glandular	(25)	(25)	(21)	(23)
Cardiovascular system				
Blood vessel	(25)	(25)	(22)	(25)
Heart	(25)	(25)	(22)	(25)

[a] Number of animals examined microscopically at the site and the number of animals with lesion. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Endocrine System				
Adrenal cortex	(25)	(25)	(22)	(25)
Accessory adrenal cortical nodule	1 (4%)			
Hypertrophy	1 (4%)			
Infiltration cellular, polymorphonuclear				1 (4%)
Vacuolization cytoplasmic	16 (64%)	15 (60%)	16 (73%)	18 (72%)
Subcapsular, hyperplasia	25 (100%)	24 (96%)	20 (91%)	24 (96%)
Adrenal medulla	(23)	(25)	(21)	(24)
Islets, pancreatic	(25)	(25)	(22)	(25)
Hyperplasia		1 (4%)		1 (4%)
Parathyroid gland	(20)	(21)	(21)	(23)
Cyst		1 (5%)		
Pituitary gland	(25)	(24)	(22)	(23)
Pars distalis, hyperplasia				1 (4%)
Thyroid gland	(23)	(25)	(22)	(25)
Cyst			1 (5%)	
Ectopic thymus	3 (13%)	1 (4%)	2 (9%)	2 (8%)
General Body System				
None				
Genital System				
Clitoral gland	(24)	(24)	(22)	(22)
Acinus, degeneration	9 (38%)	6 (25%)	5 (23%)	11 (50%)
Duct, ectasia				1 (5%)
Ovary	(25)	(25)	(22)	(25)
Atrophy	1 (4%)		1 (5%)	
Cyst	1 (4%)	3 (12%)	1 (5%)	
Hemorrhage				1 (4%)
Uterus	(25)	(25)	(22)	(25)
Endometrium, hyperplasia, cystic	22 (88%)	18 (72%)	16 (73%)	17 (68%)
Vagina	(25)	(25)	(22)	(23)
Infiltration cellular, polymorphonuclear		1 (4%)		2 (9%)
Hematopoietic System				
Bone marrow	(25)	(25)	(22)	(24)
Hyperplasia	1 (4%)			3 (13%)
Lymph node	(1)	(3)	(1)	(4)
Lumbar, hyperplasia, lymphoid		1 (33%)		
Pancreatic, hyperplasia, lymphoid		1 (33%)		
Renal, hyperplasia, lymphoid		1 (33%)		
Lymph node, mandibular	(25)	(25)	(22)	(25)
Hyperplasia, lymphoid	3 (12%)	4 (16%)	2 (9%)	1 (4%)
Lymph node, mesenteric	(25)	(25)	(21)	(24)
Hyperplasia, lymphoid	1 (4%)	3 (12%)	2 (10%)	3 (13%)
Spleen	(25)	(25)	(22)	(25)
Hematopoietic cell proliferation	2 (8%)	2 (8%)	8 (36%)	7 (28%)
Hyperplasia, lymphoid	20 (80%)	19 (76%)	16 (73%)	16 (64%)
Thymus	(25)	(25)	(21)	(24)
Atrophy	1 (4%)			
Hyperplasia, lymphoid	6 (24%)	9 (36%)	2 (10%)	5 (21%)

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Integumentary System				
Mammary gland	(25)	(25)	(22)	(25)
Skin	(25)	(25)	(22)	(25)
Musculoskeletal System				
Bone	(0)	(2)	(1)	(0)
Bone, femur	(25)	(25)	(22)	(25)
Fibro-osseous lesion	1 (4%)			
Nervous System				
Brain, brain stem	(25)	(25)	(21)	(25)
Brain, cerebellum	(25)	(25)	(21)	(25)
Brain, cerebrum	(25)	(25)	(21)	(25)
Mineralization	2 (8%)	1 (4%)	2 (10%)	
Respiratory System				
Lung	(25)	(25)	(22)	(25)
Hemorrhage		1 (4%)		
Infiltration cellular, lymphocyte		1 (4%)		3 (12%)
Metaplasia, osseous		1 (4%)		
Nose	(25)	(25)	(22)	(25)
Hyaline droplet	1 (4%)	4 (16%)	1 (5%)	6 (24%)
Trachea	(25)	(25)	(21)	(25)
Special Senses System				
Eye	(25)	(24)	(21)	(23)
Cataract				1 (4%)
Harderian gland	(25)	(25)	(22)	(24)
Infiltration cellular, lymphocyte	2 (8%)	1 (4%)		
Urinary System				
Kidney	(25)	(25)	(22)	(25)
Casts protein	14 (56%)	15 (60%)	9 (41%)	15 (60%)
Hydronephrosis				3 (12%)
Infarct	1 (4%)			
Infiltration cellular, lymphocyte	11 (44%)	13 (52%)	7 (32%)	11 (44%)
Metaplasia, osseous				1 (4%)
Urinary bladder	(25)	(25)	(22)	(25)
Infiltration cellular, lymphocyte	8 (32%)	5 (20%)	5 (23%)	3 (12%)

TABLE A10
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Disposition Summary						
Animals initially in study	25	25	25	25	25	25
Early deaths						
Moribund		2	3	2	4	2
Natural deaths		2	2	2	1	1
Survivors						
Died/moribund after day 320		1		1		1
Terminal kill	25	20	20	20	19	21
Missexed					1	
Animals examined microscopically	25	25	25	25	24	25
Alimentary System						
Esophagus	(25)	(25)	(25)	(25)	(22)	(25)
Gallbladder	(25)	(23)	(23)	(22)	(23)	(24)
Intestine large, cecum	(25)	(23)	(23)	(22)	(23)	(23)
Intestine large, colon	(25)	(23)	(23)	(22)	(23)	(24)
Intestine large, rectum	(25)	(23)	(23)	(22)	(23)	(23)
Intestine small, duodenum	(25)	(23)	(23)	(22)	(23)	(24)
Sarcoma						1 (4%)
Intestine small, ileum	(25)	(23)	(23)	(22)	(23)	(23)
Intestine small, jejunum	(25)	(23)	(23)	(22)	(23)	(23)
Liver	(25)	(25)	(23)	(24)	(23)	(25)
Carcinoma NOS, metastatic, pancreas		1 (4%)				
Hepatocellular carcinoma					1 (4%)	
Mesentery	(0)	(1)	(0)	(0)	(0)	(2)
Carcinoma NOS, metastatic, pancreas		1 (100%)				
Pancreas	(25)	(25)	(25)	(22)	(23)	(24)
Carcinoma NOS		1 (4%)				
Salivary glands	(25)	(25)	(25)	(24)	(23)	(25)
Stomach, forestomach	(25)	(24)	(24)	(24)	(23)	(24)
Stomach, glandular	(25)	(23)	(23)	(22)	(23)	(23)
Cardiovascular System						
Blood vessel	(25)	(25)	(25)	(25)	(23)	(25)
Heart	(25)	(25)	(25)	(25)	(24)	(25)
Endocrine System						
Adrenal cortex	(25)	(24)	(24)	(25)	(23)	(25)
Adrenal medulla	(23)	(24)	(24)	(25)	(23)	(24)
Islets, pancreatic	(25)	(24)	(25)	(25)	(23)	(25)
Parathyroid gland	(20)	(23)	(21)	(20)	(18)	(23)
Pituitary gland	(25)	(25)	(24)	(25)	(23)	(23)
Pars intermedia, adenoma						1 (4%)
Thyroid gland	(23)	(24)	(25)	(23)	(22)	(25)
C-cell, adenoma				1 (4%)		
General Body System						
Tissue NOS	(0)	(0)	(0)	(1)	(0)	(0)

TABLE A10
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Genital System						
Clitoral gland	(24)	(24)	(23)	(21)	(21)	(22)
Ovary	(25)	(24)	(25)	(24)	(23)	(25)
Uterus	(25)	(25)	(25)	(23)	(23)	(25)
Polyp stromal						1 (4%)
Vagina	(25)	(24)	(24)	(24)	(23)	(23)
Hematopoietic System						
Bone marrow	(25)	(24)	(24)	(23)	(23)	(24)
Lymph node	(1)	(3)	(1)	(3)	(4)	(4)
Mediastinal, carcinoma NOS, metastatic, pancreas		1 (33%)				
Renal, carcinoma NOS, metastatic, pancreas		1 (33%)				
Lymph node, mandibular	(25)	(25)	(24)	(24)	(23)	(25)
Lymph node, mesenteric	(25)	(24)	(23)	(24)	(23)	(24)
Carcinoma NOS, metastatic, pancreas		1 (4%)				
Spleen	(25)	(25)	(24)	(24)	(23)	(25)
Thymus	(25)	(21)	(22)	(22)	(24)	(24)
Integumentary System						
Mammary gland	(25)	(25)	(25)	(23)	(23)	(25)
Adenoacanthoma		1 (4%)	1 (4%)			1 (4%)
Adenocarcinoma	1 (4%)		3 (12%)		2 (9%)	
Skin	(25)	(25)	(25)	(25)	(23)	(25)
Hemangiosarcoma				1 (4%)		
Musculoskeletal System						
Bone	(0)	(0)	(2)	(0)	(0)	(0)
Mandible, osteosarcoma			1 (50%)			
Pelvis, osteosarcoma			1 (50%)			
Bone, femur	(25)	(25)	(25)	(25)	(24)	(25)
Skeletal muscle	(0)	(1)	(2)	(0)	(0)	(0)
Rhabdomyosarcoma			1 (50%)			
Sarcoma		1 (100%)				
Nervous System						
Brain, brain stem	(25)	(24)	(25)	(24)	(23)	(25)
Brain, cerebellum	(25)	(24)	(25)	(24)	(23)	(25)
Brain, cerebrum	(25)	(24)	(25)	(24)	(23)	(25)
Peripheral nerve	(0)	(0)	(1)	(0)	(0)	(0)
Spinal cord, cervical	(0)	(0)	(1)	(0)	(0)	(0)
Spinal cord, lumbar	(0)	(0)	(1)	(0)	(0)	(0)
Spinal cord, thoracic	(0)	(0)	(1)	(0)	(0)	(0)

TABLE A10
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Respiratory System						
Lung	(25)	(24)	(24)	(24)	(24)	(25)
Adenocarcinoma, metastatic, mammary gland	1 (4%)				1 (4%)	
Alveolar/bronchiolar adenoma	1 (4%)					1 (4%)
Osteosarcoma, metastatic, bone			1 (4%)			
Nose	(25)	(25)	(25)	(24)	(24)	(25)
Neuroblastoma				1 (4%)		
Trachea	(25)	(24)	(24)	(24)	(23)	(25)
Special Senses System						
Eye	(25)	(23)	(23)	(22)	(23)	(23)
Harderian gland	(25)	(24)	(25)	(25)	(23)	(24)
Adenoma		1 (4%)		2 (8%)		1 (4%)
Urinary System						
Kidney	(25)	(24)	(24)	(24)	(23)	(25)
Urinary bladder	(25)	(24)	(24)	(23)	(23)	(25)
Systemic Lesions						
Multiple organs[b]	(25)	(25)	(25)	(25)	(24)	(25)
Histiocytic sarcoma				1 (4%)	1 (4%)	1 (4%)
Leukemia granulocytic				1 (4%)		
Lymphoma malignant	2 (8%)	2 (8%)	1 (4%)	5 (20%)	4 (17%)	4 (16%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	4	6	8	12	8	9
Total primary neoplasms	4	6	8	12	8	11
Total animals with benign neoplasms	1	1		3		4
Total benign neoplasms	1	1		3		4
Total animals with malignant neoplasms	3	4	8	9	8	6
Total malignant neoplasms	3	4	8	9	8	7
Total animals with metastatic neoplasms	1	1	1		1	
Total metastatic neoplasms	1	5	1		1	
Total animals with uncertain neoplasms-benign or malignant		1				
Total uncertain neoplasms		1				

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of animals with any tissue examined microscopically

[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A11
Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Bone: Osteosarcoma						
Overall rate[b]	0/25 (0.0%)	0/25 (0.0%)	2/25 (8.0%)	0/25 (0.0%)	0/24 (0.0%)	0/25 (0.0%)
Adjusted rate[c]	0/25.0 (0.0%)	0/23.8 (0.0%)	2/23.5 (8.5%)	0/23.2 (0.0%)	0/20.7 (0.0%)	0/23.7 (0.0%)
Terminal rate[d]	0/25 (0.0%)	0/20 (0.0%)	1/20 (5.0%)	0/20 (0.0%)	0/19 (0.0%)	0/21 (0.0%)
First incidence (days)	—[f]	—	297	—	—	—
	Vehicle Control	—[g]	P=0.221	—	—	—
		AZT-H	P=0.232	—	—	—
Poly-3 test[e]			**3TC-H**	P=0.237**N**	P=0.263**N**	P=0.232**N**
				NVP-H	—	—
					AZT/3TC-H	—
Harderian Gland: Adenoma						
Overall rate	0/25 (0.0%)	1/24 (4.2%)	0/25 (0.0%)	2/25 (8.0%)	0/23 (0.0%)	1/24 (4.2%)
Adjusted rate	0/25.0 (0.0%)	1/23.1 (4.3%)	0/23.2 (0.0%)	2/23.2 (8.6%)	0/20.7 (0.0%)	1/23.3 (4.3%)
Terminal rate	0/25 (0.0%)	1/20 (5.0%)	0/20 (0.0%)	2/20 (10.0%)	0/19 (0.0%)	1/21 (4.8%)
First incidence (days)	—	320 (T)	—	320 (T)	—	318 (T)
	Vehicle Control	P=0.484	—	P=0.218	—	P=0.486
		AZT-H	P=0.499**N**	P=0.501	P=0.522**N**	P=0.759**N**
Poly-3 test			**3TC-H**	P=0.234	—	P=0.501
				NVP-H	P=0.260**N**	P=0.498**N**
					AZT/3TC-H	P=0.523
Mammary Gland: Adenocarcinoma						
Overall rate	1/25 (4.0%)	0/25 (0.0%)	3/25 (12.0%)	0/23 (0.0%)	2/23 (8.7%)	0/25 (0.0%)
Adjusted rate	1/25.0 (4.0%)	0/23.8 (0.0%)	3/23.6 (12.7%)	0/21.8 (0.0%)	2/21.1 (9.5%)	0/23.7 (0.0%)
Terminal rate	1/25 (4.0%)	0/20 (0.0%)	2/20 (10.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	319 (T)	—	277	—	280	—
	Vehicle Control	P=0.510**N**	P=0.282	P=0.527**N**	P=0.440	P=0.510**N**
		AZT-H	P=0.112	—	P=0.208	—
Poly-3 test			**3TC-H**	P=0.128**N**	P=0.553**N**	P=0.112**N**
				NVP-H	P=0.226	—
					AZT/3TC-H	P=0.208**N**
Mammary Gland: Adenoacanthoma or Adenocarcinoma						
Overall rate	1/25 (4.0%)	1/25 (4.0%)	4/25 (16.0%)	0/23 (0.0%)	2/23 (8.7%)	1/25 (4.0%)
Adjusted rate	1/25.0 (4.0%)	1/23.9 (4.2%)	4/24.0 (16.6%)	0/21.8 (0.0%)	2/21.1 (9.5%)	1/23.9 (4.2%)
Terminal rate	1/25 (4.0%)	0/20 (0.0%)	2/20 (10.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	319 (T)	313	271	—	280	302
	Vehicle Control	P=0.750	P=0.161	P=0.527**N**	P=0.440	P=0.751
		AZT-H	P=0.174	P=0.518**N**	P=0.456	P=0.760**N**
Poly-3 test			**3TC-H**	P=0.066**N**	P=0.397**N**	P=0.173**N**
				NVP-H	P=0.226	P=0.519
					AZT/3TC-H	P=0.455**N**

TABLE A11
Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Malignant Lymphoma						
Overall rate	2/25 (8.0%)	2/25 (8.0%)	1/25 (4.0%)	5/25 (20.0%)	4/24 (16.7%)	4/25 (16.0%)
Adjusted rate	2/25.0 (8.0%)	2/24.4 (8.2%)	1/23.7 (4.2%)	5/23.7 (21.1%)	4/23.6 (16.9%)	4/24.5 (16.3%)
Terminal rate	2/25 (8.0%)	0/20 (0.0%)	0/20 (0.0%)	4/20 (20.0%)	0/19 (0.0%)	2/21 (9.5%)
First incidence (days)	316 (T)	277	258	251	102	247
	Vehicle Control	P=0.686	P=0.518**N**	P=0.186	P=0.307	P=0.324
		AZT-H	P=0.509**N**	P=0.196	P=0.319	P=0.336
Poly-3 test			**3TC-H**	P=0.092	P=0.171	P=0.182
				NVP-H	P=0.502**N**	P=0.479**N**
					AZT/3TC-H	P=0.628**N**
All Organs: Benign Neoplasms						
Overall rate	1/25 (4.0%)	1/25 (4.0%)	0/25 (0.0%)	3/25 (12.0%)	0/24 (0.0%)	4/25 (16.0%)
Adjusted rate	1/25.0 (4.0%)	1/23.8 (4.2%)	0/23.2 (0.0%)	3/23.2 (13.0%)	0/20.7 (0.0%)	4/24.1 (16.6%)
Terminal rate	1/25 (4.0%)	1/20 (5.0%)	0/20 (0.0%)	3/20 (15.0%)	0/19 (0.0%)	3/21 (14.3%)
First incidence (days)	316 (T)	320 (T)	—	320 (T)	—	275
	Vehicle Control	P=0.749	P=0.515**N**	P=0.275	P=0.537**N**	P=0.162
		AZT-H	P=0.504**N**	P=0.293	P=0.527**N**	P=0.177
Poly-3 test			**3TC-H**	P=0.112	—	P=0.059
				NVP-H	P=0.134**N**	P=0.523
					AZT/3TC-H	P=0.075
All Organs: Malignant Neoplasms						
Overall rate	3/25 (12.0%)	4/25 (16.0%)	8/25 (32.0%)	9/25 (36.0%)	8/24 (33.3%)	6/25 (24.0%)
Adjusted rate	3/25.0 (12.0%)	4/24.7 (16.2%)	8/25.0 (32.0%)	9/24.9 (36.2%)	8/24.0 (33.3%)	6/25.0 (24.0%)
Terminal rate	3/25 (12.0%)	0/20 (0.0%)	3/20 (15.0%)	5/20 (25.0%)	3/19 (15.8%)	2/21 (9.5%)
First incidence (days)	316 (T)	277	258	251	102	247
	Vehicle Control	P=0.493	P=0.084	**P=0.044**	P=0.071	P=0.233
		AZT-H	P=0.167	P=0.100	P=0.146	P=0.373
Poly-3 test			**3TC-H**	P=0.495	P=0.580	P=0.378**N**
				NVP-H	P=0.536**N**	P=0.267**N**
					AZT/3TC-H	P=0.344**N**
All Organs: Benign or Malignant Neoplasms						
Overall rate	4/25 (16.0%)	6/25 (24.0%)	8/25 (32.0%)	12/25 (48.0%)	8/24 (33.3%)	9/25 (36.0%)
Adjusted rate	4/25.0 (16.0%)	6/25.0 (24.0%)	8/25.0 (32.0%)	12/24.9 (48.2%)	8/24.0 (33.3%)	9/25.0 (36.0%)
Terminal rate	4/25 (16.0%)	1/20 (5.0%)	3/20 (15.0%)	8/20 (40.0%)	3/19 (15.8%)	5/21 (23.8%)
First incidence (days)	316 (T)	277	258	251	102	247
	Vehicle Control	P=0.364	P=0.161	**P=0.013**	P=0.141	P=0.097
		AZT-H	P=0.378	P=0.066	P=0.344	P=0.271
Poly-3 test			**3TC-H**	P=0.191	P=0.580	P=0.500
				NVP-H	P=0.223**N**	P=0.281**N**
					AZT/3TC-H	P=0.541

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the group stated to the left on the same row. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence is indicated by **N**. Significant P values are bolded.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

TABLE A12
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Disposition Summary						
Animals initially in study	25	25	25	25	25	25
Early deaths						
Moribund		2	3	2	4	2
Natural deaths		2	2	2	1	1
Survivors						
Died/moribund after day 320		1		1		1
Terminal kill	25	20	20	20	19	21
Missexed					1	
Animals examined microscopically	25	25	25	25	24	25
Alimentary System						
Esophagus	(25)	(25)	(25)	(25)	(22)	(25)
Gallbladder	(25)	(23)	(23)	(22)	(23)	(24)
Intestine large, cecum	(25)	(23)	(23)	(22)	(23)	(23)
Hyperplasia, lymphoid			1 (4%)			
Intestine large, colon	(25)	(23)	(23)	(22)	(23)	(24)
Intestine large, rectum	(25)	(23)	(23)	(22)	(23)	(23)
Intestine small, duodenum	(25)	(23)	(23)	(22)	(23)	(24)
Intestine small, ileum	(25)	(23)	(23)	(22)	(23)	(23)
Intestine small, jejunum	(25)	(23)	(23)	(22)	(23)	(23)
Liver	(25)	(25)	(23)	(24)	(23)	(25)
Basophilic focus		1 (4%)				
Clear cell focus			1 (4%)			
Hematopoietic cell proliferation		1 (4%)		1 (4%)		
Hepatodiaphragmatic nodule			1 (4%)			
Infiltration cellular, lymphocyte	1 (4%)	3 (12%)	2 (9%)	6 (25%)	3 (13%)	4 (16%)
Inflammation, chronic active	1 (4%)	2 (8%)	1 (4%)	2 (8%)	2 (9%)	3 (12%)
Tension lipidosis	2 (8%)		2 (9%)	3 (13%)	2 (9%)	3 (12%)
Vacuolization cytoplasmic	14 (56%)	13 (52%)	14 (61%)	11 (46%)	13 (57%)	15 (60%)
Centrilobular, degeneration				1 (4%)		3 (12%)
Mesentery	(0)	(1)	(0)	(0)	(0)	(2)
Fat, necrosis						2 (100%)
Pancreas	(25)	(25)	(25)	(22)	(23)	(24)
Infiltration cellular, lymphocyte	2 (8%)	4 (16%)	2 (8%)	1 (5%)	3 (13%)	2 (8%)
Necrosis	1 (4%)					
Salivary glands	(25)	(25)	(25)	(24)	(23)	(25)
Infiltration cellular, lymphocyte	22 (88%)	17 (68%)	18 (72%)	19 (79%)	16 (70%)	20 (80%)
Stomach, forestomach	(25)	(24)	(24)	(24)	(23)	(24)
Inflammation, suppurative		1 (4%)				
Inflammation, chronic active					1 (4%)	
Ulcer	1 (4%)	1 (4%)			1 (4%)	
Epithelium, hyperplasia	2 (8%)	1 (4%)			1 (4%)	1 (4%)
Stomach, glandular	(25)	(23)	(23)	(22)	(23)	(23)
Cardiovascular System						
Blood vessel	(25)	(25)	(25)	(25)	(23)	(25)
Heart	(25)	(25)	(25)	(25)	(24)	(25)
Inflammation, chronic active			1 (4%)			
Mineralization			1 (4%)	1 (4%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

TABLE A12
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Endocrine System						
Adrenal cortex	(25)	(24)	(24)	(25)	(23)	(25)
Accessory adrenal cortical nodule	1 (4%)			1 (4%)		
Hypertrophy	1 (4%)					
Infiltration cellular, polymorphonuclear						1 (4%)
Vacuolization cytoplasmic	16 (64%)	14 (58%)	16 (67%)	16 (64%)	13 (57%)	18 (72%)
Subcapsular, hyperplasia	25 (100%)	24 (100%)	24 (100%)	24 (96%)	22 (96%)	24 (96%)
Adrenal medulla	(23)	(24)	(24)	(25)	(23)	(24)
Islets, pancreatic	(25)	(24)	(25)	(25)	(23)	(25)
Hyperplasia		2 (8%)		1 (4%)		1 (4%)
Parathyroid gland	(20)	(23)	(21)	(20)	(18)	(23)
Infiltration cellular, lymphocyte					1 (6%)	
Pituitary gland	(25)	(25)	(24)	(25)	(23)	(23)
Fibrosis			1 (4%)			
Pars distalis, hyperplasia						1 (4%)
Thyroid gland	(23)	(24)	(25)	(23)	(22)	(25)
Cyst				1 (4%)		
Ectopic thymus	3 (13%)		1 (4%)		1 (5%)	2 (8%)
General Body System						
Tissue NOS	(0)	(0)	(0)	(1)	(0)	(0)
Genital System						
Clitoral gland	(24)	(24)	(23)	(21)	(21)	(22)
Acinus, degeneration	9 (38%)	12 (50%)	6 (26%)	5 (24%)	6 (29%)	11 (50%)
Duct, ectasia						1 (5%)
Ovary	(25)	(24)	(25)	(24)	(23)	(25)
Atrophy	1 (4%)	1 (4%)	1 (4%)			
Cyst	1 (4%)	3 (13%)	1 (4%)	2 (8%)	3 (13%)	
Cyst dermoid			1 (4%)			
Hemorrhage						1 (4%)
Uterus	(25)	(25)	(25)	(23)	(23)	(25)
Atrophy			1 (4%)			
Degeneration, cystic		1 (4%)				
Endometrium, hyperplasia, cystic	22 (88%)	19 (76%)	19 (76%)	19 (83%)	17 (74%)	17 (68%)
Vagina	(25)	(24)	(24)	(24)	(23)	(23)
Infiltration cellular, polymorphonuclear		2 (8%)		2 (8%)		2 (9%)
Hematopoietic System						
Bone marrow	(25)	(24)	(24)	(23)	(23)	(24)
Fibrosis			1 (4%)			
Hyperplasia	1 (4%)	2 (8%)	2 (8%)	1 (4%)	3 (13%)	3 (13%)
Lymph node	(1)	(3)	(1)	(3)	(4)	(4)
Lumbar, sinus, dilatation		1 (33%)				
Renal, angiectasis		1 (33%)				
Renal, thrombus		1 (33%)				
Lymph node, mandibular	(25)	(25)	(24)	(24)	(23)	(25)
Hyperplasia, lymphoid	3 (12%)		3 (13%)	4 (17%)	2 (9%)	1 (4%)
Lymph node, mesenteric	(25)	(24)	(23)	(24)	(23)	(24)
Angiectasis		1 (4%)				
Hyperplasia, lymphoid	1 (4%)	3 (13%)	3 (13%)	4 (17%)	3 (13%)	3 (13%)
Spleen	(25)	(25)	(24)	(24)	(23)	(25)
Hematopoietic cell proliferation	2 (8%)	8 (32%)	7 (29%)	6 (25%)	6 (26%)	7 (28%)
Hyperplasia, lymphoid	20 (80%)	16 (64%)	17 (71%)	14 (58%)	14 (61%)	16 (64%)

TABLE A12
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Hematopoietic System (continued)						
Thymus	(25)	(21)	(22)	(22)	(24)	(24)
Atrophy	1 (4%)		2 (9%)			
Hyperplasia, lymphoid	6 (24%)	2 (10%)	2 (9%)	8 (36%)	6 (25%)	5 (21%)
Integumentary System						
Mammary gland	(25)	(25)	(25)	(23)	(23)	(25)
Skin	(25)	(25)	(25)	(25)	(23)	(25)
Inflammation, suppurative		1 (4%)				
Ulcer		1 (4%)				
Musculoskeletal System						
Bone	(0)	(0)	(2)	(0)	(0)	(0)
Bone, femur	(25)	(25)	(25)	(25)	(24)	(25)
Fibro-osseous lesion	1 (4%)					
Skeletal muscle	(0)	(1)	(2)	(0)	(0)	(0)
Degeneration			1 (50%)			
Nervous System						
Brain, brain stem	(25)	(24)	(25)	(24)	(23)	(25)
Brain, cerebellum	(25)	(24)	(25)	(24)	(23)	(25)
Hemorrhage		1 (4%)				
Brain, cerebrum	(25)	(24)	(25)	(24)	(23)	(25)
Mineralization	2 (8%)			3 (13%)		
Peripheral nerve	(0)	(0)	(1)	(0)	(0)	(0)
Axon, degeneration			1 (100%)			
Spinal cord, cervical	(0)	(0)	(1)	(0)	(0)	(0)
Spinal cord, lumbar	(0)	(0)	(1)	(0)	(0)	(0)
Spinal cord, thoracic	(0)	(0)	(1)	(0)	(0)	(0)
Respiratory System						
Lung	(25)	(24)	(24)	(24)	(24)	(25)
Hemorrhage				1 (4%)		
Infiltration cellular, lymphocyte		1 (4%)				3 (12%)
Inflammation, chronic active					1 (4%)	
Metaplasia, osseous		1 (4%)				
Nose	(25)	(25)	(25)	(24)	(24)	(25)
Hyaline droplet	1 (4%)	3 (12%)	4 (16%)	1 (4%)	6 (25%)	6 (24%)
Trachea	(25)	(24)	(24)	(24)	(23)	(25)
Special Senses System						
Eye	(25)	(23)	(23)	(22)	(23)	(23)
Cataract						1 (4%)
Harderian gland	(25)	(24)	(25)	(25)	(23)	(24)
Infiltration cellular, lymphocyte	2 (8%)				1 (4%)	
Inflammation, chronic active			1 (4%)			

TABLE A12
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Urinary System						
Kidney	(25)	(24)	(24)	(24)	(23)	(25)
Casts protein	14 (56%)	14 (58%)	8 (33%)	10 (42%)	12 (52%)	15 (60%)
Hydronephrosis						3 (12%)
Infarct	1 (4%)					
Infiltration cellular, lymphocyte	11 (44%)	10 (42%)	11 (46%)	15 (63%)	11 (48%)	11 (44%)
Metaplasia, osseous						1 (4%)
Mineralization			1 (4%)			
Renal tubule, regeneration			1 (4%)			
Urinary bladder	(25)	(24)	(24)	(23)	(23)	(25)
Infiltration cellular, lymphocyte	8 (32%)	4 (17%)	8 (33%)	4 (17%)	8 (35%)	3 (12%)

APPENDIX B
GENETIC TOXICOLOGY

TABLE B1
Frequency of Micronuclei in Peripheral Blood Erythrocytes and Reticulocytes in 1-Day-Old Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP[a]

	% RET	% Micronucleated NCE	% Micronucleated RET
Male			
Vehicle Control[b]	5	5	5
Mean ± standard error	20.0 ± 1.4	0.2 ± 0.0	0.5 ± 0.1
Tukey test[c]	A	A	A
AZT-H	8	8	8
Mean ± standard error	8.7 ± 1.2	3.9 ± 0.9	6.2 ± 1.2
Tukey test	B	B	B
Dunnett's Test P value[d]	P<0.001	P<0.001	P=0.008
3TC-H	5	5	5
Mean ± standard error	20.8 ± 2.2	0.2 ± 0.0	0.5 ± 0.1
Tukey test	A	A	A
Dunnett's Test P value	NS	NS	NS
NVP-H	5	5	5
Mean ± standard error	18.9 ± 0.1	0.2 ± 0.0	0.4 ± 0.0
Tukey test	A	A	A
Dunnett's Test P value	NS	NS	NS
AZT/3TC-H	6	6	6
Mean ± standard error	5.8 ± 0.7	4.3 ± 0.4	7.3 ± 1.0
Tukey test	B	B	B
Dunnett's Test P value	P<0.001	P<0.001	P=0.003
AZT/3TC/NVP-M	3	3	3
Mean ± standard error	5.8 ± 0.7	3.5 ± 0.5	9.6 ± 4.7
Tukey test	B	B	B
Dunnett's Test P value	P<0.001	P=0.045	P=0.001
AZT/3TC/NVP-H	6	6	6
Mean ± standard error	6.2 ± 0.7	6.7 ± 0.4	7.9 ± 1.4
Tukey test	B	C	B
Dunnett's Test P value	P<0.001	P<0.001	P=0.001

TABLE B1
Frequency of Micronuclei in Peripheral Blood Erythrocytes and Reticulocytes in 1-Day-Old Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

	% RET	% Micronucleated NCE	% Micronucleated RET
Female			
Vehicle Control	5	5	5
Mean ± standard error	19.6 ± 2.4	0.2 ± 0.0	0.5 ± 0.1
Tukey test	A	A	A
AZT-H	5	5	5
Mean ± standard error	5.6 ± 0.6	3.7 ± 0.6	9.4 ± 3.6
Tukey test	B	B	AB
Dunnett's Test P value	P<0.001	P<0.001	P=0.014
3TC-H	3	3	3
Mean ± standard error	17.2 ± 1.2	0.2 ± 0.1	0.5 ± 0.0
Tukey test	A	A	AB
Dunnett's Test P value	NS	NS	NS
NVP-H	3	5	5
Mean ± standard error	17.4 ± 0.4	0.2 ± 0.0	0.4 ± 0.0
Tukey test	A	A	A
Dunnett's Test P value	NS	NS	NS
AZT/3TC-H	5	5	5
Mean ± standard error	6.3 ± 0.8	3.1 ± 0.3	6.5 ± 1.5
Tukey test	B	B	A
Dunnett's Test P value	P<0.001	P<0.001	NS
AZT/3TC/NVP-M	4	4	4
Mean ± standard error	5.6 ± 0.5	3.8 ± 0.4	8.3 ± 2.2
Tukey test	B	B	AB
Dunnett's Test P value	P<0.001	P<0.001	P=0.043
AZT/3TC/NVP-H	5	5	5
Mean ± standard error	6.2 ± 0.9	6.4 ± 0.6	10.3 ± 3.0
Tukey test	B	C	B
Dunnett's Test P value	P<0.001	P<0.001	P=0.007

[a] NCE = normochromatic erythrocytes; RET = reticulocytes. Dams were dosed from gestational day (GD) 12 through GD 18 with the listed doses; AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-M = 160/100/112 mg/kg AZT/3TC/NVP; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number examined

[c] Letters associated with an exposure group represent significance of Tukey test evaluation. Groups with different letters are significantly different from each other (P<0.05).

[d] Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that exposed group. Two-tailed Dunnett's tests were used for the % RET values and one-tailed Dunnett's tests were used for the % micronucleated NCE and % micronucleated RET values. NS = Not significant

TABLE B2
Frequency of Micronuclei in Peripheral Blood Erythrocytes and Reticulocytes in 28-Day-Old Heterozygous F1 p53$^{+/-}$ Male Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP[a]

	% RET	% Micronucleated NCE	% Micronucleated RET
Vehicle Control[b]	8	8	8
Mean ± standard error	6.80 ± 0.2	0.23 ± 0.01	0.27 ± 0.02
Tukey test[c]	A	A	A
AZT-H	7	7	7
Mean ± standard error	6.19 ± 0.3	5.02 ± 0.5	2.47 ± 0.40
Tukey test	A	B	B
3TC-H	8	8	8
Mean ± standard error	7.03 ± 0.2	0.27 ± 0.01	0.29 ± 0.01
Tukey test	A	A	A
AZT/3TC-H	7	7	7
Mean ± standard error	7.290 ± 0.7	6.50 ± 0.35	4.11 ± 0.46
Tukey test	A	C	C

[a] NCE = normochromatic erythrocytes; RET = reticulocytes; AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; AZT/3TC-H = 240/150 mg/kg AZT/3TC

[b] Number examined

[c] Letters associated with a dose group represent significance of Tukey test evaluation. Groups with different letters are significantly different from each other ($P<0.05$).

APPENDIX C
CLINICAL PATHOLOGY RESULTS

TABLE C1
Hematology and Clinical Chemistry Data for Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Male				
n	20	24	21	22
Hematology				
Hematocrit (%)	45.7 ± 1.5	46.0 ± 1.9	47.5 ± 1.6	48.8 ± 1.2
Erythrocytes (10^6/mm^3)	9.4 ± 0.3	9.5 ± 0.4	9.8 ± 0.3	10.1 ± 0.2
Mean cell volume (µm^3)	48.7 ± 0.3	48.4 ± 0.3	48.5 ± 0.2	48.4 ± 0.3
Mean cell hemoglobin (pg)	16.0 ± 0.1	15.8 ± 0.1	15.8 ± 0.1	15.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.7 ± 0.1	32.6 ± 0.2	32.4 ± 0.1
Platelets (10^3/mm^3)	780.4 ± 30.5	785.9 ± 37.8	861.2 ± 38.0	842.0 ± 43.5
Leukocytes (10^3/mm^3)	3.5 ± 0.7	3.3 ± 0.4	3.9 ± 0.4	3.8 ± 0.4
Neutrophils (%)	5.4 ± 0.9	4.4 ± 0.6	3.4 ± 0.4	4.4 ± 0.8
Lymphocytes (%)	85.8 ± 1.4	85.0 ± 1.3	89.5 ± 0.9	87.8 ± 1.2
Monocytes (%)	5.0 ± 0.3	6.3 ± 0.6	4.7 ± 0.5	4.8 ± 0.4
Basophils (%)	2.2 ± 0.3	2.6 ± 0.4	1.5 ± 0.2	2.0 ± 0.3
Eosinophils (%)	1.5 ± 0.3	1.8 ± 0.5	0.8 ± 0.1	1.0 ± 0.2
Clinical Chemistry				
Glucose (mg/dL)	196.9 ± 11.4	197.4 ± 7.2	191.8 ± 8.5	196.9 ± 11.5
Total protein (g/dL)	6.8 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.2
Alanine aminotransferase (U/L)	38.7 ± 3.1*	38.9 ± 2.6	48.7 ± 5.3	55.6 ± 6.3**
Alkaline phosphatase (U/L)	104.4 ± 22.1	77.5 ± 5.1	97.0 ± 6.7	81.5 ± 5.1
Female				
n	25	22	19	20
Hematology				
Hematocrit (%)	47.1 ± 0.7	46.8 ± 1.1	47.2 ± 1.2	45.4 ± 1.0
Erythrocytes (10^6/mm^3)	9.7 ± 0.1	9.6 ± 0.2	9.7 ± 0.2	9.4 ± 0.2
Mean cell volume (µm^3)	48.6 ± 0.2	48.5 ± 0.2	49.0 ± 0.2	48.4 ± 0.2
Mean cell hemoglobin (pg)	16.0 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	44.7 ± 11.8	32.9 ± 0.1	32.7 ± 0.1	32.8 ± 0.1
Platelets (10^3/mm^3)	724.7 ± 26.7	711.7 ± 34.2	700.1 ± 46.7	751.0 ± 31.5
Leukocytes (10^3/mm^3)	4.3 ± 0.6	3.2 ± 0.2	4.5 ± 0.4	3.9 ± 0.4
Neutrophils (%)	4.9 ± 0.6	4.2 ± 0.5	4.7 ± 0.8	4.0 ± 0.5
Lymphocytes (%)	84.9 ± 1.2	85.4 ± 1.5	86.6 ± 1.6	86.5 ± 1.2
Monocytes (%)	5.9 ± 0.6	6.0 ± 0.8	5.2 ± 0.5	6.2 ± 0.5
Basophils (%)	2.6 ± 0.4	2.8 ± 0.5	2.2 ± 0.4	1.9 ± 0.3
Eosinophils (%)	1.7 ± 0.3	1.6 ± 0.4	1.3 ± 0.3	1.4 ± 0.3

TABLE C1
Hematology and Clinical Chemistry Data for Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Female (continued)				
n	25	22	19	20
Clinical Chemistry				
Glucose (mg/dL)	156.8 ± 8.3	163.7 ± 6.2	175.0 ± 9.0	170.7 ± 6.8
Total protein (g/dL)	6.7 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.5 ± 0.1
Alanine aminotransferase (U/L)	30.7 ± 6.7	23.8 ± 1.2	22.7 ± 1.4	29.9 ± 3.8
Alkaline phosphatase (U/L)	138.7 ± 22.8	116.1 ± 7.1	113.4 ± 7.3	128.4 ± 7.3

* Significant (P≤0.05) dose trend
** Significantly different (P≤0.01) from the vehicle control group by Dunnett's test
[a] Data are presented as mean ± standard error. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

TABLE C2
Hematology and Clinical Chemistry Data for Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Male						
n	20	17	24	22	21	22
Hematology						
Hematocrit (%)	45.7 ± 1.5	50.1 ± 1.0	45.6 ± 1.4	48.3 ± 1.2	50.1 ± 1.4	48.8 ± 1.2
Erythrocytes (10^6/mm^3)	9.4 ± 0.3	10.3 ± 0.2	9.4 ± 0.3	9.8 ± 0.2	10.2 ± 0.3	10.1 ± 0.2
Mean cell volume (μm^3)	48.7 ± 0.3	48.9 ± 0.3	48.7 ± 0.2	49.2 ± 0.2	49.0 ± 0.2	48.4 ± 0.3
Mean cell hemoglobin (pg)	16.0 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	15.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.5 ± 0.1	32.7 ± 0.1	32.4 ± 0.1	32.5 ± 0.1	32.4 ± 0.1
Platelets (10^3/mm^3)	780.4 ± 30.5	881.5 ± 26.1	854.2 ± 38.2	853.2 ± 32.7	913.1 ± 27.9	842.0 ± 43.5
Leukocytes (10^3/mm^3)	3.5 ± 0.7	3.0 ± 0.4	3.7 ± 0.6	3.1 ± 0.4	3.8 ± 0.4	3.8 ± 0.4
Neutrophils (%)	5.4 ± 0.9	3.6 ± 0.4	5.4 ± 1.0	3.7 ± 0.4	4.3 ± 1.3	4.4 ± 0.8
Lymphocytes (%)	85.8 ± 1.4	87.8 ± 0.9	85.4 ± 1.5	87.2 ± 1.0	87.1 ± 2.2	87.8 ± 1.2
Monocytes (%)	5.0 ± 0.3	5.2 ± 0.3	5.6 ± 0.4	6.3 ± 0.6	6.1 ± 0.8	4.8 ± 0.4
Basophils (%)	2.2 ± 0.3	2.1 ± 0.4	2.2 ± 0.4	1.7 ± 0.2	1.6 ± 0.3	2.0 ± 0.3
Eosinophils (%)	1.5 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	1.1 ± 0.3	0.9 ± 0.2	1.0 ± 0.2
Clinical Chemistry						
Glucose (mg/dL)	196.9 ± 11.4	209.5 ± 13.0	205.9 ± 10.8	172.1 ± 9.7	184.4 ± 10.9	196.9 ± 11.5
Total protein (g/dL)	6.8 ± 0.1	6.9 ± 0.2	6.8 ± 0.2	6.9 ± 0.1	7.0 ± 0.2	7.1 ± 0.2
Alanine aminotransferase (U/L)	38.7 ± 3.1	43.8 ± 3.3	59.3 ± 26.1	35.9 ± 2.7	44.0 ± 3.1	55.6 ± 6.3*
Alkaline phosphatase (U/L)	104.4 ± 22.1	98.1 ± 5.9	105.7 ± 12.3	91.2 ± 9.2	90.4 ± 4.1	81.5 ± 5.1
Female						
n	25	20	19	20	19	20
Hematology						
Hematocrit (%)	47.1 ± 0.7	45.9 ± 1.1	45.0 ± 0.9	46.1 ± 1.8	46.8 ± 1.5	45.4 ± 1.0
Erythrocytes (10^6/mm^3)	9.7 ± 0.1	9.5 ± 0.2	9.2 ± 0.2	9.4 ± 0.4	9.6 ± 0.3	9.4 ± 0.2
Mean cell volume (μm^3)	48.6 ± 0.2	48.6 ± 0.2	48.6 ± 0.2	48.8 ± 0.3	48.6 ± 0.3	48.4 ± 0.2
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	15.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	44.7 ± 11.8	33.0 ± 0.1	32.7 ± 0.1	32.8 ± 0.1	32.7 ± 0.1	32.8 ± 0.1
Platelets (10^3/mm^3)	724.7 ± 26.7	689.9 ± 24.2	699.6 ± 27.3	757.0 ± 33.5	756.9 ± 40.2	751.0 ± 31.5
Leukocytes (10^3/mm^3)	4.3 ± 0.6	3.6 ± 0.3	3.5 ± 0.3	3.8 ± 0.4	4.8 ± 0.6	3.9 ± 0.4
Neutrophils (%)	4.9 ± 0.6	4.6 ± 0.7	4.4 ± 0.5	4.5 ± 0.8	4.0 ± 1.0	4.0 ± 0.5
Lymphocytes (%)	84.9 ± 1.2	84.4 ± 1.6	84.5 ± 1.2	87.0 ± 1.3	87.6 ± 1.5	86.5 ± 1.2
Monocytes (%)	5.9 ± 0.6	6.9 ± 0.9	6.5 ± 0.6	6.0 ± 0.5	5.4 ± 0.4	6.2 ± 0.5
Basophils (%)	2.6 ± 0.4	2.6 ± 0.3	2.9 ± 0.4	1.8 ± 0.2	1.8 ± 0.3	1.9 ± 0.3
Eosinophils (%)	1.7 ± 0.3	1.5 ± 0.2	1.7 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.3

TABLE C2
Hematology and Clinical Chemistry Data for Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Female (continued)						
n	25	20	19	20	19	20
Clinical Chemistry						
Glucose (mg/dL)	156.8 ± 8.3	154.4 ± 7.5	168.5 ± 7.8	158.5 ± 6.6	169.8 ± 7.5	170.7 ± 6.8
Total Protein (g/dL)	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Alanine aminotransferase (U/L)	30.7 ± 6.7	26.9 ± 1.0	23.7 ± 1.6	27.4 ± 3.4	28.6 ± 2.2	29.9 ± 3.8
Alkaline phosphatase (U/L)	138.7 ± 22.8	120.7 ± 6.9	184.6 ± 62.0	130.1 ± 24.1	126.7 ± 11.5	128.4 ± 7.3

* Significantly different (P≤0.05) from the vehicle control group by Tukey's test
[a] Data are presented as mean ± standard error. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

APPENDIX D
BODY WEIGHT ANALYSES

TABLE D1
Body Weights of Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control		AZT/3TC/NVP-L		AZT/3TC/NVP-M		AZT/3TC/NVP-H	
Week	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight
2	25/18	8.0 ± 1.1	25/16	7.7 ± 0.8	23/14	7.6 ± 1.6	24/18	7.3 ± 1.1
3	25/18	10.8 ± 1.4	25/16	10.4 ± 1.0	23/14	10.2 ± 2.1	24/18	9.8 ± 1.7
4	25/18	15.8 ± 1.8**[b]	25/16	15.5 ± 1.4	23/14	14.5 ± 2.6	24/18	14.1 ± 2.2*
5	25/18	20.1 ± 1.4***	25/16	19.5 ± 1.2	23/14	18.3 ± 2.4*	24/18	18.2 ± 2.1**
6	25/18	23.1 ± 1.4***	25/16	22.2 ± 1.2	23/14	20.9 ± 2.4**	24/18	20.6 ± 2.0***
7	25/18	25.5 ± 1.4***	25/16	24.4 ± 1.2	23/14	23.2 ± 2.4***	24/18	22.7 ± 2.0***
8	25/18	27.1 ± 1.5***	25/16	25.9 ± 1.3	23/14	24.7 ± 2.5**	24/18	24.1 ± 2.1***
9	25/18	28.6 ± 1.7***	25/16	27.3 ± 1.5	23/14	26.2 ± 2.7**	24/18	25.6 ± 2.2***
10	25/18	29.9 ± 1.9***	25/16	28. 6 ± 1.7	23/14	27.5 ± 2.9**	24/18	26.8 ± 2.4***
11	25/18	31.1 ± 2.1***	25/16	29.8 ± 1.9	23/14	28.8 ± 3.2*	23/18	28.0 ± 2.6***
12	25/18	32.4 ± 2.3***	25/16	30.9 ± 2.1	23/14	30.0 ± 3.5*	23/18	29.1 ± 2.8**
13	25/18	33.5 ± 2.5***	25/16	32.0 ± 2.4	23/14	31.2 ± 3.8*	23/18	30.2 ± 2.9**
14	25/18	34.7 ± 2.7***	25/16	33.0 ± 2.6	23/14	32.4 ± 4.1*	23/18	31.2 ± 3.1**
15	25/18	35.8 ± 2.9***	25/16	34.1 ± 2.8	23/14	33.5 ± 4.3	23/18	32.3 ± 3.2**
16	25/18	36.9 ± 3.2**	25/16	35.2 ± 2.9	23/14	34.5 ± 4.6	23/18	33.3 ± 3.4**
17	25/18	37.9 ± 3.4**	25/16	36.2 ± 3.0	23/14	35.5 ± 4.8	23/18	34.3 ± 3.6**
18	25/18	38.8 ± 3.7**	25/16	37.2 ± 3.1	23/14	36.4 ± 5.0	23/18	35.3 ± 3.7*
19	25/18	39.6 ± 4.0**	25/16	38.2 ± 3.2	23/14	37.3 ± 5.1	23/18	36.3 ± 3.9*
20	25/18	40.4 ± 4.3*	25/16	39.1 ± 3.3	23/14	38.2 ± 5.2	23/18	37.3 ± 4.1*
21	25/18	41.2 ± 4.7*	25/16	39.9 ± 3.5	23/14	39.0 ± 5.3	23/18	38.3 ± 4.3
22	25/18	41.9 ± 5.1*	25/16	40.8 ± 3.6	23/14	39.8 ± 5.4	23/18	39.2 ± 4.5
23	25/18	42.6 ± 5.4*	25/16	41.5 ± 3.6	23/14	40.6 ± 5.3	23/18	40.1 ± 4.6
24	24/18	44.0 ± 4.5*	25/16	42.3 ± 3.7	23/14	41.4 ± 5.3	23/18	41.0 ± 4.8
25	24/18	44.7 ± 4.5*	25/16	42.9 ± 3.8	23/14	42.0 ± 5.3	23/18	41.8 ± 4.9
26	24/18	45.3 ± 4.5*	25/16	43.5 ± 3.8	23/14	42.7 ± 5.2	23/18	42.5 ± 5.0
27	24/18	45.8 ± 4.4	25/16	44.1 ± 3.8	23/14	43.2 ± 5.2	23/18	43.3 ± 5.0
28	24/18	46.3 ± 4.4	25/16	44.6 ± 3.8	23/14	43.7 ± 5.2	23/18	43.9 ± 5.1
29	23/18	46.7 ± 4.4	25/16	44.9 ± 3.8	23/14	44.2 ± 5.2	23/18	44.5 ± 5.1
30	23/18	47.1 ± 4.3	25/16	45.3 ± 3.7	23/14	44.6 ± 5.2	23/18	45.1 ± 5.1
31	23/18	47.4 ± 4.3	25/16	45.6 ± 3.7	23/14	44.9 ± 5.2	23/18	45.5 ± 5.2
32	23/18	47.8 ± 4.3	25/16	45.8 ± 3.6	23/14	45.2 ± 5.1	23/18	45.9 ± 5.2
33	23/18	48.1 ± 4.3	25/16	46.0 ± 3.6	23/14	45.5 ± 5.1	23/18	46.3 ± 5.3
34	23/18	48.5 ± 4.4	25/16	46.2 ± 3.6	23/14	45.7 ± 5.0	23/18	46.6 ± 5.3
35	23/18	48.8 ± 4.4	25/16	46.4 ± 3.6	23/14	45.9 ± 5.0	23/18	46.9 ± 5.4
36	23/18	49.1 ± 4.4	25/16	46.7 ± 3.7	23/14	46.1 ± 4.9	23/18	47.1 ± 5.4
37	23/18	49.4 ± 4.4	25/16	46.9 ± 3. 7	23/14	46.3 ± 4.9	23/18	47.3 ± 5.4
38	23/18	49.7 ± 4.4	25/16	47.1 ± 3.7	23/14	46.5 ± 4.8	23/18	47.4 ± 5.5
39	23/18	49.8 ± 4.4	25/16	47.4 ± 3.7	23/14	46.6 ± 4.8	23/18	47.5 ± 5.4
40	22/18	49.9 ± 4.4	25/16	47.6 ± 3.6	23/14	46.7 ± 4.7	23/18	47.6 ± 5.4
41	22/18	49.9 ± 4.4	24/16	47.8 ± 3.7	23/14	46.6 ± 4.9*	23/18	47.6 ± 5.4
42	22/18	50.0 ± 4.4	24/16	48.1 ± 3.8	23/14	46.6 ± 5.2*	23/18	47.6 ± 5.5
43	21/18	49.8 ± 4.5	24/16	48.3 ± 3.8	23/14	46.5 ± 5.6	23/18	47.6 ± 5.6
44	20/18	49.8 ± 4.8	22/15	48.3 ± 3.8	22/14	46.4 ± 6.2	22/17	48.0 ± 5.6

[a] Unadjusted weights are given as mean ± standard error in grams. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

[b] Asterisks represent significance of comparisons of body weights adjusted for litter correlation by Dunnett's test; * P<0.05, ** P<0.01, *** P<0.001. Asterisks appearing in the vehicle control column represent the trend. Asterisks in the dosed group columns represent pairwise comparisons with the vehicle control group.

TABLE D2
Body Weights of Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control		AZT-H		3TC-H		NVP-H		AZT/3TC-H		AZT/3TC/NVP-H	
Week	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight
2	25/18	8.0 ± 1.1	24/18	7.4 ± 1.0	25/16	8.3 ± 1.0	26/17	7.9 ± 1.0	25/17	7.5 ± 0.7	24/18	7.3 ± 1.1
3	25/18	10.8 ± 1.4	24/18	10.0 ± 1.4	25/16	11.1 ± 1.2	26/17	10.7 ± 1.2	25/17	10.0 ± 0.8	24/18	9.8 ± 1.7
4	25/18	15.8 ± 1.8	24/18	14.6 ± 2.0	25/16	16.5 ± 1.6	26/17	15.6 ± 1.9	25/17	14.7 ± 1.2	24/18	14.1 ± 2.2
5	25/18	20.1 ± 1.4	23/17	18.8 ± 1.8	25/16	21.0 ± 1.4*[b]	25/16	19.9 ± 1.4	25/17	19.1 ± 1.4	24/18	18.2 ± 2.1**
6	25/18	23.1 ± 1.4	23/17	21.6 ± 1.8*	25/16	23.7 ± 1.5	25/16	22.5 ± 1.6	25/17	22.0 ± 1.5	24/18	20.6 ± 2.0***
7	25/18	25.5 ± 1.4	23/17	23.7 ± 2.1**	25/16	26.2 ± 1.6	25/16	24.7 ± 1.7	25/17	24.4 ± 1.7	24/18	22.7 ± 2.0***
8	25/18	27.1 ± 1.5	23/17	25.3 ± 2.4**	25/16	27.9 ± 1.7	25/16	26.2 ± 1.9	25/17	26.1 ± 1.9	24/18	24.1 ± 2.1***
9	25/18	28.6 ± 1.7	23/17	26.7 ± 2.5**	25/16	29.5 ± 1.9	25/16	27.6 ± 2.0	25/17	27.5 ± 2.2	24/18	25.6 ± 2.2***
10	25/18	29.9 ± 1.9	23/17	28.0 ± 2.6**	25/16	30.9 ± 2.1	25/16	28.8 ± 2.2	25/17	28.8 ± 2.5	24/18	26.8 ± 2.4***
11	25/18	31.1 ± 2.1	23/17	29.2 ± 2.7*	25/16	32.2 ± 2.3	25/16	30.0 ± 2.4	25/17	30.1 ± 2. 9	23/18	28.0 ± 2.6***
12	25/18	32.4 ± 2.3	23/17	30.4 ± 3.0*	25/16	33.5 ± 2.5	25/16	31.2 ± 2.6	25/17	31.4 ± 3.2	23/18	29.1 ± 2.8***
13	25/18	33.5 ± 2.5	23/17	31.5 ± 3.3*	25/16	34.7 ± 2.7	25/16	32.3 ± 2.8	25/17	32.6 ± 3.6	23/18	30.2 ± 2.9***
14	25/18	34.7 ± 2.7	23/17	32.6 ± 3.6	25/16	35.9 ± 3.0	25/16	33.3 ± 3.1	24/16	34.1 ± 3.6	23/18	31.2 ± 3.1***
15	25/18	35.8 ± 2.9	23/17	33.7 ± 3.9	25/16	37.0 ± 3.2	25/16	34.4 ± 3.3	24/16	35.3 ± 3.8	23/18	32.3 ± 3.2***
16	25/18	36.9 ± 3.2	23/17	34.7 ± 4.1	25/16	38.1 ± 3.4	25/16	35.4 ± 3.5	24/16	36.5 ± 3.9	23/18	33.3 ± 3.4***
17	25/18	37.9 ± 3.4	23/17	35.7 ± 4.4	25/16	39.0 ± 3.5	25/16	36.4 ± 3.7	24/16	37.6 ± 3.9	23/18	34.3 ± 3.6**
18	25/18	38.8 ± 3.7	23/17	36.7 ± 4.5	25/16	39.9 ± 3.7	25/16	37.3 ± 3.9	24/16	38.6 ± 3.9	23/18	35.3 ± 3.7**
19	25/18	39.6 ± 4.0	23/17	37.6 ± 4.6	25/16	40.7 ± 3.9	25/16	38.2 ± 4.1	24/16	39.5 ± 3.8	23/18	36.3 ± 3.9*
20	25/18	40.4 ± 4.3	23/17	38.4 ± 4.8	25/16	41.6 ± 4.0	25/16	39.0 ± 4.3	24/16	40.4 ± 3.8	23/18	37.3 ± 4.1*
21	25/18	41.2 ± 4.7	23/17	39.2± 4.9	25/16	42.3 ± 4.1	25/16	39.8 ± 4.5	24/16	41.3 ± 3.7	23/18	38.3 ± 4.3
22	25/18	41.9 ± 5.1	23/17	39.9 ± 5.1	25/16	43.1 ± 4.1	25/16	40.6 ± 4.6	23/16	42.3 ± 3.5	23/18	39.2 ± 4.5
23	25/18	42.6 ± 5.4	22/16	41.2 ± 4.8	25/16	43.8 ± 4.2	25/16	41.4 ± 4.7	23/16	43.0 ± 3.4	23/18	40.1 ± 4.6
24	24/18	44.0 ± 4.5	22/16	41.9 ± 4.7	25/16	44.5 ± 4.2	24/15	41.9 ± 4.8	23/16	43.8 ± 3.3	23/18	41.0 ± 4.8
25	24/18	44.7 ± 4.5	21/16	43.0 ± 4.1	25/16	45.2 ± 4.1	24/15	42.5 ± 4.8	23/16	44.4 ± 3.1	23/18	41.8 ± 4.9
26	24/18	45.3 ± 4.5	21/16	43.6 ± 3.9	24/16	46.2 ± 4.0	24/15	43.1 ± 4.9	23/16	44.9 ± 3.0	23/18	42.5 ± 5.0
27	24/18	45.8 ± 4.4	21/16	44.1 ± 3.8	24/16	46.8 ± 3.9	24/15	43.6 ± 4.8	23/16	45.4 ± 2.9	23/18	43.3 ± 5.0
28	24/18	46.3 ± 4.4	21/16	44.5 ± 3.7	24/16	47.4 ± 3.9	24/15	44.0 ± 4.8	22/15	45.9 ± 2.9	23/18	43.9 ± 5.1
29	23/18	46.7 ± 4.4	21/16	44.9 ± 3.6	24/16	47.9 ± 3.8	24/15	44.5 ± 4.7	22/15	46.3 ± 2.9	23/18	44.5 ± 5.1
30	23/18	47.1 ± 4.3	21/16	45.3 ± 3.6	24/16	48.3 ± 3.8	24/15	44.9 ± 4.7	22/15	46.7 ± 2.8	23/18	45.1 ± 5.1
31	23/18	47.4 ± 4.3	21/16	45.7 ± 3.5	24/16	48.7 ± 3.7	23/15	45.7 ± 4.4	22/15	47.1 ± 2.8	23/18	45.5 ± 5.2
32	23/18	47.8 ± 4.3	21/16	46.0 ± 3.4	24/16	49.1 ± 3.7	23/15	46.0 ± 4.5	22/15	47.4 ± 2.8	23/18	45.9 ± 5.2
33	23/18	48.1 ± 4.3	21/16	46.3 ± 3.4	24/16	49.4 ± 3.6	23/15	46.4 ± 4.5	22/15	47.7 ± 2.8	23/18	46.3 ± 5.3
34	23/18	48.5 ± 4.4	20/15	46.7 ± 3.4	24/16	49.6 ± 3.7	22/15	46.9 ± 4.6	22/15	48.0 ± 2.8	23/18	46.6 ± 5.3
35	23/18	48.8 ± 4.4	20/15	46.8 ± 3.7	23/16	50.3 ± 3.2	22/15	47.3 ± 4.7	22/15	48.3 ± 2.8	23/18	46.9 ± 5.4
36	23/18	49.1 ± 4.4	20/15	47.0 ± 4.0	23/16	50.5 ± 3.3	22/15	47.7 ± 4.7	22/15	48.6 ± 2.9	23/18	47.1 ± 5.4

TABLE D2
Body Weights of Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control		AZT-H		3TC-H		NVP-H		AZT/3TC-H		AZT/3TC/NVP-H	
Week	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight
37	23/18	49.4 ± 4.4	19/14	47.8 ± 3.3	23/16	50.7 ± 3.4	22/15	48.0 ± 4.7	22/15	48.9 ± 2.9	23/18	47.3 ± 5.4
38	23/18	49.7 ± 4.4	19/14	48.0 ± 3.7	23/16	50.9 ± 3.5	22/15	48.4 ± 4.6	22/15	49.2 ± 3.0	23/18	47.4 ± 5.5
39	23/18	49.8 ± 4.4	19/14	48.1 ± 4.2	23/16	51.1 ± 3.6	22/15	48.7 ± 4.5	22/15	49.5 ± 3.0	23/18	47.5 ± 5.4
40	22/18	49.9 ± 4.4	18/13	49.2 ± 2.6	23/16	51.3 ± 3.6	22/15	48.9 ± 4.5	22/15	49.7 ± 3.0	23/18	47.6 ± 5.4
41	22/18	49.9 ± 4.4	18/13	49.5 ± 2.5	23/16	51.6 ± 3.6	22/15	49.2 ± 4.4	22/15	49.9 ± 3.0	23/18	47.6 ± 5.4
42	22/18	50.0 ± 4.4	18/13	49.7 ± 2.5	23/16	51.8 ± 3.5	22/15	49.4 ± 4.3	22/15	50.0 ± 3.0	23/18	47.6 ± 5.5
43	21/18	49.8 ± 4.5	18/13	49.9 ± 2.5	23/16	52.0 ± 3.5	22/15	49.6 ± 4.2	21/14	50.0 ± 3.0	23/18	47.6 ± 5.6
44	20/18	49.8 ± 4.8	18/13	50.2 ± 2.5	23/16	52.3 ± 3.4	22/15	49.9 ± 4.1	19/13	50.3 ± 3.2	22/17	48.0 ± 5.6

[a] Unadjusted weights are given as mean ± standard error in grams. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Asterisks represent significance of pairwise comparisons with the vehicle control group for body weights adjusted for litter correlation by Dunnett's test; * P<0.05, ** P<0.01, *** P<0.001.

TABLE D3
Body Weights of Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control		AZT/3TC/NVP-L		AZT/3TC/NVP-M		AZT/3TC/NVP-H	
Week	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight
2	25/18	7.9 ± 0.9**[b]	25/15	7.7 ± 0.9	23/12	7.5 ± 1.1	25/17	7.1 ± 0.9**
3	25/18	10.5 ± 0.9**	25/15	10.0 ± 0.9	23/12	9.8 ± 1.3	25/17	9.5 ± 1.0**
4	25/18	13.8 ± 1.2***	25/15	13.6 ± 1.2	23/12	13.3 ± 1.4	25/17	12.5 ± 1.3**
5	25/18	17.2 ± 1.2***	25/15	16.6 ± 1.4	23/12	16.3 ± 1.5	25/17	15.3 ± 1.2***
6	25/18	19.3 ± 1.2***	25/15	18.6 ± 1.5	23/12	18.1 ± 1.6	25/17*	17.2 ± 1.3***
7	25/18	21.1 ± 1.2***	25/15	20.3 ± 1.6	23/12	19.6 ± 1.7	25/17**	18.8 ± 1.3***
8	25/18	22.2 ± 1.4***	25/15	21.4 ± 1.6	23/12	20.9 ± 1.8	25/17*	20.0 ± 1.5***
9	25/18	23.2 ± 1.5***	25/15	22.4 ± 1.7	23/12	22.0 ± 1.8	25/17	21.2 ± 1.7**
10	25/18	24.0 ± 1.7**	25/15	23.3 ± 1.8	23/12	23.1 ± 2.0	25/17	22.3 ± 1.9**
11	25/18	24.9 ± 1.9*	25/15	24.1 ± 1.9	23/12	24.1 ± 2.2	25/17	23.3 ± 2.2*
12	25/18	25.7 ± 2.1	25/15	24.9 ± 2.0	23/12	25.0 ± 2.4	25/17	24.4 ± 2.5
13	25/18	26.5 ± 2.3	25/15	25.7 ± 2.2	23/12	26.0 ± 2.6	25/17	25.4 ± 2.8
14	25/18	27.3 ± 2.5	25/15	26.5 ± 2.4	22/12	26.9 ± 2.9	25/17	26.4 ± 3.2
15	25/18	28.1 ± 2.7	25/15	27.3 ± 2.7	22/12	27.8 ± 3.2	25/17	27.8 ± 3.6
16	25/18	28.9 ± 2.9	25/15	28.1 ± 2.9	22/12	28. 7 ± 3.5	25/17	28.2 ± 4.1
17	25/18	29.7 ± 3.2	25/15	28.9 ± 3.1	22/12	29.5 ± 3.8	25/17	29.0 ± 4.5
18	25/18	30.5 ± 3.5	25/15	29.6 ± 3.4	22/12	30.3 ± 4.1	25/17	29.8 ± 4.9
19	25/18	31.2 ± 3.8	25/15	30.4 ± 3.6	21/12	30.9 ± 4.3	25/17	30.6 ± 5.3
20	25/18	31.9 ± 4.1	25/15	31.1 ± 3.8	21/12	31.5 ± 4.6	25/17	31.4 ± 5.7
21	25/18	32.5 ± 4.3	25/15	31.7 ± 3.9	21/12	32.1 ± 4.8	25/17	32.1 ± 6.0
22	25/18	33.2 ± 4.6	25/15	32.3 ± 4.1	21/12	32.7 ± 4.9	25/17	32.9 ± 6.3
23	25/18	33.9 ± 4.8	25/15	32.8 ± 4.2	21/12	33.3 ± 5.1	25/17	33.6 ± 6.5
24	25/18	34.5 ± 5.1	25/15	33.3 ± 4.4	21/12	33.9 ± 5.2	25/17	34.3 ± 6.8
25	25/18	35.2 ± 5.3	25/15	33.8 ± 4.7	21/12	34.5 ± 5.3	25/17	34.9 ± 6.9
26	25/18	35.9 ± 5.5	24/15	34.7 ± 4.6	21/12	35.0 ± 5.4	25/17	35.6 ± 7.0
27	25/18	36.6 ± 5.7	24/15	35.4 ± 4.7	21/12	35.6 ± 5.5	25/17	36.1 ± 7.0
28	25/18	37.3 ± 5.9	24/15	36.0 ± 4.8	21/12	36.2 ± 5.5	25/17	36.7 ± 7.1
29	25/18	37.8 ± 6.0	24/15	36.6 ± 4.8	21/12	36.8 ± 5.6	25/17	37.2 ± 7.1
30	25/18	38.4 ± 6.1	24/15	37.2 ± 4.9	21/12	37.3 ± 5.7	25/17	37.7 ± 7.1
31	25/18	38.9 ± 6.1	24/15	37.7 ± 4.8	21/12	37.8 ± 5.8	25/17	38.1 ± 7.2
32	25/18	39.4 ± 6.2	24/15	38.2 ± 4.8	21/12	38.3 ± 5.9	25/17	38.5 ± 7.2
33	25/18	39.8 ± 6.2	24/15	38.7 ± 4.8	21/12	38.7 ± 6.0	25/17	39.0 ± 7.3
34	25/18	40.2 ± 6.3	23/15	39.1 ± 4.9	21/12	39.1 ± 6.1	25/17	39.3 ± 7.4
35	25/18	40.6 ± 6.5	23/15	39.6 ± 5.0	21/12	39.4 ± 6.2	24/17	39.9 ± 7.7
36	25/18	41.0 ± 6.6	23/15	39.9 ± 5.0	21/12	39.8 ± 6.3	24/17	40.4 ± 7.8
37	25/18	41.4 ± 6.7	23/15	40.3 ± 5.1	20/12	40.8 ± 5.7	24/17	40.9 ± 7.8
38	25/18	41.8 ± 6.9	23/15	40.7 ± 5.1	20/12	41.2 ± 5.7	23/17	41.9 ± 7.6
39	25/18	42.2 ± 6.9	23/15	41.0 ± 5.0	20/12	41.6 ± 5.7	23/17	42.1 ± 7.6
40	25/18	42.6 ± 7.0	23/15	41.4 ± 5.0	20/12	42.0 ± 5.6	23/17	42.4 ± 7.7
41	25/18	43.0 ± 7.0	22/15	42.1 ± 4.7	20/12	42.5 ± 5.6	23/17	42.5 ± 7.8
42	25/18	43.4 ± 7.0	22/15	42.5 ± 4.6	20/12	42.9 ± 5.7	23/17	42.5 ± 8.0
43	25/18	43.8 ± 7.0	22/15	42.9 ± 4.5	20/12	43.3 ± 5.7	22/16	43.3 ± 7.7
44	25/18	44.3 ± 7.0	21/14	43.3 ± 4.6	19/11	43.3 ± 5.6	21/15	43.9 ± 7.7

[a] Unadjusted weights are given as mean ± standard error in grams. AZT/3TC/NVP-L = 80/50/56 mg/kg; AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

[b] Asterisks represent significance of comparisons of body weights adjusted for litter correlation by Dunnett's test; * P<0.05, ** P<0.01, *** P<0.001. Asterisks appearing in the vehicle control column represent the trend. Asterisks in the dosed group columns represent pairwise comparisons with the vehicle control group.

TABLE D4
Body Weights of Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control		AZT-H		3TC-H		NVP-H		AZT/3TC-H		AZT/3TC/NVP-H	
Week	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight
2	25/18	7.9 ± 0.9	25/19	7.1 ± 1.1*[b]	25/16	8.1 ± 0.8	25/16	8.1 ± 1.0	24/16	7.5 ± 0.7	25/17	7.1 ± 0.9**
3	25/18	10.5 ± 0.9	25/19	9.5 ± 1.4*	25/16	10.6 ± 1.0	25/16	10.6 ± 1.2	24/16	9.9 ± 1.0	25/17	9.5 ± 1.0***
4	25/18	13.8 ± 1.2	25/19	13.0 ± 1.5	25/16	14.3 ± 1.0	25/16	14.3 ± 1.5	24/16	13.5 ± 0.8	25/17	12.5 ± 1.3***
5	25/18	17.2 ± 1.2	25/19	16.1 ± 1.6*	25/16	17.6 ± 1.0	25/16	17.4 ± 1.5	24/16	16.7 ± 0.9	25/17	15.3 ± 1.2***
6	25/18	19.3 ± 1.2	25/19	18.1 ± 1.6**	25/16	19.6 ± 1.1	25/16	19.4 ± 1.7	24/16	18.8 ± 0.9	25/17	17.2 ± 1.3***
7	25/18	21.1 ± 1.2	25/19	19.7 ± 1.6**	25/16	21.3 ± 1.2	25/16	21.1 ± 1.9	24/16	20.5 ± 1.0	25/17	18.8 ± 1.3***
8	25/18	22.2 ± 1.4	25/19	20.9 ± 1.7*	25/16	22.4 ± 1.3	25/16	22.2 ± 2.1	24/16	21.7 ± 1.1	25/17	20.0 ± 1.5***
9	25/18	23.2 ± 1.5	25/19	22.0 ± 1.8*	25/16	23.5 ± 1.5	25/16	23.2 ± 2.4	24/16	22.9 ± 1.2	25/17	21.2 ± 1.7***
10	25/18	24.0 ± 1.7	25/19	23.0 ± 1.9	25/16	24.4 ± 1.6	25/16	24.2 ± 2.7	24/16	23.9 ± 1.4	25/17	22.3 ± 1.9**
11	25/18	24.9 ± 1.9	25/19	23.9 ± 2.0	25/16	25.3 ± 1.8	25/16	25.2 ± 3.1	24/16	24.9 ± 1.6	25/17	23.3 ± 2.2
12	25/18	25.7 ± 2.1	25/19	24.7 ± 2.1	25/16	26.1 ± 2.1	25/16	26.1 ± 3.4	24/16	25.9 ± 1.8	25/17	24.4 ± 2.5
13	25/18	26.5 ± 2.3	25/19	25.5 ± 2.3	25/16	27.0 ± 2.3	25/16	27.0 ± 3.8	24/16	26.6 ± 2.3	25/17	25.4 ± 2.8
14	25/18	27.3 ± 2.5	25/19	26.3 ± 2.5	25/16	27.7 ± 2.5	25/16	27.9 ± 4.1	23/16	27.7 ± 2.6	25/17	26.4 ± 3.2
15	25/18	28.1 ± 2.7	25/19	27.1 ± 2.8	25/16	28.5 ± 2.7	25/16	28.8 ± 4.4	23/16	28.5 ± 3.1	25/17	27.3 ± 3.6
16	25/18	28.9 ± 2.9	25/19	28.0 ± 3.0	25/16	29.2 ± 2.9	25/16	29.6 ± 4.7	22/16	29.7 ± 2.7	25/17	28.2 ± 4.1
17	25/18	29.7 ± 3.2	25/19	28.9 ± 3.3	25/16	29.8 ± 3.1	25/16	30.4 ± 4.9	22/16	30.6 ± 2.8	25/17	29.0 ± 4.5
18	25/18	30.5 ± 3.5	25/19	29.8 ± 3.6	25/16	30.5 ± 3.3	25/16	31.2 ± 5.1	22/16	31.4 ± 3.0	25/17	29.8 ± 4.9
19	25/18	31.2 ± 3.8	25/19	30.6 ± 3.8	25/16	31.1 ± 3.4	25/16	31.9 ± 5.2	22/16	32.1 ± 3.1	25/17	30.6 ± 5.3
20	25/18	31.9 ± 4.1	25/19	31.5 ± 4.1	25/16	31.8 ± 3.6	25/16	32.6 ± 5.3	22/16	32.9 ± 3.2	25/17	31.4 ± 5.7
21	25/18	32.5 ± 4.3	25/19	32.3 ± 4.3	25/16	32.4 ± 3.7	25/16	33.3 ± 5.4	22/16	33.6 ± 3.3	25/17	32.1 ± 6.0
22	25/18	33.2 ± 4.6	25/19	33.0 ± 4.5	25/16	33.1 ± 3.8	25/16	34.0 ± 5.6	22/16	34.4 ± 3.4	25/17	32.9 ± 6.3
23	25/18	33.9 ± 4.8	25/19	33.7 ± 4.7	25/16	33.8 ± 3.8	25/16	34.6 ± 5.6	22/16	35.1 ± 3.6	25/17	33.6 ± 6.5
24	25/18	34.5 ± 5.1	25/19	34.4 ± 4.9	25/16	34.5 ± 4.0	25/16	35.3 ± 5.7	22/16	35.9 ± 3.7	25/17	34.3 ± 6.8
25	25/18	35.2 ± 5.3	25/19	34.9 ± 5.1	25/16	35.2 ± 4.1	25/16	35.9 ± 5.8	22/16	36.6 ± 3.9	25/17	34.9 ± 6.9
26	25/18	35.9 ± 5.5	25/19	35.4 ± 5.3	25/16	35.9 ± 4.2	25/16	36.4 ± 5.9	22/16	37.3 ± 4.1	25/17	35.6 ± 7.0
27	25/18	36.6 ± 5.7	25/19	35.8 ± 5.5	25/16	36.6 ± 4.3	25/16	37.0 ± 5.9	22/16	38.0 ± 4.3	25/17	36.1 ± 7.0
28	25/18	37.3 ± 5.9	25/19	36.3 ± 5.7	25/16	37.3 ± 4.4	25/16	37.5 ± 5.9	22/16	38.6 ± 4.5	25/17	36.7 ± 7.1
29	25/18	37.8 ± 6.0	25/19	36.7 ± 5.8	25/16	38.0 ± 4.5	25/16	38.1 ± 5.9	22/16	39.1 ± 4.6	25/17	37.2 ± 7.1
30	25/18	38.4 ± 6.1	25/19	37.2 ± 6.0	25/16	38.6 ± 4.6	25/16	38.7 ± 6.0	22/16	39.6 ± 4.7	25/17	37.7 ± 7.1
31	25/18	38.9 ± 6.1	25/19	37.7 ± 6.1	25/16	39.2 ± 4.6	25/16	39.2 ± 6.0	22/16	40.1 ± 4.7	25/17	38.1 ± 7.2
32	25/18	39.4 ± 6.2	25/19	38.2 ± 6.3	25/16	39.6 ± 4.6	25/16	39.7 ± 6.1	22/16	40.5 ± 4.7	25/17	38.5 ± 7.2
33	25/18	39.8 ± 6.2	25/19	38.7 ± 6.4	25/16	40.0 ± 4.6	25/16	40.2 ± 6.2	22/16	40.8 ± 4.8	25/17	39.0 ± 7.3
34	25/18	40.2 ± 6.3	25/19	39.1 ± 6.6	25/16	40.4 ± 4.6	25/16	40.6 ± 6.4	22/16	41.1 ± 5.0	25/17	39.3 ± 7.4
35	25/18	40.6 ± 6.5	25/19	39.4 ± 6.8	25/16	40.8 ± 4.7	24/16	41.3 ± 6.5	22/16	41.3 ± 5.3	24/17	39.9 ± 7.7
36	25/18	41.0 ± 6.6	25/19	39.7 ± 7.1	24/15	41.3 ± 4.9	24/16	41.6 ± 6.7	21/16	41.9 ± 5.7	24/17	40.4 ± 7.8

TABLE D4
Body Weights of Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control		AZT-H		3TC-H		NVP-H		AZT/3TC-H		AZT/3TC/NVP-H	
Week	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight	Pups/ Litters	Weight
37	25/18	41.4 ± 6.7	25/19	40.0 ± 7.3	24/15	41.6 ± 5.1	22/16	42.3 ± 6.9	20/15	43.1 ± 4.9	24/17	40.9 ± 7.8
38	25/18	41.8 ± 6.9	25/19	40.3 ± 7.6	24/15	41.8 ± 5.5	22/16	42.5 ± 7.2	20/15	43.6 ± 5.0	23/17	41.9 ± 7.6
39	25/18	42.2 ± 6.9	24/19	41.1 ± 7.6	22/14	41.7 ± 5.3	22/16	42.6 ± 7.5	20/15	44.2 ± 5.2	23/17	42.1 ± 7.6
40	25/18	42.6 ± 7.0	23/18	41.2 ± 8.0	22/14	41.7 ± 5.7	22/16	42.7 ± 7.9	19/14	45.2 ± 5.1	23/17	42.4 ± 7.7
41	25/18	43.0 ± 7.0	23/18	41.6 ± 8.2	21/13	42.7 ± 4.7	22/16	42.8 ± 8.4	19/14	45.8 ± 5.3	23/17	42.5 ± 7.8
42	25/18	43.4 ± 7.0	21/17	42.1 ± 8.5	20/12	42.9 ± 5.1	21/15	43.9 ± 8.0	19/14	46.4 ± 5.5	23/17	42.5 ± 8.0
43	25/18	43.8 ± 7.0	21/17	42.5 ± 8.7	20/12	43.2 ± 5.5	20/14	44.7 ± 8.1	19/14	47.0 ± 5.7	22/16	43.3 ± 7.7
44	25/18	44.3 ± 7.0	21/17	42.9 ± 8.8	20/12	43.4 ± 5.9	20/14	45.1 ± 8.3	18/13	48.1 ± 5.6	21/15	43.9 ± 7.7

[a] Unadjusted weights are given as mean ± standard error in grams. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Asterisks represent significance of pairwise comparisons with the vehicle control group for body weights adjusted for litter correlation by Dunnett's test; * P<0.05, ** P<0.01, *** P<0.001.

APPENDIX E
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE E1
Organ Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	R[b]	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
Male					
n		20	24	22	22
Necropsy body wt		46.0 ± 1.0	44.6 ± 0.7	41.4 ± 1.4	44.0 ± 1.2
Brain					
Absolute	0.38	515.3 ± 4.1**	502.3 ± 3.7	487.0 ± 6.2**	465.3 ± 5.7**
Relative	0.06	11.3 ± 0.3	11.3 ± 0.2	12.2 ± 0.6	10.8 ± 0.4
Heart					
Absolute	–0.19	234.9 ± 6.9	231.0 ± 4.3	216.2 ± 7.9	231.7 ± 5.7
Relative	–0.24	5.1 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.3 ± 0.1
Kidney					
Absolute	0.15	672.0 ± 14.4*	631.1 ± 12.9	583.0 ± 22.9**	619.7 ± 21.7[c]
Relative	0.15	14.6 ± 0.2	14.2 ± 0.3	14.1 ± 0.3	14.2 ± 0.3[c]
Liver					
Absolute	0.16	2,039.5 ± 81.1	2,082.2 ± 66.1	2,124.2 ± 169.9	2,338.4 ± 135.7
Relative	0.06	44.0 ± 1.1*	46.9 ± 1.7	51.5 ± 4.7	52.8 ± 2.3
Lung					
Absolute	0.44	253.8 ± 8.6	262.0 ± 7.3	252.6 ± 7.3	237.7 ± 7.7
Relative	0.18	5.6 ± 0.2	5.9 ± 0.2	6.3 ± 0.3	5.5 ± 0.2
Female					
n		25	22	19	21
Necropsy body wt		41.3 ± 1.4	40.7 ± 1.0	41.2 ± 1.4	40.3 ± 1.6
Brain					
Absolute	0.29	534.6 ± 7.3**	512.6 ± 4.1*	494.3 ± 6.3**	472.4 ± 3.3**
Relative	0.57	13.4 ± 0.5*	12.8 ± 0.3	12.2 ± 0.4	12.1 ± 0.5
Heart					
Absolute	0.29	170.0 ± 4.6**	171.7 ± 3.4	174.0 ± 4.2	188.1 ± 4.5*
Relative	0.50	4.2 ± 0.1*	4.3 ± 0.1	4.3 ± 0.1	4.8 ± 0.2
Kidney					
Absolute	0.39	448.5 ± 9.5	429.1 ± 8.7	410.9 ± 9.1	436.5 ± 13.5
Relative	–0.08	11.1 ± 0.3	10.6 ± 0.2	10.1 ± 0.2*	11.0 ± 0.3
Liver					
Absolute	0.32	1,462.3 ± 51.3[d]	1,507.1 ± 46.8	1,447.7 ± 49.2	1,494.2 ± 71.3
Relative	–0.22	35.5 ± 0.9[d]	37.1 ± 0.9	35.3 ± 0.6	37.1 ± 0.9
Lung					
Absolute	–0.11	239.8 ± 8.2[d]	246.7 ± 8.6	223.5 ± 7.0	235.6 ± 6.5[e]
Relative	0.39	5.9 ± 0.2[d]	6.1 ± 0.2	5.6 ± 0.3	6.1 ± 0.3[e]

* Significant trend (P≤0.05, vehicle control column) or significantly different (P≤0.05, dosed group columns) from the vehicle control group by Dunnett's test

** P≤0.01

[a] Body weights are given in grams; organ weights (absolute weights) are given in milligrams; organ-weight-to-body-weight ratios (relative weights) are given as the organ weight/body weight; AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg

[b] R = Correlation within littermates

[c] n=21

[d] n=24

[e] n=20

TABLE E2
Organ Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	R[b]	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Male							
n		20	18	23	22	21	22
Necropsy body wt		46.0 ± 1.0	45.8 ± 0.6	47.9 ± 0.6	46.0 ± 0.7	45.8 ± 0.7	44.0 ± 1.2
Brain							
Absolute	0.32	515.3 ± 4.1††	493.0 ± 3.8*††	531.0 ± 4.8††	509.5 ± 4.8††	498.1 ± 4.1††[c]	465.3 ± 5.7**
Relative	0.36	11.3 ± 0.3	10.8 ± 0.1	11.1 ± 0.2	11.1 ± 0.2	11.0 ± 0.2[c]	10.8 ± 0.4
Heart							
Absolute	0.15	234.9 ± 6.9	232.3 ± 6.5	250.1 ± 6.4	223.0 ± 6.3	232.1 ± 5.8[c]	231.7 ± 5.7
Relative	0.12	5.1 ± 0.1	5.1 ± 0.1	5.2 ± 0.2	4.8 ± 0.1	5.1 ± 0.1[c]	5.3 ± 0.1
Kidney							
Absolute	0.23	672.0 ± 14.4	653.0 ± 18.2	721.1 ± 18.0††	630.9 ± 20.0	666.8 ± 13.5	619.7 ± 21.7[d]
Relative	0.04	14.6 ± 0.2	14.2 ± 0.3	15.0 ± 0.3	13.7 ± 0.3	14.6 ± 0.2	14.2 ± 0.3[d]
Liver							
Absolute	0.35	2,039.5 ± 81.1	2,290.2 ± 98.4	2,164.6 ± 83.7	2,005.3 ± 81.7	2,276.6 ± 99.9	2,338.4 ± 135.7
Relative	0.18	44.0 ± 1.1††	49.8 ± 1.7	44.9 ± 1.3††	43.3 ± 1.3††	49.3 ± 1.5	52.8 ± 2.3**
Lung							
Absolute	–0.02	253.8 ± 8.6	241.9 ± 9.4[e]	288.7 ± 9.5*††	255.5 ± 8.5	244.5 ± 9.1	237.7 ± 7.7
Relative	0.01	5.6 ± 0.2	5.3 ± 0.2[e]	6.0 ± 0.2	5.6 ± 0.2	5.3 ± 0.2	5.5 ± 0.2
Female							
n		25	20	20	20	19	21
Necropsy body wt		41.3 ± 1.4	40.4 ± 2.1	40.7 ± 1.5	42.3 ± 1.8	44.9 ± 1.3	40.3 ± 1.6
Brain							
Absolute	0.37	534.6 ± 7.3††	491.6 ± 4.2**	542.8 ± 5.0††	542.0 ± 5.9††	513.1 ± 6.0††	472.4 ± 3.3**
Relative	0.43	13.4 ± 0.5	12.8 ± 0.7	13.8 ± 0.6	13.2 ± 0.5	11.6 ± 0.4	12.1 ± 0.5
Heart							
Absolute	0.11	170.0 ± 4.6	171.0 ± 4.1	176.4 ± 4.8	169.5 ± 7.1	190.6 ± 4.9*	188.1 ± 4.5
Relative	0.18	4.2 ± 0.1	4.4 ± 0.2	4.5 ± 0.2	4.0 ± 0.1†	4.3 ± 0.1	4.8 ± 0.2
Kidney							
Absolute	0.27	448.5 ± 9.5	415.2 ± 10.6	455.7 ± 5.8	433.3 ± 14.0	451.9 ± 9.6	436.5 ± 13.5
Relative	0.07	11.1 ± 0.3	10.6 ± 0.4	11.5 ± 0.5	10.4 ± 0.3	10.2 ± 0.2	11.0 ± 0.3
Liver							
Absolute	0.18	1,462.3 ± 51.3[f]	1,435.9 ± 50.9	1,450.8 ± 31.0	1,564.5 ± 90.0	1,681.7 ± 79.7	1,494.2 ± 71.3
Relative	–0.14	35.5 ± 0.9[f]	36.3 ± 1.1	36.3 ± 1.0	37.3 ± 1.7	37.4 ± 1.3	37.1 ± 0.9
Lung							
Absolute	0.12	239.8 ± 8.2[f]	216.1 ± 4.7	242.9 ± 10.2	219.2 ± 7.3	250.7 ± 9.9	235.6 ± 6.5[c]
Relative	0.34	5.9 ± 0.2[f]	5.6 ± 0.3	6.1 ± 0.3	5.3 ± 0.2	5.7 ± 0.3	6.1 ± 0.3[c]

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
** P≤0.01
† Significantly different (P≤0.05) from the AZT/3TC/NVP=H group by Dunnett's test
†† P≤0.01
[a] Body weights are given in grams; organ weights (absolute weights) are given in milligrams; organ-weight-to-body-weight ratios (relative weights) are given as the organ weight/body weight; AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP
[b] R = Correlation within littermates
[c] n=20 [d] n=21 [e] n=17 [f] n=24

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

AZT, 3TC, and NVP were obtained from Cipla Ltd., Mumbai Central (Mumbai, India) in single lots F60731, B10250, and FX1009, respectively. Identity and purity analyses were conducted by the study laboratory at the National Center for Toxicological Research (NCTR; Jefferson, AR) and Galbraith Laboratories, Inc. (Knoxville, TN). To ensure stability, the bulk chemicals were stored in sealed amber jars at room temperature. Reports on analyses performed in support of the AZT, 3TC, and NVP *in utero/*postnatal gavage study are on file at the NCTR.

AZT

Lot F60731 of the chemical, a white-to-beige crystalline solid, was identified as AZT by the study laboratory using proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe/electron ionization (DEP/EI) mass spectrometry (MS), and liquid chromatography combined with mass spectrometry (LC-MS). All spectra were consistent with the structure of AZT, reference spectra, and spectra of AZT samples obtained from Sigma-Aldrich® Corporation (St. Louis, MO) or spectra of a previously characterized lot (Cipla lot FX4159) used in preceding studies (NTP, 2013a,b). A representative proton NMR spectrum is presented in Figure F1.

The study laboratory assessed the purity of the bulk chemical by proton and carbon-13 NMR spectroscopy and high-performance liquid chromatography (HPLC). HPLC was conducted with a Waters Millennium^{32} system using photodiode array (PDA) detection at 254 nm (Waters Corporation, Milford, MA). The analytical column was a Nova-Pak® (3.9 mm × 150 mm, 4 µm particle size, and 60 Å pore size) C18 column (Waters Corporation). The mobile phase (1 mL/minute) was held at 5% acetonitrile:95% water for 5 minutes and then linearly changed to 95% acetonitrile:5% water over 20 minutes, followed by a final 5-minute hold.

No impurity resonances were detected by either proton or carbon-13 NMR analyses other than those associated with the solvent. HPLC-PDA detected no impurities with an overall purity of approximately 100%. The overall purity of lot F60731 was determined to be at least 99.9%.

3TC

Lot B10250 of the chemical, a white-to-off-white crystalline solid, was identified as 3TC by the study laboratory using proton NMR spectroscopy, DEP/EI-MS, and LC-MS. All spectra were consistent with the structure of 3TC and/or the spectra of a 3TC sample obtained from GlaxoWellcome (Research Triangle Park, NC). A representative proton NMR spectrum is presented in Figure F2.

The study laboratory assessed the purity of the bulk chemical by proton NMR spectroscopy and the same HPLC-PDA system used to estimate the purity of lot F60731 of AZT.

Total impurity was estimated at 0.5% by proton NMR spectroscopy. HPLC-PDA detected one impurity with a peak area of 1.1% of the total peak area and estimated a purity of approximately 98.9%. The overall purity of lot B10250 was estimated to be approximately 99%.

NVP

Lot FX1009 of the chemical, a white-to-off-white crystalline powder, was identified as NVP by the study laboratory using proton NMR spectroscopy, DEP/EI-MS, gas chromatography/electron ionization MS, and LC-MS. All spectra were consistent with the structure of NVP, literature spectra, and/or the spectra of an NVP sample obtained from Boehringer/Ingelheim (Ridgefield, CT). A representative proton NMR spectrum is presented in Figure F3.

Karl Fischer titration and elemental analyses of lot FX1009 were performed by Galbraith Laboratories, Inc., and the study laboratory assessed the purity of the bulk chemical by proton NMR spectroscopy and the same HPLC-PDA system used to estimate the purity of lot F60731 of AZT.

For lot FX1009, Karl Fischer titration indicated less than 0.14% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for NVP. Total impurity was estimated at 0.2% by proton NMR. HPLC-PDA detected a single peak, indicating that the test article was 100.0% pure. The overall purity of lot FX1009 was estimated to be at least 99.5%.

Dosing Vehicle

The vehicle used for dose formulations in this study was a 0.2% methylcellulose/0.1% Tween® 80 aqueous solution. This vehicle was selected based upon preliminary experiments to find a vehicle that gave homogeneous formulations of the individual test articles and the test article combinations. Methylcellulose was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in two lots (084K0065 and 125K0055) and Tween® 80 was obtained from the same source in three lots (073K00643, 064K00631 and 125K01921). Proton and carbon-13 NMR analyses of methylcellulose lot 084K0065 were performed by the study laboratory, and spectra were consistent with those obtained previously for a methylcellulose standard obtained from Fischer Scientific (Fair Lawn, NJ).

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by admixing the test chemicals with an aqueous solution of 0.2% methylcellulose/0.1% Tween® 80 to give the required concentrations (Table F1). AZT and 3TC formed true solutions at the concentrations used, whereas NVP was insoluble and formed stable suspensions. The dose formulations were stored (with constant stirring) in sealed amber glass bottles at room temperature for up to 21 days.

Homogeneity and stability studies of 0.05 and 0.20 mg/mL formulations of AZT in the methylcellulose/Tween® 80 vehicle and of high- and low-concentration mixtures of the three test chemicals in the dosing vehicle were conducted by the study laboratory using HPLC. For these analyses, the same Waters HPLC-PDA system was used as for the bulk chemical purity determinations except that the solvent program was a 3-minute linear gradient from 100% mobile phase A (methanol:water, 5:95; 0.005 M sodium phosphate monobasic, 0.003 M sodium pentanesulfonic acid; pH 2.5) to 100% mobile phase B (methanol:water, 90:10; 0.005 M sodium phosphate monobasic, 0.003 M sodium pentanesulfonic acid; pH 2.5) followed by a 10.5-minute hold. The high-concentration mixture was composed of AZT (20 mg/mL), 3TC (10 mg/mL), and NVP (13.3 mg/mL), and the low-concentration mixture was composed of AZT (6.66 mg/mL), 3TC (3.33 mg/mL), and NVP (4.4 mg/mL). Homogeneity was confirmed, and stability was confirmed for up to 29 days for the AZT formulations and for up to 21 days for the three-component formulations stored at room temperature in clear glass vials sealed with Teflon® lined silicone rubber septa, that were stirred constantly.

At fifteen time points, analyses of the dose formulations of the antiretroviral drugs were conducted by the study laboratory using HPLC-PDA by the system described above for the homogeneity and stability studies. Of the 161 measured concentrations of test chemical, 139 were within 10% of the target concentration; all preparations that were accepted for use were within 15% of their target concentrations (Table F2).

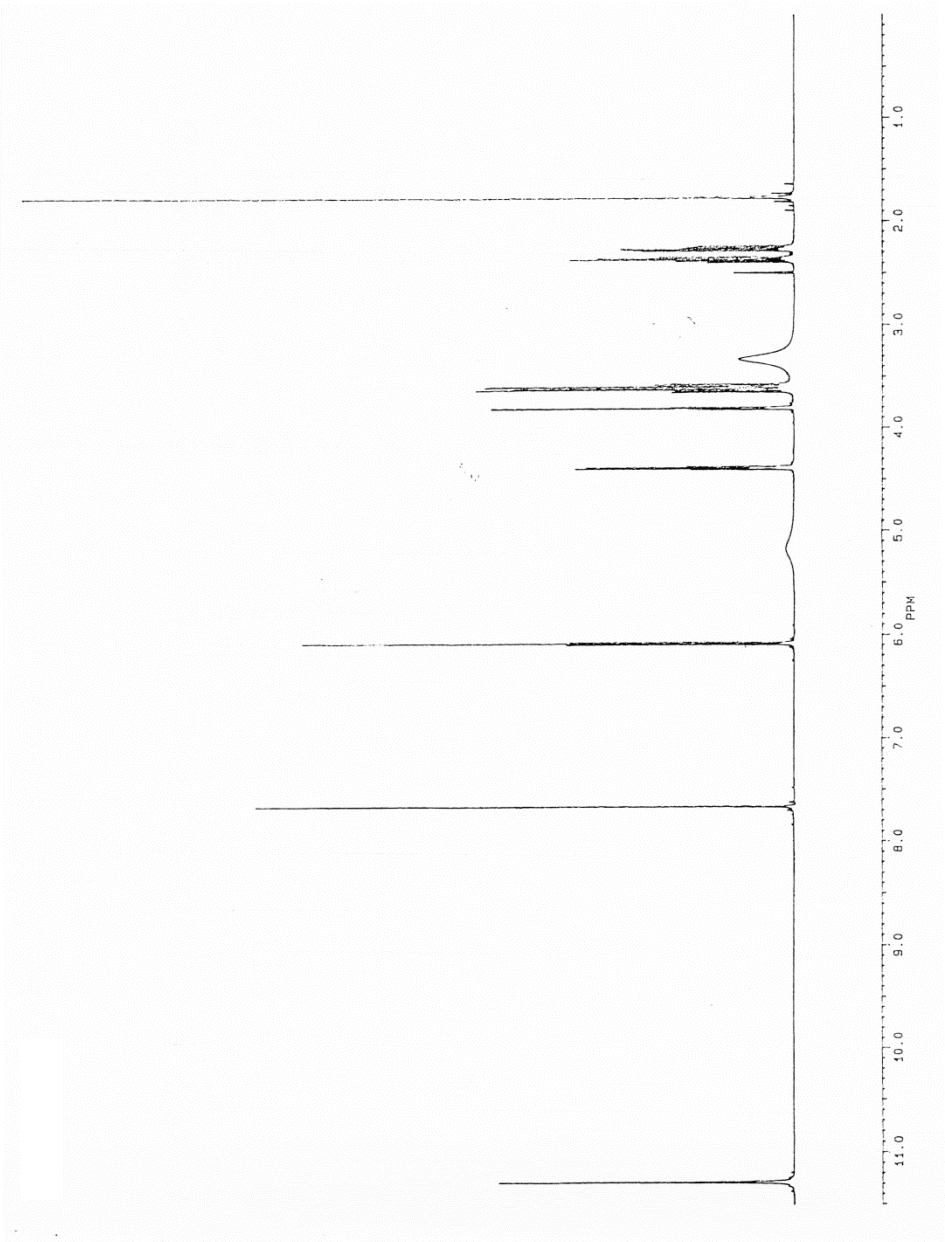

FIGURE F1
Proton Nuclear Magnetic Resonance Spectrum of AZT

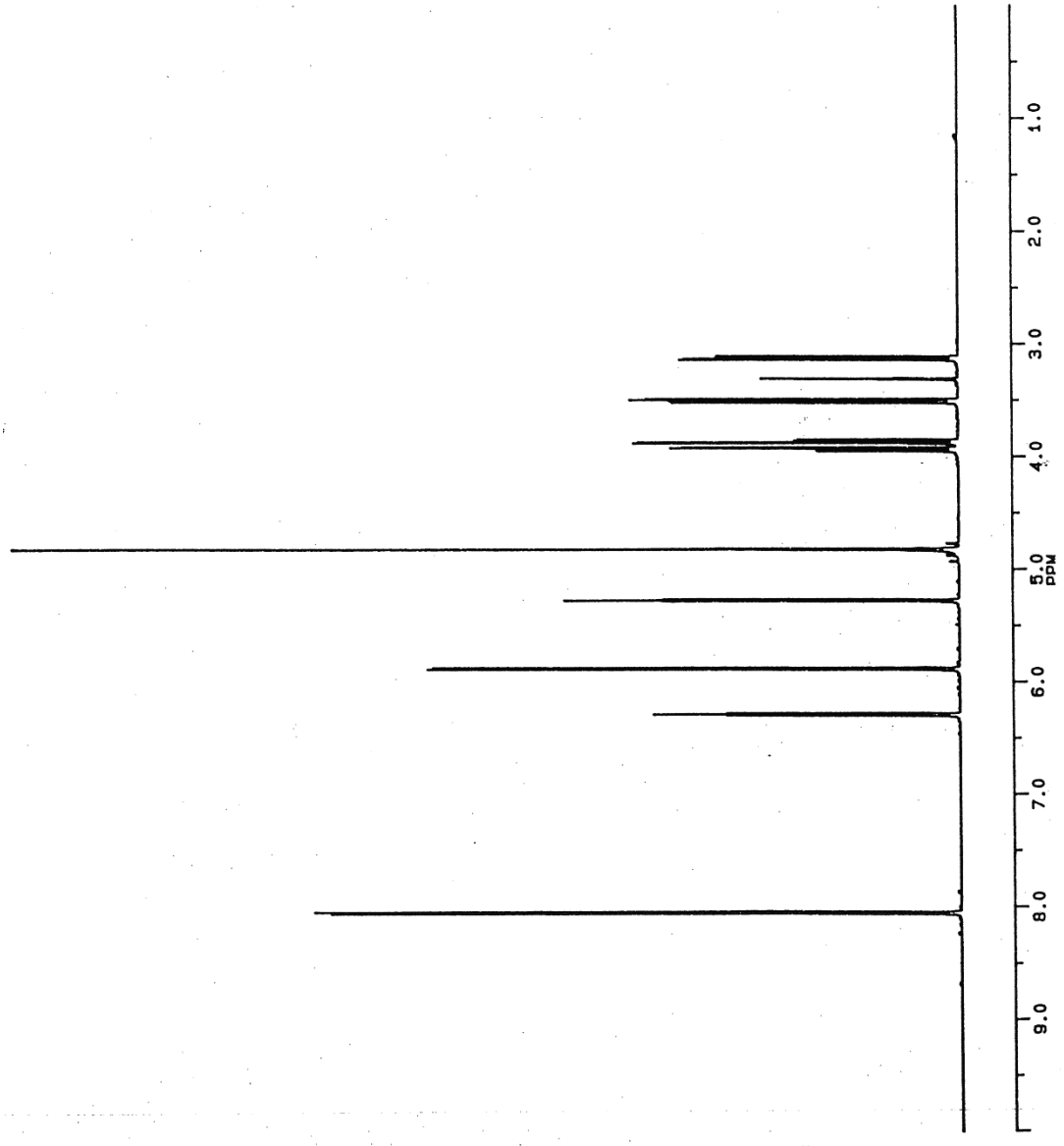

FIGURE F2
Proton Nuclear Magnetic Resonance Spectrum of 3TC

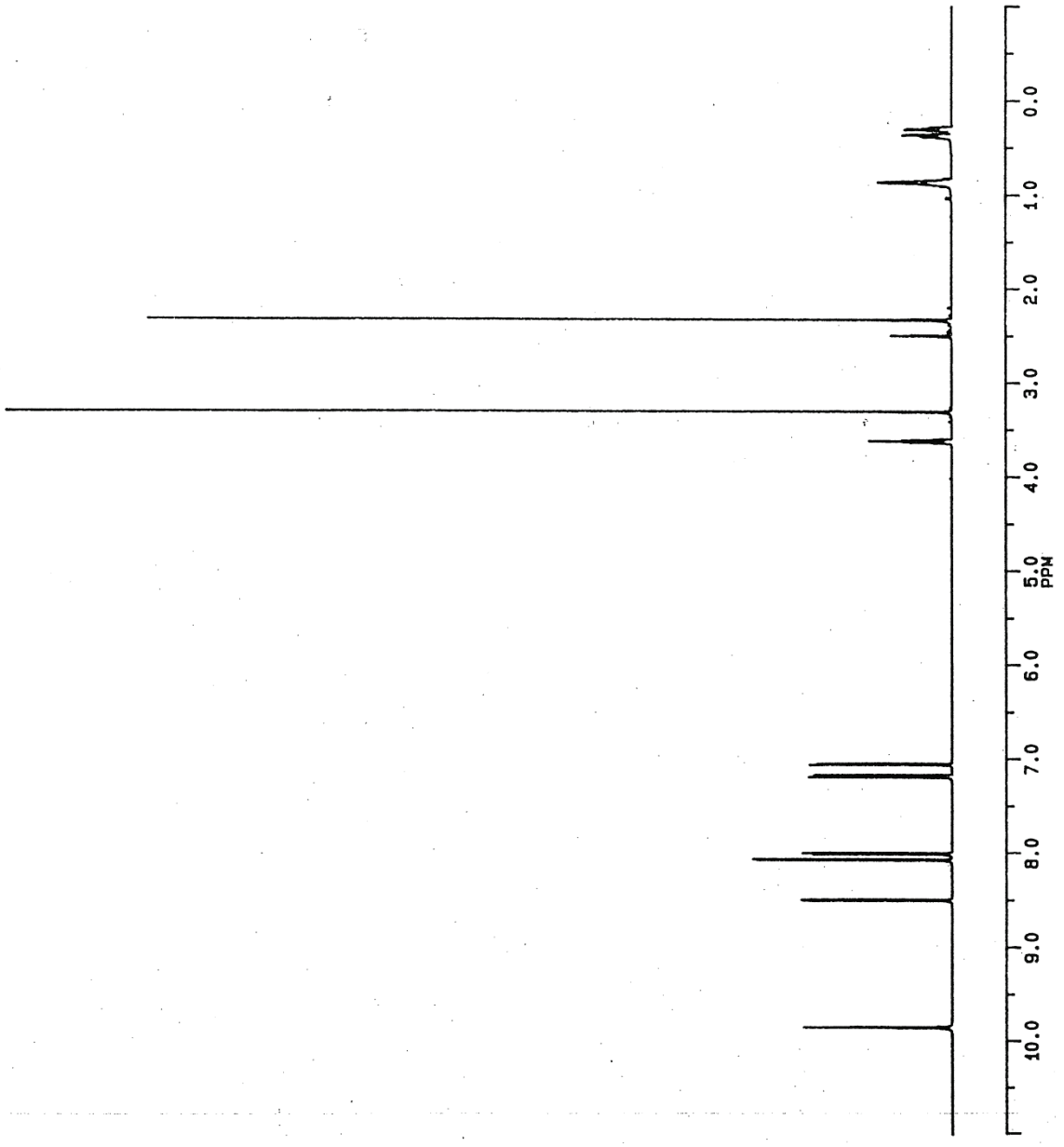

FIGURE F3
Proton Nuclear Magnetic Resonance Spectrum of NVP

TABLE F1
Preparation and Storage of Dose Formulations in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Preparation
Weighed amounts of the test chemicals were homogenized into an even suspension in 80% of the required volume of an aqueous solution of 0.2% methylcellulose and 0.1% Tween® 80 that was then completely transferred into a volumetric flask and adjusted to the required final volume. The dose formulations were prepared every 2 weeks or as required.

Chemical Lot Numbers
AZT, F60731
3TC, B10250
NVP, FX1009

Maximum Storage Time
21 days

Storage Conditions
Stored (with constant stirring) in sealed amber glass bottles at room temperature

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
AZT					
	December 27, 2006		12	11.6 ± 0.1	–3
	January 4, 2007		20	18.7 ± 0.3	–7
	May 3, 2007		12	11.0 ± 0.3	–8
			20	18.2 ± 0.1	–9
	July 12, 2007		12	12.5 ± 0.3	+4
3TC					
	December 27, 2006		7.5	7.05 ± 0.04	–6
	January 4, 2007		12.5	11.4 ± 0.1	–9
	May 3, 2007		7.5	6.86 ± 0.03	–9
	May 4, 2007		12.5	11.7 ± 0.0	–6
	July 12, 2007		7.5	7.44 ± 0.24	–1
	July 13, 2007		12.5	12.5 ± 0.6	0
NVP					
	December 19, 2006		8.4	7.62 ± 0.12	–9
	January 4, 2007		3.5	3.70 ± 0.35	+6
			14	14.9 ± 0.1	+6
	May 10, 2007		8.4	7.63 ± 0.17	–9
			3.5	3.47 ± 0.03	–1
			14	12.7 ± 0.2	–9
	July 12, 2007		8.4	8.37 ± 0.38	0
	July 13, 2007		3.5	3.86 ± 0.01	+10
			14	14.4 ± 0.3	+3
	October 29, 2007		8.4	8.47 ± 0.36	+1
			14	14.2 ± 0.7	+1
AZT and 3TC					
	December 27, 2006				
		AZT	12	11.7 ± 0.1	–3
		3TC	7.5	7.09 ± 0.05	–5
	January 4, 2007				
		AZT	20	18.8 ± 0.1	–6
		3TC	12.5	11.4 ± 0.1	–9
	May 3, 2007				
		AZT	12	11.3 ± 0.0	–6
		3TC	7.5	6.77 ± 0.05	–10

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT and 3TC (continued)					
	May 4, 2007				
		AZT	20	18.4 ± 0.1	–8
		3TC	12.5	11.2 ± 0.0	–10
	July 12, 2007				
		AZT	12	12.0 ± 0.9	0
		3TC	7.5	7.40 ± 0.55	–1
	July 13, 2007				
		AZT	20	21.1 ± 1.0	+6
		3TC	12.5	13.4 ± 0.7	+7
AZT, 3TC, and NVP					
	December 19, 2006				
		AZT	4	4.03 ± 0.04	+1
		3TC	2.5	2.52 ± 0.01	+1
		NVP	2.8	2.67 ± 0.02	–5
	December 19, 2006				
		AZT	8	7.90 ± 0.15	–1
		3TC	5	4.63 ± 0.08	–7
		NVP	5.6	5.18 ± 0.10	–8
	December 19, 2006				
		AZT	12	11.1 ± 0. 1	–8
		3TC	7.5	6.79 ± 0.04	–9
		NVP	8.4	8.04 ± 0.02	–4
	January 4, 2007				
		AZT	6.67	6.62 ± 0.13	–1
		3TC	4.17	3.94 ± 0.06	–6
		NVP	1.17	1.09 ± 0.32[b]	–7
	January 4, 2007				
		AZT	6.67	6.74 ± 0.10	+1
		3TC	4.17	3.95 ± 0.05	–5
		NVP	4.67	4.72 ± 1.58[b]	+1
	January 4, 2007				
		AZT	13.33	12.5 ± 0.1	–6
		3TC	8.33	7.94 ± 0.12	–5
		NVP	2.33	2.28 ± 0.36	–2

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT, 3TC, and NVP (continued)					
	January 4, 2007				
		AZT	13.33	12.7 ± 0.1	–5
		3TC	8.33	7.97 ± 0.10	–4
		NVP	9.33	8.96 ± 0.34	–4
	January 4, 2007				
		AZT	20	18.6 ± 0.1	–7
		3TC	12.5	11.4 ± 0.1	–9
		NVP	3.5	3.31 ± 0.17	–5
	January 4, 2007				
		AZT	20	18.7 ± 0.4	–7
		3TC	12.5	11.6 ± 0.2	–7
		NVP	14	11.8 ± 0.3[c]	–16
	January 10, 2007				
		AZT	20	19.0 ± 0.1	–5
		3TC	12.5	11.7 ± 0.3	–6
		NVP	14	13.9 ± 0.2	–1
	May 10, 2007				
		AZT	4	4.03 ± 0.02	+1
		3TC	2.5	2.41 ± 0.03	–4
		NVP	2.8	2.69 ± 0.02	–4
	May 10, 2007				
		AZT	8	7.74 ± 0.10	–3
		3TC	5	4.57 ± 0.06	–9
		NVP	5.6	5.53 ± 0.03	–1
	May 10, 2007				
		AZT	12	10.7 ± 0.0	–11
		3TC	7.5	6.87 ± 0.05	–8
		NVP	8.4	7.93 ± 0.09	–6
	May 10, 2007				
		AZT	6.67	6.66 ± 0.12	0
		3TC	4.17	3.79 ± 0.04	–9
		NVP	1.17	1.20 ± 0.01	+3

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT, 3TC, and NVP (continued)					
	May 10, 2007				
		AZT	6.67	6.54 ± 0.04	–2
		3TC	4.17	3.88 ± 0.04	–7
		NVP	4.67	4.32 ± 0.04	–7
	May 10, 2007				
		AZT	13.33	12.5 ± 0.1	–6
		3TC	8.33	7.69 ± 0.05	–8
		NVP	2.33	2.38 ± 0.01	+2
	May 10, 2007				
		AZT	13.33	12.7 ± 0.5	–5
		3TC	8.33	7.74 ± 0.30	–7
		NVP	9.33	8.36 ± 1.1	–10
	May 10, 2007				
		AZT	20	18.4 ± 0.4	–8
		3TC	12.5	11.3 ± 0.2	–10
		NVP	3.5	3.49 ± 0.07	0
	May 10, 2007				
		AZT	20	19.1 ± 0.1	–5
		3TC	12.5	11.0 ± 0.0	–12
		NVP	14	13.0 ± 0.1	–7
	June 21, 2007				
		AZT	4	3.93 ± 0.09	–2
		3TC	2.5	2.42 ± 0.05	–3
		NVP	2.8	2.59 ± 0.06	–8
	June 21, 2007				
		AZT	12	10.5 ± 0.7	–13
		3TC	7.5	6.34 ± 0.43	–15
		NVP	8.4	7.14 ± 0.03	–15
	June 21, 2007				
		AZT	6.67	5.98 ± 0.03	–10
		3TC	4.17	3.72 ± 0.03	–11
		NVP	1.17	1.07 ± 0.0	–9

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT, 3TC, and NVP (continued)					
	June 21, 2007				
		AZT	20	17.5 ± 0.9	–13
		3TC	12.5	10.5 ± 0.6[c]	–16
		NVP	14	8.47 ± 0.15[c]	–40
	June 28, 2007				
		AZT	6.67	6.44 ± 0.04	–3
		3TC	4.17	3.73 ± 0.02	–11
		NVP	1.17	1.03 ± 0.02	–12
	June 28, 2007				
		AZT	12	11.0 ± 0.0	–8
		3TC	7.5	6.73 ± 0.01	–10
		NVP	8.4	7.00 ± 0.01[c]	–17
	June 28, 2007				
		AZT	20	18.0 ± 0.4	–10
		3TC	12.5	10.7 ± 0.2	–14
		NVP	14	12.1 ± 1.8	–14
	July 5, 2007				
		AZT	6.67	6.26 ± 0.15	–6
		3TC	4.17	3.72 ± 0.11	–11
		NVP	1.17	0.77 ± 0.05[c]	–34
	July 5, 2007				
		AZT	12	10.9 ± 0.0	–9
		3TC	7.5	6.65 ± 0.03	–11
		NVP	8.4	8.12 ± 0.12	–3
	July 5, 2007				
		AZT	20	18.8 ± 0.2	–6
		3TC	12.5	11.3 ± 0.2	–10
		NVP	14	12.3 ± 0.3	–12
	July 12, 2007				
		AZT	4	4.31 ± 0.22	+8
		3TC	2.5	2.57 ± 0.12	+3
		NVP	2.8	2.71 ± 0.05	–3

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT, 3TC, and NVP (continued)					
	July 12, 2007				
		AZT	8	8.14 ± 0.23	+2
		3TC	5	5.01 ± 0.20	0
		NVP	5.6	5.44 ± 0.13	–3
	July 12, 2007				
		AZT	12	12.2 ± 0.3	+2
		3TC	7.5	7.56 ± 0.24	+1
		NVP	8.4	8.32 ± 0.09	–1
	July 13, 2007				
		AZT	6.67	7.33 ± 0.05	+10
		3TC	4.17	4.59 ± 0.04	+10
		NVP	1.17	1.33 ± 0.06	+14
	July 13, 2007				
		AZT	6.67	7.25 ± 0.12	+9
		3TC	4.17	4.60 ± 0.10	+10
		NVP	4.67	4.79 ± 0.31	+3
	July 13, 2007				
		AZT	13.33	14.4 ± 0.1	+8
		3TC	8.33	8.96 ± 0.05	+8
		NVP	2.33	2.74 ± 0.13[c]	+18
	July 13, 2007				
		AZT	13.33	14.6 ± 0.1	+10
		3TC	8.33	9.00 ± 0.02	+8
		NVP	9.33	9.87 ± 0.27	+6
	July 13, 2007				
		AZT	20	21.0 ± 0.1	+5
		3TC	12.5	13.1 ± 0.2	+5
		NVP	3.5	4.06 ± 0.18	+16
	July 13, 2007				
		AZT	20	20.8 ± 0.2	+4
		3TC	12.5	13.0 ± 0.1	+4
		NVP	14	14.9 ± 0.4	+6

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT, 3TC, and NVP (continued)					
	September 27, 2007				
		AZT	4	4.40 ± 0.15[d]	+10
		3TC	2.5	2.73 ± 0.00[d]	+9
		NVP	2.8	2.95 ± 0.19[d]	+5
	September 27, 2007				
		AZT	6.67	7.09 ± 0.27	+6
		3TC	4.17	4.27 ± 0.14	+2
		NVP	1.17	1.18 ± 0.04	+1
	September 27, 2007				
		AZT	12	12.7 ± 0.6	+6
		3TC	7.5	7.54 ± 0.22	+1
		NVP	8.4	8.95 ± 0.24	+7
	September 27, 2007				
		AZT	20	21.0 ± 0.8	+5
		3TC	12.5	12.8 ± 0.3	+2
		NVP	14	15.2 ± 0.4	+9
	November 20, 2007				
		AZT	12	11.9 ± 0.4	–1
		3TC	7.5	7.40 ± 0.09	–1
		NVP	8.4	8.71 ± 0.20	+4

[a] Results of triplicate analyses (mean ± standard deviation). For dams (gestational days 12 to 18) and pups [postnatal days (PNDs) 11 to 28], dosing volume=10 mL/kg twice daily; 2.5 mg/mL=50 mg/kg, 2.8 mg/mL=56 mg/kg, 4 mg/mL=80 mg/kg, 5 mg/mL=100 mg/kg, 5.6 mg/mL=112 mg/kg, 7.5 mg/mL=150 mg/kg, 8 mg/mL=160 mg/kg, 8.4 mg/mL=168 mg/kg, 12 mg/mL=240 mg/kg. For pups (PNDs 1 to 10), dosing volume=3 mL/kg twice daily; 1.17 mg/mL=7 mg/kg, 2.33 mg/mL=14 mg/kg, 3.5 mg/mL=21 mg/kg, 4.17 mg/mL=25 mg/kg, 4.67 mg/mL=28 mg/kg, 6.67 mg/mL=40 mg/kg, 8.33 mg/mL=50 mg/kg, 9.33 mg/mL=56 mg/kg, 12.5 mg/mL=75 mg/kg, 13.33 mg/mL=80 mg/kg, 14 mg/mL=84 mg/kg, 20 mg/mL=120 mg/kg.
[b] n=6
[c] Dose preparation was out of specification and not used for study
[d] n=2

APPENDIX G
LITTER EFFECTS ON SURVIVAL, BODY WEIGHT, AND PATHOLOGY

LITTER EFFECTS ON SURVIVAL, BODY WEIGHT, AND PATHOLOGY

BACKGROUND

The study design consisted of eight dose groups which were compared in two evaluations: (1) the combination dose comparison compared the vehicle control group to the different dose level group of the AZT/3TC/NVP-L, AZT/3TC/NVP-M, and AZT/3TC/NVP-H groups; and (2) the high dose comparison compared the vehicle control group with the AZT-H, 3TC-H, NVP-H, AZT/3TC-H and AZT/3TC/NVP-H groups. There were 25 heterozygous F1 $p53^{+/-}$ mice per sex initially assigned to each dose group.

Female C3H/HeNTac wild-type mice were mated with male homozygous, p53-null C57BL/6(N12)*Trp53*$^{(-/-)}$ mice in order to produce heterozygous F1 $p53^{+/-}$ offspring. Each male was mated consecutively with up to eight females. The pregnant females were randomized to the treatment groups and their heterozygous F1 $p53^{+/-}$ pups were bred into the study. The mice were dosed by gavage. The pregnant females were dosed daily from gestational day (GD) 12 through GD 18. On postnatal day (PND) 1, heterozygous F1 $p53^{+/-}$ mice were culled to six pups (three males and three females, where possible) and postnatal dosing was initiated. The pups were dosed with half the adult dose in half the dosing volume until PND 10, then the full adult volume and dose until PND 28. In addition, the NVP component of the dose was further reduced fourfold for doses administered on PNDs 1 to 3 because of neonatal toxicity considerations. A full description of the doses is given in Table 1 of the main Materials and Methods section of this Report.

The heterozygous F1 $p53^{+/-}$ mice were weaned on PND 21 and assigned to either stay on study or to be culled on PND 28 for genotoxicity evaluation (see Appendix B). Either one or two mice of each sex from the same litter were assigned to each study. The litter and sire assignments of all pups in the study are shown in Tables G1 and G2. The primary experimental unit in this study was the heterozygous F1 $p53^{+/-}$ pup. However, because the male C57BL/6(N12)*Trp53*$^{(-/-)}$ mice used in these studies sired multiple litters and more than one pup per litter was loaded into the studies, two potential clustering factors existed: common litter origin and common sire. While all pups within a litter received the same treatment (i.e., litter implies treatment group), pups from the same sire were not restricted to the same litter (i.e. sire does not imply treatment group).

Additional analyses were performed as outlined below to determine whether these clustering factors had any significant influence on the outcomes of the survival, body weight, and pathology evaluations.

METHODS

Survival

A proportional hazards model (Cox, 1972) was run by Sex and Group using Days-On-Study, the censor flag, and AZT dose level as the predictor. For this study, a linear trend estimate was formed using contrasts.

The proportional hazards assumption was checked using the method of Lin *et al.* (1993). The litter or sire effect clustering was adjusted for by using the aggregated sandwich estimate of variance (Lin and Wei, 1989; Binder, 1992). Ties were handled by Efron's (1977) method.

All tests of interest were single degree-of-freedom tests and were transformed into Estimate ± Standard Error and given a Z-score testing against zero and a single-sided P value following the NTP convention of appending an "**N**" for negative dose effects.

The combination dose comparison compared the vehicle control group to the different dose level group of the AZT/3TC/NVP-L, AZT/3TC/NVP-M, and AZT/3TC/NVP-H groups and the high dose comparison compared the vehicle control group with the AZT-H, 3TC-H, NVP-H, AZT/3TC-H, and AZT/3TC/NVP-H groups.

Body Weight

The basic body weight analysis typically consisted of a repeated-measures mixed model predicting body weight based on a fixed treatment group effect and age (Appendix D). This study required the addition of a random litter effect to account for correlation among littermates. Furthermore, since the correlation can be negative within litters, the mixed model should specify the litter effect in the "R" matrix rather than the "Z" matrix. In this study, pups share the dam and so littermates (implicitly dam-mates) are one possible correlation. However, multiple litters can share a common sire and so sire-mates are another possible correlation. Therefore, three correlation models were run: unadjusted (assumes pup independence), litter-adjusted (assumes correlation among dam/littermates but independence among litters), and sire-adjusted (assumes pups from different sires are independent). The combination of the heteroscedastic variance over time coupled with the litter correlation makes a repeated-measures model difficult. Therefore, since the time course is not really of great concern, a basic mixed model was run by time point. Dunnett's adjustment was used to compare to vehicle controls.

The combination dose comparison compared the vehicle control group to the different dose level group of the AZT/3TC/NVP-L, AZT/3TC/NVP-M, and AZT/3TC/NVP-H dose groups. The levels were referred to as low, medium, and high since the levels of all components in the combination were raised proportionally. Combined with vehicle, this formed a standard control+3 design and is referred to below as the Dose Level sub-design or the Trio Dose Level sub-design. The high dose comparison compared the vehicle control group with the AZT-H, 3TC-H, NVP-H, AZT/3TC-H, and AZT/3TC/NVP-H dose groups.

The body weight data for each animal were rasterized to evenly-spaced time points (every week) via LOESS (Locally Weighted Scatterplot Smoothing) scoring (Cleveland, 1979; Cleveland *et al.,* 1988). This process reduces the number of time points for the mixed-effects model, reduces the effects of outliers, and creates a grid of regularly spaced time points. The scored data were then treated as primary data for the mixed effects model in order to correct for possible correlation among littermates and this study was long enough to demonstrate variance heterogeneity over time. Therefore, a choice was necessary because of the difficulty in simultaneously adjusting for litters and for variance heterogeneity. The analysis opted to keep litter adjustments and to estimate the model at each time point. These models were run separately for each sex. The model treated body weight as a function of treatment group. Observations within each litter (dam- or sire-determined) were presumed to be correlated at a given time point. Since separate analyses were performed at each time point, the variance was allowed to change.

In order to better summarize the results, a two-stage analysis was also conducted for each Sex × Study Duration combination on two aspects of the growth curve: early growth rate (slope between 2 to 5 weeks for all studies) and late growth rate (slope between 38 to 44 weeks for 45-week studies). These analyses determined whether there was any treatment-related effects on early growth or late growth.

Dunnett's (1955) method was used to compare dose levels to control within each drug combination at each age. A polynomial contrast was used to test for linear trend with dose at each age. Contrasts were used to compare drug combinations within dose levels at each age.

Pathology

For neoplastic lesions, the Poly-k method of Bailer and Portier (1988) as modified by Bieler and Williams (1993) and NIEHS (continuity-correction) was used to analyze age-adjusted incidences. Bieler and Williams (1993), in the derivation of their variance correction, used the fact that the Cochran-Armitage test can be envisioned as a binomial-weighted regression. If we begin with a weighted regression paradigm with binomial weights, we can generalize this framework and view the Cochran-Armitage test as a generalized linear model with binomial variation and an identity link function. If this analysis is performed with the Poly-k weights, then the resulting analysis can be used with more complex designs, including litter correlations and factorial effects as well as alternative link functions.

Correlation among littermates (litter-adjusted) was achieved by using the generalized linear model described above with estimation using generalized estimating equations (Liang and Zeger, 1986) and an exchangeable correlation among littermates. Sire-adjusted analyses were generated in the same manner differing only in the specification of the correlation group variable.

It should be noted that the implementation details of this method are different from the Bieler and Williams variance-adjusted Poly-k test (Bieler and Williams, 1993). Particularly, the variance is not quantal-adjusted and all

comparisons are estimated within a single analysis of variance model rather than multiple regression models. Suitable contrasts were used to test the relevant hypotheses. One-sided results were generated and, per NTP custom, an "**N**" was suffixed to indicate negative trends. Since the variance structure is group specific, rather than estimated from the null hypothesis, uniform treatment groups were dealt with by adding an uncorrelated dummy lesion observation to all groups (if necessary for any group) with value=0.005 and Poly-3 weight=0.005.

The presented results include the usual unadjusted Bieler-and-Williams quantal-adjusted Poly-3, Poly-3 weighted binomial/identity-link GLIM with litter-adjusted GEE correlation, and Poly-3 weighted binomial/identity-link GLIM with sire-adjusted GEE correlation.

For nonneoplastic lesions, the Poly-k method was used with k=3 to analyze age-adjusted incidence and the non-zero severity score averages were computed. The cluster-adjusted model was used here, also. In addition, to incorporate lesion severity scores, the distribution-free (but unadjusted for age) method of Jonckheere (1954) and Terpstra (1952) was used to compute monotonic trend tests, and the method of Shirley (1977) as modified by Williams (1986) was used to compute comparisons to controls. No attempt was made to adjust JT/SW for correlation among clusters.

RESULTS

Survival

There are mildly significant negative results when comparing the low and medium doses of AZT/3TC/NVP to the vehicle control group in males. In females, the unadjusted standard analysis indicates no significant differences but the use of the empirical variance probably understates the variability due to the uniform vehicle group and finds significant positive results when comparing all dose levels to the vehicle control group. As above, a single event would destroy the significance. Cluster analysis for either litter or sire effects did not alter the empirical variance or its significance (Table G3). Likewise, cluster analysis for either litter or sire effects did not alter the empirical variance or its significance for the high dose comparison.

Body Weight

The unadjusted body weight Tables are shown in Appendix D. The two adjusted analyses were generally identical to the unadjusted analysis.

Pathology

The following results refer to the litter-adjusted analysis unless otherwise stated. The only nonsystemic neoplasm found within male subjects in sufficient quantity to justify analysis was liver hepatocellular adenoma. Hepatocellular carcinomas were also found in males (although in insufficient numbers to justify its own analysis). Therefore, the hepatocellular adenoma or carcinoma pool was also analyzed. The only neoplastic lesion found within female subjects in sufficient quantity to justify analysis was malignant lymphoma, a systemic lesion.

Neoplasms

Major neoplasms occurring in individual heterozygous F1 $p53^{+/-}$ mice in the study (hepatocellular tumors in males and lymphomas and sarcomas in females) are listed in Tables G1 and G2. In both males and females, incidences of lesions did not appear to cluster with common litters or common sires. Conversely, there was some evidence that lesions were negatively associated with common litters or sires, which resulted in increased statistical significance when common litter or common sire was used as a covariant in the Poly-3 evaluations of incidences. For example, the incidence of hepatocellular adenoma was increased in the male AZT/3TC/NVP-L group relative to the vehicle control group with a Poly-3 significance that increased from 0.03 to 0.005 or <0.001 when the evaluation was adjusted for dam or sire, respectively (Table G4). In males, adjusting for dam resulted in significant differences in the incidences of malignant lymphoma between the 3TC-H (0/25) and NVP-H (0/26) dose groups and the AZT-H (3/24) and AZT/3TC-H (3/25) dose groups. These differences did not reach statistical significance in the naive evaluation (Table G5).

The male AZT/3TC/NVP-H dose group provides an example where the negative association of hepatic lesions with common litters can be easily visualized. In this group, there are 12 mice out of 24 that expressed a liver lesion (hepatocellular adenoma, hepatocellular carcinoma, or foci) and 12 that did not. Half the mice (12) were derived as

pairs from six litters, whereas the other 12 mice represented individual litters. The lesions were distributed between the two groups so that five lesions were expressed in the six pairs of mice that were derived from common litters. A totally neutral effect of litter clustering would predict that the liver lesions would be distributed evenly across the litters so that there would be a similar number of litters with both pups expressing either a lesion or no lesion, and those where one pup expressed a liver lesion and the other did not. Clustering would produce a greater proportion of litters exhibiting either a lesion present in both pups or absence of lesions from both pups. However, as shown in Table G1, five of six litters had only one of the two pups exhibiting a liver lesion and one litter has neither pup exhibiting a lesion.

A similar negative association of hepatic lesions with common sire is also apparent in the AZT/3TC/NVP-H group where only one sire contributed to more than one litter. Of the three F1 $p53^{+/-}$ mice sharing this common sire, two exhibited a liver lesion and one did not.

In females, where only low rates of neoplasm incidences were observed, adjusting for litter or sire in the combination dose comparison did not change the statistical significance of the incidences of any neoplastic lesion in any dose group relative to the vehicle controls (Table G6), but the incidence of mammary gland adenocarcinoma did become significantly greater in the 3TC-H group (3/25) than those in the AZT-H (0/25) and NVP-H (0/23) groups when adjusted for litter (Table G7).

Nonneoplastic Lesions

In general, exposure to the AZT/3TC/NVP drug combinations or their high dose components significantly changed the incidence or severity of several nonneoplastic endpoints, when analyzed by naive Poly-3 tests not adjusting for litter or sire correlation. Because in most cases nonneoplastic lesions were not clustered in litters, adjusting for either litter or sire increased the number of significant lesions.

For the drug combinations in males, these changes included incidences of hepatic basophilic foci and inflammation, adrenal hypoplasia, nasal hyaline droplets, and renal hydronephrosis, where adjusting for litter or sire clustering produced statistically significant ($P<0.05$) increases in individual dose comparisons or in dose trends that had not reached statistical significance in the evaluation that did not adjust for litter or sire clustering effects; and incidences of bone marrow hyperplasia, pancreatic hyperplasia, and thymic atrophy, where adjusting for litter or sire clustering produced statistically significant ($P<0.05$) decreases in individual dose comparisons or in dose trends that had not reached statistical significance in the evaluation that did not adjust for litter or sire clustering effects (Table G8). Similar changes in statistical significance were observed in the high dose comparison evaluations (Table G9).

For the drug combinations in females, these changes included hepatic centrilobular degeneration and cellular infiltration, pancreatic cellular infiltration, infiltration of hemopoietic cells into the spleen, and renal hydronephrosis, where adjusting for litter or sire clustering produced statistically significant ($P<0.05$) increases in individual dose comparisons or in dose trends that had not reached statistical significance in the evaluation that did not adjust for litter or sire clustering effects; incidences of cellular infiltration of the salivary glands, where adjusting for litter or sire clustering produced statistically significant ($P<0.05$) decreases in individual dose comparisons or in dose trends that had not reached statistical significance in the evaluation that did not adjust for litter or sire clustering effects; and incidences of nasal hyaline droplets and cellular infiltration of the lung, where adjusting for litter or sire clustering eliminated statistically significant ($P<0.05$) increases in individual dose comparisons or in dose trends that were observed in the evaluation that did not adjust for litter or sire clustering effects (Table G10). Similar changes in statistical significance were observed in the high dose comparison evaluations (Table G11).

CONCLUSIONS

The use of common sires and more than one pup per litter to populate dose groups did not confound the sensitivity of these studies to detect neoplastic lesions. There appeared to be a slightly negative rather than positive correlation between littermates and pathological endpoints. This suggests that in these mice, which are derived from inbred parent strains, the epigenetic effects of intrauterine position override genetic differences between litters.

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment[a]	F1 Mouse UIN[b]	Dam UIN	Sire UIN[c]	Litter UIN	Lesion[d]	CID[e]
Vehicle control						
	7G000002304	7F000000444	7E000000057	7G000002303		138
	7G000002303	7F000000444	7E000000057	7G000002303		86
	7G000002025	7F000000369	7E000000060	7G000002022		6
	7G000002024	7F000000369	7E000000060	7G000002022		28
	7G000002050	7F000000382	7E000000061	7G000002048		51
	7G000002168	7F000000401	7E000000062	7G000002162		74
	7G000002082	7F000000389	7E000000065	7G000002079		12
	7G000002084	7F000000389	7E000000065	7G000002079		52
	7G000002417	7F000000434	7E000000067	7G000002413		189
	7G000002418	7F000000434	7E000000067	7G000002413		190
	7G000002349	7F000000455	7E000000068	7G000002349		172
	7G000002227	7F000000381	7E000000070	7G000002226		97
	7G000002228	7F000000381	7E000000070	7G000002226		43
	7G000002909	7F000000529	7E000000072	7G000002906		340
	7G000002474	7F000000428	7E000000075	7G000002471		208
	7G000002475	7F000000428	7E000000075	7G000002471		209
	7G000002551	7F000000467	7E000000076	7G000002550		239
	7G000003077	7F000000546	7E000000078	7G000003074		350
	7G000003415	7F000000495	7E000000079	7G000003413		382
	7G000003401	7F000000592	7E000000081	7G000003400		360
	7G000002869	7F000000446	7E000000082	7G000002865		307
	7G000002870	7F000000446	7E000000082	7G000002865	L	297
	7G000002786	7F000000486	7E000000082	7G000002780		308
	7G000003068	7F000000555	7E000000083	7G000003066		351
	7G000003493	7F000000585	7E000000087	7G000003485		401
Mice per group	**25**			**Number with lesions**	**1**	**=4.0%**
AZT-H						
	7G000002186	7F000000417	7E000000057	7G000002185		10
	7G000002187	7F000000417	7E000000057	7G000002185		102
	7G000001986	7F000000367	7E000000058	7G000001983	L	33
	7G000001987	7F000000367	7E000000058	7G000001983		34
	7G000002319	7F000000422	7E000000060	7G000002318		154
	7G000002318	7F000000422	7E000000060	7G000002318		153
	7G000002492	7F000000380	7E000000061	7G000002488		206
	7G000002032	7F000000385	7E000000063	7G000002028		16
	7G000002091	7F000000371	7E000000066	7G000002088		60
	7G000002090	7F000000371	7E000000066	7G000002088	L	59
	7G000002777	7F000000516	7E000000068	7G000002773		198
	7G000002423	7F000000427	7E000000069	7G000002422		184
	7G000002424	7F000000427	7E000000069	7G000002422		188
	7G000002571	7F000000497	7E000000071	7G000002569		237
	7G000002239	7F000000405	7E000000072	7G000002233	L	104
	7G000002238	7F000000405	7E000000072	7G000002233		103
	7G000002808	7F000000521	7E000000074	7G000002806	L	303
	7G000002482	7F000000458	7E000000076	7G000002480		88
	7G000003501	7F000000562	7E000000080	7G000003495		221
	7G000002946	7F000000475	7E000000082	7G000002944	L	345
	7G000002932	7F000000484	7E000000083	7G000002925	L	329

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
AZT-H (continued)						
	7G000003006	7F000000522	7E000000085	7G000003004	L	344
	7G000003353	7F000000599	7E000000086	7G000003348	L	363
	7G000003362	7F000000574	7E000000093	7G000003357		26
Mice per group	**24**			**Number with lesions**	**8**	**=33.3%**
3TC-H						
	7G000002451	7F000000462	7E000000057	7G000002451		199
	7G000002452	7F000000462	7E000000057	7G000002451		200
	7G000002764	7F000000395	7E000000058	7G000002763		275
	7G000002763	7F000000395	7E000000058	7G000002763	L	274
	7G000002098	7F000000370	7E000000059	7G000002096		8
	7G000002099	7F000000370	7E000000059	7G000002096		71
	7G000001991	7F000000402	7E000000063	7G000001991		24
	7G000002671	7F000000503	7E000000066	7G000002669	L	262
	7G000002670	7F000000503	7E000000066	7G000002669		261
	7G000002264	7F000000368	7E000000067	7G000002260		92
	7G000002263	7F000000368	7E000000067	7G000002260		91
	7G000002655	7F000000505	7E000000068	7G000002653		260
	7G000002654	7F000000505	7E000000068	7G000002653		259
	7G000002193	7F000000379	7E000000071	7G000002191	L	90
	7G000002374	7F000000421	7E000000075	7G000002370		163
	7G000002373	7F000000421	7E000000075	7G000002370		162
	7G000002894	7F000000498	7E000000079	7G000002891	L	333
	7G000002939	7F000000452	7E000000081	7G000002936		335
	7G000002938	7F000000452	7E000000081	7G000002936		334
	7G000002948	7F000000404	7E000000082	7G000002948		332
	7G000003549	7F000000564	7E000000086	7G000003545		355
	7G000003548	7F000000564	7E000000086	7G000003545		406
	7G000003449	7F000000583	7E000000087	7G000003447		396
	7G000003423	7F000000577	7E000000089	7G000003421		395
	7G000003372	7F000000578	7E000000095	7G000003366		377
Mice per group	**25**			**Number with lesions**	**4**	**=16.0%**
NVP-H						
	7G000002323	7F000000420	7E000000059	7G000002323		157
	7G000002829	7F000000514	7E000000060	7G000002823		310
	7G000002179	7F000000425	7E000000061	7G000002179	L	107
	7G000002180	7F000000425	7E000000061	7G000002179	L	108
	7G000002733	7F000000453	7E000000062	7G000002729		277
	7G000002732	7F000000453	7E000000062	7G000002729		276
	7G000002114	7F000000376	7E000000063	7G000002110		80
	7G000002115	7F000000376	7E000000063	7G000002110		15
	7G000001997	7F000000373	7E000000064	7G000001996		36
	7G000002039	7F000000413	7E000000065	7G000002034		64
	7G000002038	7F000000413	7E000000065	7G000002034	L	63
	7G000002390	7F000000435	7E000000067	7G000002388		173
	7G000002391	7F000000435	7E000000067	7G000002388		174

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
NVP-H (continued)						
	7G000002875	7F000000515	7E000000068	7G000002874		21
	7G000002645	7F000000511	7E000000071	7G000002643		263
	7G000002295	7F000000441	7E000000072	7G000002294		142
	7G000002747	7F000000509	7E000000073	7G000002747		2
	7G000002439	7F000000451	7E000000075	7G000002434		213
	7G000002438	7F000000451	7E000000075	7G000002434		212
	7G000002196	7F000000388	7E000000076	7G000002196		109
	7G000002197	7F000000388	7E000000076	7G000002196		19
	7G000003408	7F000000551	7E000000083	7G000003406		387
	7G000003409	7F000000551	7E000000083	7G000003406		388
	7G000002795	7F000000478	7E000000085	7G000002788		309
	7G000003337	7F000000561	7E000000088	7G000003335		370
	7G000003338	7F000000561	7E000000088	7G000003335		371
Mice per group	**26**			**Number with lesions**	**3**	**=11.5%**
AZT/3TC-H						
	7G000002204	7F000000416	7E000000057	7G000002201		134
	7G000002205	7F000000416	7E000000057	7G000002201		135
	7G000002682	7F000000501	7E000000060	7G000002682		265
	7G000002683	7F000000501	7E000000060	7G000002682	L	266
	7G000002269	7F000000424	7E000000061	7G000002267		149
	7G000002270	7F000000424	7E000000061	7G000002267		150
	7G000002834	7F000000525	7E000000062	7G000002831	L	318
	7G000002005	7F000000374	7E000000065	7G000002001		42
	7G000002004	7F000000374	7E000000065	7G000002001	L	41
	7G000002055	7F000000390	7E000000066	7G000002055	L	68
	7G000002802	7F000000517	7E000000067	7G000002799		11
	7G000002335	7F000000415	7E000000068	7G000002335	L	151
	7G000002336	7F000000415	7E000000068	7G000002335		152
	7G000002758	7F000000400	7E000000069	7G000002758	L	271
	7G000002382	7F000000412	7E000000070	7G000002379	L	177
	7G000002383	7F000000412	7E000000070	7G000002379		178
	7G000002701	7F000000480	7E000000075	7G000002699	L	281
	7G000002700	7F000000480	7E000000075	7G000002699	L	280
	7G000002744	7F000000496	7E000000075	7G000002738		282
	7G000003057	7F000000553	7E000000082	7G000003052		354
	7G000002915	7F000000460	7E000000085	7G000002913	L	69
	7G000003329	7F000000567	7E000000089	7G000003326		85
	7G000003330	7F000000567	7E000000089	7G000003326	L	379
	7G000003481	7F000000597	7E000000092	7G000003475		380
	7G000003470	7F000000575	7E000000095	7G000003467		397
Mice per group	**25**			**Number with lesions**	**11**	**=44.0%**
AZT/3TC/NVP-L						
	7G000002010	7F000000366	7E000000057	7G000002008	L	30
	7G000002011	7F000000366	7E000000057	7G000002008		31
	7G000002820	7F000000526	7E000000057	7G000002814		311
	7G000002841	7F000000513	7E000000058	7G000002837		313
	7G000002840	7F000000513	7E000000058	7G000002837		312
	7G000002396	7F000000457	7E000000059	7G000002396		191

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
AZT/3TC/NVP-L (continued)						
	7G000002397	7F000000457	7E000000059	7G000002396	L	192
	7G000002300	7F000000449	7E000000060	7G000002297	L	139
	7G000002299	7F000000449	7E000000060	7G000002297		50
	7G000002067	7F000000407	7E000000062	7G000002062		57
	7G000002213	7F000000429	7E000000064	7G000002209		99
	7G000002212	7F000000429	7E000000064	7G000002209		98
	7G000002129	7F000000408	7E000000066	7G000002127	L	78
	7G000002128	7F000000408	7E000000066	7G000002127		77
	7G000002466	7F000000440	7E000000069	7G000002461		216
	7G000002465	7F000000440	7E000000069	7G000002461		215
	7G000002581	7F000000499	7E000000075	7G000002579	L	247
	7G000002580	7F000000499	7E000000075	7G000002579		222
	7G000002966	7F000000500	7E000000079	7G000002963	L	336
	7G000003015	7F000000539	7E000000082	7G000003012	L	337
	7G000003048	7F000000558	7E000000084	7G000003044	L	353
	7G000003460	7F000000540	7E000000088	7G000003455		391
	7G000003343	7F000000570	7E000000090	7G000003341		375
	7G000003344	7F000000570	7E000000090	7G000003341	L	376
	7G000003392	7F000000573	7E000000093	7G000003387		374
Mice per group	**25**			**Number with lesions**	**9**	**=36.0%**
AZT/3TC/NVP-M						
	7G000002147	7F000000397	7E000000059	7G000002143	L	83
	7G000002148	7F000000397	7E000000059	7G000002143		84
	7G000002280	7F000000423	7E000000060	7G000002278		130
	7G000002281	7F000000423	7E000000060	7G000002278	L	131
	7G000002694	7F000000450	7E000000062	7G000002688		288
	7G000002693	7F000000450	7E000000062	7G000002688	L	287
	7G000002314	7F000000431	7E000000065	7G000002314		146
	7G000002248	7F000000432	7E000000066	7G000002243	L	127
	7G000002247	7F000000432	7E000000066	7G000002243		126
	7G000002604	7F000000488	7E000000068	7G000002604		249
	7G000002501	7F000000410	7E000000069	7G000002497	L	218
	7G000002500	7F000000410	7E000000069	7G000002497	L	205
	7G000002537	7F000000465	7E000000070	7G000002537		233
	7G000002538	7F000000465	7E000000070	7G000002537	L	234
	7G000002409	7F000000459	7E000000071	7G000002408		194
	7G000002408	7F000000459	7E000000071	7G000002408		193
	7G000002254	7F000000409	7E000000073	7G000002252		128
	7G000002255	7F000000409	7E000000073	7G000002252	L	129
	7G000002999	7F000000538	7E000000079	7G000002996		347
	7G000002857	7F000000375	7E000000080	7G000002855		320
	7G000002856	7F000000375	7E000000080	7G000002855		300
	7G000003431	7F000000584	7E000000087	7G000003431	L	385
	7G000003463	7F000000590	7E000000096	7G000003463	L	386
Mice per group	**23**			**Number with lesions**	**10**	**=43.5%**

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
AZT/3TC/NVP-H						
	7G000002344	7F000000384	7E000000057	7G000002342	L	168
	7G000002343	7F000000384	7E000000057	7G000002342		167
	7G000002273	7F000000419	7E000000058	7G000002273		114
	7G000002521	7F000000483	7E000000060	7G000002521		223
	7G000002403	7F000000443	7E000000061	7G000002402		187
	7G000002402	7F000000443	7E000000061	7G000002402	L	186
	7G000002564	7F000000477	7E000000062	7G000002562		243
	7G000002565	7F000000477	7E000000062	7G000002562	L	244
	7G000002526	7F000000476	7E000000063	7G000002524	L	232
	7G000002073	7F000000414	7E000000066	7G000002071	L	66
	7G000002577	7F000000481	7E000000066	7G000002576	L	246
	7G000002576	7F000000481	7E000000066	7G000002576		245
	7G000002220	7F000000399	7E000000068	7G000002218		115
	7G000002221	7F000000399	7E000000068	7G000002218	L	116
	7G000002888	7F000000520	7E000000069	7G000002887	L	319
	7G000002310	7F000000445	7E000000071	7G000002308	L	144
	7G000002557	7F000000494	7E000000073	7G000002556		4
	7G000002556	7F000000494	7E000000073	7G000002556		242
	7G000002518	7F000000490	7E000000074	7G000002517		220
	7G000002534	7F000000468	7E000000076	7G000002531	L	231
	7G000002921	7F000000528	7E000000077	7G000002918		346
	7G000003525	7F000000589	7E000000085	7G000003521	L	405
	7G000003308	7F000000571	7E000000092	7G000003307		367
	7G000003557	7F000000600	7E000000093	7G000003553	L	407
Mice per group	**24**			**Number with lesions**	**12**	**=50.0%**

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; AZT/3TC/NVP-M = 160/100/112 mg/kg AZT/3TC/NVP; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] UIN = unique identification number

[c] Adjacent cells within a column with the same shading share the same sire or litter.

[d] L = hepatocellular adenoma, hepatocellular carcinoma or basophilic foci

[e] CID = carcass identification number for pathological evaluation

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment[a]	F1 Mouse UIN[b]	Dam UIN	Sire UIN[c]	Litter UIN	Lesion[d]	CID[e]
Vehicle control						
	7G000002306	7F000000444	7E000000057	7G000002303	Ma	155
	7G000002307	7F000000444	7E000000057	7G000002303		156
	7G000002027	7F000000369	7E000000060	7G000002022		29
	7G000002054	7F000000382	7E000000061	7G000002048		54
	7G000002053	7F000000382	7E000000061	7G000002048		53
	7G000002169	7F000000401	7E000000062	7G000002162		75
	7G000002170	7F000000401	7E000000062	7G000002162		76
	7G000002086	7F000000389	7E000000065	7G000002079		55
	7G000002087	7F000000389	7E000000065	7G000002079	L	56
	7G000002354	7F000000455	7E000000068	7G000002349		166
	7G000002360	7F000000426	7E000000069	7G000002355		171
	7G000002232	7F000000381	7E000000070	7G000002226		137
	7G000002231	7F000000381	7E000000070	7G000002226		136
	7G000002911	7F000000529	7E000000072	7G000002906		341
	7G000002477	7F000000428	7E000000075	7G000002471		207
	7G000002552	7F000000467	7E000000076	7G000002550		238
	7G000002994	7F000000532	7E000000077	7G000002988		342
	7G000002995	7F000000532	7E000000077	7G000002988		343
	7G000003537	7F000000591	7E000000078	7G000003528		402
	7G000002873	7F000000446	7E000000082	7G000002865		306
	7G000002872	7F000000446	7E000000082	7G000002865	L	305
	7G000002787	7F000000486	7E000000082	7G000002780		304
	7G000003539	7F000000595	7E000000090	7G000003538		403
	7G000003297	7F000000586	7E000000091	7G000003293		362
	7G000003281	7F000000580	7E000000096	7G000003278		361
Mice per group	**25**			**Number with lesions**	**3**	**=12.0%**
AZT-H						
	7G000002190	7F000000417	7E000000057	7G000002185		105
	7G000001988	7F000000367	7E000000058	7G000001983	Mc	22
	7G000001989	7F000000367	7E000000058	7G000001983		35
	7G000002494	7F000000380	7E000000061	7G000002488		202
	7G000002495	7F000000380	7E000000061	7G000002488	L	158
	7G000002640	7F000000487	7E000000062	7G000002637		257
	7G000002642	7F000000487	7E000000062	7G000002637		254
	7G000002093	7F000000371	7E000000066	7G000002088		61
	7G000002094	7F000000371	7E000000066	7G000002088		62
	7G000002426	7F000000427	7E000000069	7G000002422		185
	7G000002573	7F000000497	7E000000071	7G000002569		227
	7G000002572	7F000000497	7E000000071	7G000002569	L	159
	7G000002242	7F000000405	7E000000072	7G000002233		106
	7G000002813	7F000000521	7E000000074	7G000002806		301
	7G000002486	7F000000458	7E000000076	7G000002480		203
	7G000002487	7F000000458	7E000000076	7G000002480		201
	7G000002853	7F000000461	7E000000079	7G000002845		302
	7G000003502	7F000000562	7E000000080	7G000003495		404
	7G000002947	7F000000475	7E000000082	7G000002944		299
	7G000002934	7F000000484	7E000000083	7G000002925		327
	7G000003011	7F000000522	7E000000085	7G000003004		328
	7G000003351	7F000000599	7E000000086	7G000003348		364
	7G000003288	7F000000563	7E000000087	7G000003283		356

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
AZT-H (continued)						
	7G000003361	7F000000574	7E000000093	7G000003357		365
	7G000003565	7F000000541	7E000000094	7G000003561		399
Mice per Group	**25**			**Number with lesions**	**3**	**=12.0%**
3TC-H						
	7G000002765	7F000000395	7E000000058	7G000002763		252
	7G000002102	7F000000370	7E000000059	7G000002096	Ma	72
	7G000002103	7F000000370	7E000000059	7G000002096		73
	7G000002430	7F000000430	7E000000062	7G000002428		211
	7G000002429	7F000000430	7E000000062	7G000002428		210
	7G000001995	7F000000402	7E000000063	7G000001991		27
	7G000001994	7F000000402	7E000000063	7G000001991		25
	7G000002674	7F000000503	7E000000066	7G000002669		256
	7G000002675	7F000000503	7E000000066	7G000002669		258
	7G000002265	7F000000368	7E000000067	7G000002260		95
	7G000002266	7F000000368	7E000000067	7G000002260		96
	7G000002657	7F000000505	7E000000068	7G000002653	Ma	255
	7G000002658	7F000000505	7E000000068	7G000002653	Mc	160
	7G000002194	7F000000379	7E000000071	7G000002191		93
	7G000002195	7F000000379	7E000000071	7G000002191		94
	7G000002377	7F000000421	7E000000075	7G000002370		165
	7G000002376	7F000000421	7E000000075	7G000002370		164
	7G000002895	7F000000498	7E000000079	7G000002891		331
	7G000002941	7F000000452	7E000000081	7G000002936	Ma	269
	7G000002953	7F000000404	7E000000082	7G000002948		294
	7G000003552	7F000000564	7E000000086	7G000003545	L	357
	7G000003454	7F000000583	7E000000087	7G000003447		392
	7G000003424	7F000000577	7E000000089	7G000003421		393
	7G000003425	7F000000577	7E000000089	7G000003421		394
	7G000003370	7F000000578	7E000000095	7G000003366		359
Mice per group	**25**			**Number with lesions**	**5**	**=20.0%**
NVP-H						
	7G000002706	7F000000447	7E000000057	7G000002705		278
	7G000002707	7F000000447	7E000000057	7G000002705		279
	7G000002326	7F000000420	7E000000059	7G000002323	S	23
	7G000002327	7F000000420	7E000000059	7G000002323		70
	7G000002830	7F000000514	7E000000060	7G000002823		295
	7G000002184	7F000000425	7E000000061	7G000002179		111
	7G000002183	7F000000425	7E000000061	7G000002179		110
	7G000002737	7F000000453	7E000000062	7G000002729		291
	7G000002118	7F000000376	7E000000063	7G000002110		81
	7G000001999	7F000000373	7E000000064	7G000001996		37
	7G000002000	7F000000373	7E000000064	7G000001996	Lu	17
	7G000002042	7F000000413	7E000000065	7G000002034	L	65
	7G000002447	7F000000391	7E000000066	7G000002443	L	204
	7G000002448	7F000000391	7E000000066	7G000002443	L	44
	7G000002394	7F000000435	7E000000067	7G000002388		175
	7G000002395	7F000000435	7E000000067	7G000002388		176

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
NVP-H (continued)						
	7G000002647	7F000000511	7E000000071	7G000002643		264
	7G000002296	7F000000441	7E000000072	7G000002294		143
	7G000002752	7F000000509	7E000000073	7G000002747		293
	7G000002751	7F000000509	7E000000073	7G000002747	L	292
	7G000002440	7F000000451	7E000000075	7G000002434	L	214
	7G000002200	7F000000388	7E000000076	7G000002196		113
	7G000002199	7F000000388	7E000000076	7G000002196		112
	7G000003339	7F000000561	7E000000088	7G000003335		368
	7G000003340	7F000000561	7E000000088	7G000003335		369
Mice per group	**25**			**Number with lesions**	**7**	**=28.0%**
AZT/3TC-H						
	7G000002206	7F000000416	7E000000057	7G000002201		132
	7G000002207	7F000000416	7E000000057	7G000002201	Ma	133
	7G000002687	7F000000501	7E000000060	7G000002682		268
	7G000002272	7F000000424	7E000000061	7G000002267		148
	7G000002271	7F000000424	7E000000061	7G000002267	S	147
	7G000002007	7F000000374	7E000000065	7G000002001		40
	7G000002059	7F000000390	7E000000066	7G000002055	L	18
	7G000002060	7F000000390	7E000000066	7G000002055		67
	7G000002804	7F000000517	7E000000067	7G000002799		321
	7G000002339	7F000000415	7E000000068	7G000002335	L	3
	7G000002340	7F000000415	7E000000068	7G000002335	Ma	49
	7G000002762	7F000000400	7E000000069	7G000002758		286
	7G000002761	7F000000400	7E000000069	7G000002758		285
	7G000002386	7F000000412	7E000000070	7G000002379		179
	7G000002387	7F000000412	7E000000070	7G000002379		180
	7G000002666	7F000000512	7E000000071	7G000002659		267
	7G000002703	7F000000480	7E000000075	7G000002699		283
	7G000002704	7F000000480	7E000000075	7G000002699		284
	7G000002746	7F000000496	7E000000075	7G000002738	L	9
	7G000002745	7F000000496	7E000000075	7G000002738	L	161
	7G000002652	7F000000489	7E000000076	7G000002649		253
	7G000002917	7F000000460	7E000000085	7G000002913		348
	7G000003570	7F000000508	7E000000087	7G000003567		410
	7G000003333	7F000000567	7E000000089	7G000003326		378
Mice per group	**24**			**Number with lesions**	**7**	**=29.2%**
AZT/3TC/NVP-L						
	7G000002012	7F000000366	7E000000057	7G000002008		32
	7G000002822	7F000000526	7E000000057	7G000002814		315
	7G000002821	7F000000526	7E000000057	7G000002814		314
	7G000002843	7F000000513	7E000000058	7G000002837		316
	7G000002844	7F000000513	7E000000058	7G000002837		317
	7G000002399	7F000000457	7E000000059	7G000002396		183
	7G000002398	7F000000457	7E000000059	7G000002396		182
	7G000002301	7F000000449	7E000000060	7G000002297		140
	7G000002302	7F000000449	7E000000060	7G000002297		141
	7G000002070	7F000000407	7E000000062	7G000002062	L	7

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
AZT/3TC/NVP-L (continued)						
	7G000002069	7F000000407	7E000000062	7G000002062		58
	7G000002216	7F000000429	7E000000064	7G000002209		100
	7G000002217	7F000000429	7E000000064	7G000002209		101
	7G000002132	7F000000408	7E000000066	7G000002127		79
	7G000002470	7F000000440	7E000000069	7G000002461		217
	7G000002469	7F000000440	7E000000069	7G000002461		45
	7G000002585	7F000000499	7E000000075	7G000002579		248
	7G000002584	7F000000499	7E000000075	7G000002579	L	181
	7G000002971	7F000000500	7E000000079	7G000002963		339
	7G000003016	7F000000539	7E000000082	7G000003012		338
	7G000003050	7F000000558	7E000000084	7G000003044		352
	7G000003461	7F000000540	7E000000088	7G000003455		389
	7G000003462	7F000000540	7E000000088	7G000003455		390
	7G000003345	7F000000570	7E000000090	7G000003341		372
	7G000003346	7F000000570	7E000000090	7G000003341		373
Mice per Group	**25**			**Number with lesions**	**2**	**=8.0%**
AZT/3TC/NVP-M						
	7G000002014	7F000000394	7E000000058	7G000002013	S	38
	7G000002015	7F000000394	7E000000058	7G000002013	L	39
	7G000002150	7F000000397	7E000000059	7G000002143	L	20
	7G000002151	7F000000397	7E000000059	7G000002143		82
	7G000002259	7F000000409	7E000000073	7G000002252		123
	7G000002257	7F000000409	7E000000073	7G000002252		122
	7G000002505	7F000000410	7E000000069	7G000002497	L	5
	7G000002504	7F000000410	7E000000069	7G000002497		219
	7G000002285	7F000000423	7E000000060	7G000002278		125
	7G000002284	7F000000423	7E000000060	7G000002278		124
	7G000002251	7F000000432	7E000000066	7G000002243		121
	7G000002250	7F000000432	7E000000066	7G000002243		120
	7G000002698	7F000000450	7E000000062	7G000002688		290
	7G000002697	7F000000450	7E000000062	7G000002688		289
	7G000002412	7F000000459	7E000000071	7G000002408		195
	7G000002540	7F000000465	7E000000070	7G000002537		235
	7G000002541	7F000000465	7E000000070	7G000002537		236
	7G000002606	7F000000488	7E000000068	7G000002604		251
	7G000002605	7F000000488	7E000000068	7G000002604		250
	7G000003435	7F000000584	7E000000087	7G000003431		383
	7G000003465	7F000000590	7E000000096	7G000003463	L	87
	7G000003466	7F000000590	7E000000096	7G000003463		384
Mice per group	**22**			**Number with lesions**	**5**	**=22.7%**

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP

Treatment	F1 Mouse UIN	Dam UIN	Sire UIN	Litter UIN	Lesion	CID
AZT/3TC/NVP-H						
	7G000002347	7F000000384	7E000000057	7G000002342		169
	7G000002348	7F000000384	7E000000057	7G000002342		170
	7G000002277	7F000000419	7E000000058	7G000002273		119
	7G000002523	7F000000483	7E000000060	7G000002521	L	225
	7G000002522	7F000000483	7E000000060	7G000002521		224
	7G000002406	7F000000443	7E000000061	7G000002402		196
	7G000002407	7F000000443	7E000000061	7G000002402		197
	7G000002529	7F000000476	7E000000063	7G000002524	L	230
	7G000002528	7F000000476	7E000000063	7G000002524		229
	7G000002578	7F000000481	7E000000066	7G000002576		240
	7G000002224	7F000000399	7E000000068	7G000002218		117
	7G000002225	7F000000399	7E000000068	7G000002218		118
	7G000002633	7F000000466	7E000000070	7G000002629		241
	7G000002312	7F000000445	7E000000071	7G000002308		145
	7G000002757	7F000000463	7E000000072	7G000002753		273
	7G000002756	7F000000463	7E000000072	7G000002753		272
	7G000002520	7F000000490	7E000000074	7G000002517		228
	7G000002548	7F000000473	7E000000075	7G000002543	S	89
	7G000002547	7F000000473	7E000000075	7G000002543		226
	7G000002924	7F000000528	7E000000077	7G000002918		330
	7G000003519	7F000000587	7E000000080	7G000003513	L,Mc	381
	7G000003316	7F000000593	7E000000082	7G000003313		366
	7G000003309	7F000000571	7E000000092	7G000003307	L	298
	7G000003310	7F000000571	7E000000092	7G000003307		358
	7G000003573	7F000000566	7E000000095	7G000003571		400
Mice per group	**25**			**Number with lesions**	**5**	**=20.0%**

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; AZT/3TC/NVP-M = 160/100/112 mg/kg AZT/3TC/NVP; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] UIN = unique identification number

[c] Adjacent cells within a column with the same shading share the same sire or litter.

[d] L = malignant lymphoma, Lu = granulocytic leukemia, , Ma = mammary gland adenocarcinoma, Mc = mammary gland adenoacanthoma, S = histocytic sarcoma

[e] CID = carcass identification number for pathological evaluation

TABLE G3
Effects of Combination Doses on the Survival of Heterozygous $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Studies of AZT, 3TC, and NVP[a]

	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
Male				
Linear trend	–2.1	—	—	—
Relative hazard[b]	—	16%	16%	49%
Unadjusted standard	0.206**N**	0.043**N***	0.047**N***	0.157**N**
Unadjusted empirical	0.203**N**	0.041**N***	0.039**N***	0.158**N**
Litter-adjusted empirical	0.185**N**	0.034**N***	0.032**N***	0.135**N**
Sire-adjusted empirical	0.201**N**	0.023**N***	0.035**N***	0.138**N**
Female				
Linear trend	6.5	—	—	—
Relative hazard	—	675%	776%	820%
Unadjusted standard	0.078	0.104	0.088	0.079
Unadjusted empirical	0.030*	0.046*	0.037*	0.030*
Litter-adjusted empirical	0.036*	0.037*	0.032*	0.035*
Sire-adjusted empirical	0.033*	0.040*	0.033*	0.033*

* $P \leq 0.05$

[a] P values are presented unadjusted for litter clusters using the standard model-based variance estimate, unadjusted for litter clusters using the empirical variance estimate, litter-adjusted where the empirical variance is aggregated over litters, and sire-adjusted where the empirical variance is aggregated over sires. Significant negative trends or changes in hazard comparisons are appended with **N**.

[b] The relative hazard represents the treatment group hazard relative to vehicle controls. Vehicle control group results represent linear dose trend in the hazards and not the relative hazard estimate, which is implicitly 100% for this group.

TABLE G4
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Bone: Osteosarcoma				
Overall rate[b]	3/25 (12.0%)	0/25 (0.0%)	0/23 (0.0%)	1/24 (4.2%)
Adjusted rate[c]	3/23.9 (12.6%)	0/24.8 (0.0%)	0/22.9 (0.0%)	1/22.9 (4.4%)
Terminal rate[d]	1/20 (5.0%)	0/24 (0.0%)	0/22 (0.0%)	1/22 (4.5%)
First incidence (days)	207	—[f]	—	326 (T)
Poly-3 test[e]	0.137**N**	0.107**N**	0.121**N**	0.319**N**
Litter-adjusted correlation	–0.12			
Litter-adjusted Poly-3 rate	13.3%	0.0%	0.0%	4.7%
Litter-adjusted Poly-3 test	0.129**N**	0.017**N***	0.017**N***	0.129**N**
Sire-adjusted correlation	–0.03			
Sire-adjusted Poly-3 rate	12.7%	0.0%	0.0%	4.3%
Sire-adjusted Poly-3 test	0.133**N**	0.019**N***	0.019**N***	0.132**N**
Liver: Hepatocellular Adenoma				
Overall rate	1/25 (4.0%)	7/25 (28.0%)	7/23 (30.4%)	9/23 (39.1%)
Adjusted rate	1/22.9 (4.4%)	7/24.8 (28.3%)	7/22.9 (30.5%)	9/22.8 (39.4%)
Terminal rate	0/20 (0.0%)	7/24 (29.2%)	7/22 (31.8%)	9/22 (40.9%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	0.006**	0.030*	0.021*	0.003**
Litter-adjusted correlation	–0.30			
Litter-adjusted Poly-3 rate	5.1%	28.1%	29.9%	36.4%
Litter-adjusted Poly-3 test	0.003 **	0.005 **	0.004 **	0.002 **
Sire-adjusted correlation	0.02			
Sire-adjusted Poly-3 rate	4.3%	28.1%	30.6%	39.7%
Sire-adjusted Poly-3 test	0.001**	<0.001 ***	0.002 **	<0.001 ***
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	1/25 (4.0%)	9/25 (36.0%)	8/23 (34.8%)	10/23 (43.5%)
Adjusted rate	1/22.9 (4.4%)	9/24.8 (36.4%)	8/22.9 (34.9%)	10/22.8 (43.8%)
Terminal rate	0/20 (0.0%)	9/24 (37.5%)	8/22 (36.4%)	10/22 (45.5%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	0.004**	0.006**	0.009**	0.001**
Litter-adjusted correlation	–0.25			
Litter-adjusted Poly-3 rate	5.0%	35.7%	34.6%	42.0%
Litter-adjusted Poly-3 test	<0.001***	<0.001***	0.004**	<0.001***
Sire-adjusted correlation	–0.01			
Sire-adjusted Poly-3 rate	4.5%	36.5%	34.8%	43.6%
Sire-adjusted Poly-3 test	0.001**	<0.001***	0.002**	<0.001***
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	0/25 (0.0%)	0/25 (0.0%)	2/23 (8.7%)	0/23 (0.0%)
Adjusted rate	0/22.8 (0.0%)	0/24.8 (0.0%)	2/22.9 (8.7%)	0/22.8 (0.0%)
Terminal rate	0/20 (0.0%)	0/24 (0.0%)	2/22 (9.1%)	0/22 (0.0%)
First incidence (days)	—	—	318 (T)	—
Poly-3 test	0.379	—[g]	0.236	—
Litter-adjusted correlation	–0.05			
Litter-adjusted Poly-3 rate	0.0%	0.0%	8.8%	0.0%
Litter-adjusted Poly-3 test	0.057	—	0.057	—
Sire-adjusted correlation	–0.01			
Sire-adjusted Poly-3 rate	0.0%	0.0%	8.8%	0.0%
Sire-adjusted Poly-3 test	0.058	—	0.058	—

TABLE G4
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
All Organs: Lymphoma Malignant				
Overall rate	1/25 (4.0%)	0/25 (0.0%)	1/23 (4.3%)	2/24 (8.3%)
Adjusted rate	1/23.6 (4.2%)	0/24.8 (0.0%)	1/22.9 (4.4%)	2/23.0 (8.7%)
Terminal rate	0/20 (0.0%)	0/24 (0.0%)	1/22 (4.5%)	1/22 (4.5%)
First incidence (days)	176	—	325 (T)	307
Poly-3 test	0.238	0.491**N**	0.754	0.491
Litter-adjusted correlation	–0.08			
Litter-adjusted Poly-3 rate	4.4%	0.0%	4.5%	8.7%
Litter-adjusted Poly-3 test	0.210	0.144**N**	0.497	0.270
Sire-adjusted correlation	–0.06			
Sire-adjusted Poly-3 rate	4.0%	0.0%	5.0%	9.2%
Sire-adjusted Poly-3 test	0.166	0.165**N**	0.433	0.231
All Organs: Malignant Neoplasms				
Overall rate	7/25 (28.0%)	4/25 (16.0%)	5/23 (21.7%)	4/24 (16.7%)
Adjusted rate	7/25.0 (28.0%)	4/25.0 (16.0%)	5/23.0 (21.7%)	4/23.0 (17.4%)
Terminal rate	2/20 (10.0%)	3/24 (12.5%)	4/22 (18.2%)	3/22 (13.6%)
First incidence (days)	176	297	318 (T)	307
Poly-3 test	0.278**N**	0.249**N**	0.435**N**	0.300**N**
Litter-adjusted correlation	–0.47			
Litter-adjusted Poly-3 rate	32.9%	14.1%	24.2%	20.6%
Litter-adjusted Poly-3 test	0.195**N**	0.020**N***	0.186**N**	0.107**N**
Sire-adjusted correlation	–0.14			
Sire-adjusted Poly-3 rate	33.7%	20.3%	28.6%	23.2%
Sire-adjusted Poly-3 test	0.245**N**	0.056**N**	0.291**N**	0.159**N**
All Organs: Osteosarcoma				
Overall rate	3/25 (12.0%)	0/25 (0.0%)	0/23 (0.0%)	1/24 (4.2%)
Adjusted rate	3/23.9 (12.6%)	0/24.8 (0.0%)	0/22.9 (0.0%)	1/22.9 (4.4%)
Terminal rate	1/20 (5.0%)	0/24 (0.0%)	0/22 (0.0%)	1/22 (4.5%)
First incidence (days)	207	—	—	326 (T)
Poly-3 test	0.137**N**	0.107**N**	0.121**N**	0.319**N**
Litter-adjusted correlation	–0.12			
Litter-adjusted Poly-3 rate	13.3%	0.0%	0.0%	4.7%
Litter-adjusted Poly-3 test	0.129**N**	0.017**N***	0.017**N***	0.129**N**
Sire-adjusted correlation	–0.03			
Sire-adjusted Poly-3 rate	12.7%	0.0%	0.0%	4.3%
Sire-adjusted Poly-3 test	0.133**N**	0.019**N***	0.019**N***	0.132**N**
All Organs: Benign Neoplasms				
Overall rate	1/25 (4.0%)	7/25 (28.0%)	11/23 (47.8%)	10/24 (41.7%)
Adjusted rate	1/22.9 (4.4%)	7/24.8 (28.3%)	11/23.0 (47.8%)	10/22.9 (43.8%)
Terminal rate	0/20 (0.0%)	7/24 (29.2%)	10/22 (45.5%)	10/22 (45.5%)
First incidence (days)	313	315 (T)	318 (T)	316 (T)
Poly-3 test	<0.001***	0.030*	<0.001***	0.001***
Litter-adjusted correlation	–0.18			
Litter-adjusted Poly-3 rate	4.8%	28.2%	48.1%	41.5%
Litter-adjusted Poly-3 test	<0.001***	0.004**	<0.001***	<0.001***
Sire-adjusted correlation	0.05			
Sire-adjusted Poly-3 rate	4.2%	27.7%	47.7%	44.4%
Sire-adjusted Poly-3 test	<0.001***	<0.001***	<0.001***	<0.001***

TABLE G4
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
All Organs: Benign or Malignant Neoplasms				
Overall rate	7/25 (28.0%)	11/25 (44.0%)	13/23 (56.5%)	14/24 (58.3%)
Adjusted rate	7/25.0 (28.0%)	11/25.0 (44.0%)	13/23.0 (56.5%)	14/23.0 (60.8%)
Terminal rate	2/20 (10.0%)	10/24 (41.7%)	12/22 (54.5%)	13/22 (59.1%)
First incidence (days)	176	297	318 (T)	307
Poly-3 test	0.009**	0.190	0.040*	0.019*
Litter-adjusted correlation	–0.49			
Litter-adjusted Poly-3 rate	33.1%	41.9%	58.5%	56.8%
Litter-adjusted Poly-3 test	0.010*	0.218	0.003**	0.024*
Sire-adjusted correlation	–0.02			
Sire-adjusted Poly-3 rate	28.3%	44.4%	57.3%	61.0%
Sire-adjusted Poly-3 test	0.003**	0.074	<0.001***	0.003**

(T) Terminal kill
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg; AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically.
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

TABLE G5
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Bone: Osteosarcoma						
Overall rate[b]	3/25 (12.0%)	0/24 (0.0%)	1/25 (4.0%)	1/26 (3.8%)	0/25 (0.0%)	1/24 (4.2%)
Adjusted rate[c]	3/23.9 (12.6%)	0/19.9 (0.0%)	1/23.6 (4.2%)	1/22.9 (4.4%)	0/22.2 (0.0%)	1/22.9 (4.4%)
Terminal rate[d]	1/20 (5.0%)	0/18 (0.0%)	1/23 (4.3%)	1/22 (4.5%)	0/21 (0.0%)	1/22 (4.5%)
First incidence (days)	207	—[f]	318 (T)	322 (T)	—	326 (T)
	Vehicle Control	0.150**N**	0.307**N**	0.319**N**	0.127**N**	0.319**N**
		AZT-H	0.534	0.528	—[g]	0.528
Poly-3 test[e]			**3TC-H**	0.753	0.512**N**	0.753
				NVP-H	0.506**N**	0.761
					AZT/3TC-H	0.506
Litter-adjusted correlation	0.10					
Litter-adjusted Poly-3 rate	13.2%	0.0%	3.9%	4.5%	0.0%	4.7%
	Vehicle Control	0.017**N***	0.104**N**	0.124**N**	0.017**N***	0.129**N**
		AZT-H	0.158	0.145	—	0.143
Litter-adjusted Poly-3 test			**3TC-H**	0.461	0.158**N**	0.452
				NVP-H	0.145**N**	0.492
					AZT/3TC-H	0.143
Sire-adjusted correlation	—[g]					
Sire-adjusted Poly-3 rate	—	—	—	—	—	—
	Vehicle Control	—	—	—	—	—
		AZT-H	—	—	—	—
Sire-adjusted Poly-3 test			**3TC-H**	—	—	—
				NVP-H	—	—
					AZT/3TC-H	—
Liver: Hepatocellular Adenoma						
Overall rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	9/25 (36.0%)	9/23 (39.1%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	3/23.6 (12.7%)	2/22.4 (8.9%)	9/22.2 (40.5%)	9/22.8 (39.4%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	3/23 (13.0%)	2/22 (9.1%)	9/21 (42.9%)	9/22 (40.9%)
First incidence (days)	313	316 (T)	318 (T)	322 (T)	316 (T)	316 (T)
	Vehicle Control	0.004**	0.313	0.493	0.003**	0.003**
		AZT-H	0.038**N***	0.017**N***	0.615	0.601**N**
Poly-3 test			**3TC-H**	0.524**N**	0.031*	0.036*
				NVP-H	0.014*	0.016*
					AZT/3TC-H	0.589**N**
Litter-adjusted correlation	0.23					
Litter-adjusted Poly-3 rate	3.9%	41.6%	13.6%	8.3%	40.3%	41.1%
	Vehicle Control	<0.001***	0.118	0.307	<0.001***	<0.001***
		AZT-H	0.013**N***	0.005**N****	0.464**N**	0.488**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.309**N**	0.015*	0.015*
				NVP-H	0.006**	0.006**
					AZT/3TC-H	0.476
Sire-adjusted correlation	0.05					
Sire-adjusted Poly-3 rate	4.0%	41.5%	12.3%	8.3%	38.6%	38.6%
	Vehicle Control	<0.001***	0.154	0.324	<0.001***	0.001**
		AZT-H	0.009**N****	0.009**N****	0.410**N**	0.420**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.355**N**	0.008**	0.016*
				NVP-H	0.014*	0.020*
					AZT/3TC-H	0.499

TABLE G5
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Liver: Hepatocellular Adenoma or Carcinoma						
Overall rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	10/25 (40.0%)	10/23 (43.5%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	3/23.6 (12.7%)	2/22.4 (8.9%)	10/22.2 (45.0%)	10/22.8 (43.8%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	3/23 (13.0%)	2/22 (9.1%)	10/21 (47.6%)	10/22 (45.5%)
First incidence (days)	313	316 (T)	318 (T)	322 (T)	316 (T)	316 (T)
	Vehicle Control	0.004**	0.313	0.493	<0.001***	0.001**
		AZT-H	0.038N*	0.017N*	0.499	0.529
Poly-3 test			**3TC-H**	0.524N	0.014*	0.017*
				NVP-H	0.006**	0.007**
					AZT/3TC-H	0.585N
Litter-adjusted correlation	0.19					
Litter-adjusted Poly-3 rate	4.0%	41.3%	13.5%	8.4%	45.3%	44.8%
	Vehicle Control	<0.001***	0.122	0.309	<0.001***	<0.001***
		AZT-H	0.013N*	0.005N**	0.390	0.404
Litter-adjusted Poly-3 test			**3TC-H**	0.319N	0.005**	0.005**
				NVP-H	0.002**	0.002**
					AZT/3TC-H	0.486N
Sire-adjusted correlation	–0.04					
Sire-adjusted Poly-3 rate	4.1%	41.1%	12.1%	8.7%	43.5%	43.5%
	Vehicle Control	<0.001***	0.160	0.313	<0.001***	<0.001***
		AZT-H	0.009N**	0.011N*	0.427	0.436
Sire-adjusted Poly-3 test			**3TC-H**	0.377N	0.002**	0.004**
				NVP-H	0.008**	0.002**
					AZT/3TC-H	0.500N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma						
Overall rate	0/25 (0.0%)	1/24 (4.2%)	2/25 (8.0%)	1/25 (4.0%)	0/24 (0.0%)	0/23 (0.0%)
Adjusted rate	0/22.8 (0.0%)	1/19.9 (5.0%)	2/24.2 (8.3%)	1/22.4 (4.5%)	0/21.4 (0.0%)	0/22.8 (0.0%)
Terminal rate	0/20 (0.0%)	1/18 (5.6%)	1/23 (4.3%)	1/22 (4.5%)	0/21 (0.0%)	0/22 (0.0%)
First incidence (days)	—	319 (T)	250	320 (T)	—	—
	Vehicle Control	0.473	0.248	0.497	—	—
		AZT-H	0.568	0.734N	0.486N	0.473N
Poly-3 test			**3TC-H**	0.526N	0.263N	0.248N
				NVP-H	0.510N	0.497N
					AZT/3TC-H	—
Litter-adjusted correlation	–0.05					
Litter-adjusted Poly-3 rate	0.0%	4.9%	8.4%	4.6%	0.0%	0.0%
	Vehicle Control	0.156	0.057	0.145	—	—
		AZT-H	0.312	0.478N	0.156N	0.156N
Litter-adjusted Poly-3 test			**3TC-H**	0.286N	0.057N	0.057N
				NVP-H	0.145N	0.145N
					AZT/3TC-H	—
Sire-adjusted correlation	0.00					
Sire-adjusted Poly-3 rate	0.0%	5.1%	8.3%	4.5%	0.0%	0.0%
	Vehicle Control	0.155	0.057	0.145	—	—
		AZT-H	0.326	0.464N	0.155N	0.155N
Sire-adjusted Poly-3 test			**3TC-H**	0.282N	0.057N	0.057N
				NVP-H	0.145N	0.145N
					AZT/3TC-H	—

TABLE G5
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Lymphoma Malignant						
Overall rate	1/25 (4.0%)	3/24 (12.5%)	0/25 (0.0%)	0/26 (0.0%)	3/25 (12.0%)	2/24 (8.3%)
Adjusted rate	1/23.6 (4.2%)	3/22.1 (13.6%)	0/23.6 (0.0%)	0/22.9 (0.0%)	3/24.8 (12.1%)	2/23.0 (8.7%)
Terminal rate	0/20 (0.0%)	0/18 (0.0%)	0/23 (0.0%)	0/22 (0.0%)	0/21 (0.0%)	1/22 (4.5%)
First incidence (days)	176	168	—	—	99	307
	Vehicle Control	0.277	0.500**N**	0.507**N**	0.321	0.491
		AZT-H	0.101**N**	0.106**N**	0.609**N**	0.480**N**
Poly-3 test			**3TC-H**	—	0.123	0.229
				NVP-H	0.130	0.236
					AZT/3TC-H	0.535**N**
Litter-adjusted correlation	–0.04					
Litter-adjusted Poly-3 rate	4.3%	13.5%	0.0%	0.0%	11.9%	8.7%
	Vehicle Control	0.133	0.144**N**	0.144**N**	0.160	0.266
		AZT-H	0.030**N***	0.030**N***	0.436**N**	0.302**N**
Litter-adjusted Poly-3 test			**3TC-H**	—	0.032*	0.064
				NVP-H	0.032*	0.064
					AZT/3TC-H	0.355**N**
Sire-adjusted correlation	—					
Sire-adjusted Poly-3 rate	—	—	—	—	—	—
	Vehicle Control	—	—	—	—	—
		AZT-H	—	—	—	—
Sire-adjusted Poly-3 test			**3TC-H**	—	—	—
				NVP-H	—	—
					AZT/3TC-H	—
All Organs: Osteosarcoma						
Overall rate	3/25 (12.0%)	1/24 (4.2%)	2/25 (8.0%)	1/26 (3.8%)	0/25 (0.0%)	1/24 (4.2%)
Adjusted rate	3/23.9 (12.6%)	1/19.9 (5.0%)	2/23.6 (8.5%)	1/22.9 (4.4%)	0/22.2 (0.0%)	1/22.9 (4.4%)
Terminal rate	1/20 (5.0%)	1/18 (5.6%)	2/23 (8.7%)	1/22 (4.5%)	0/21 (0.0%)	1/22 (4.5%)
First incidence (days)	207	318 (T)	318 (T)	322 (T)	—	326 (T)
	Vehicle Control	0.371**N**	0.505**N**	0.319**N**	0.127**N**	0.319**N**
		AZT-H	0.560	0.730**N**	0.478**N**	0.729**N**
Poly-3 test			**3TC-H**	0.512**N**	0.249**N**	0.512**N**
				NVP-H	0.506**N**	0.761
					AZT/3TC-H	0.506
Litter-adjusted correlation	–0.11					
Litter-adjusted Poly-3 rate	13.3%	4.7%	8.3%	4.5%	0.0%	4.7%
	Vehicle Control	0.136**N**	0.274**N**	0.124**N**	0.017**N***	0.129**N**
		AZT-H	0.310	0.488**N**	0.157**N**	0.498**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.295**N**	0.067**N**	0.304**N**
				NVP-H	0.145**N**	0.490
					AZT/3TC-H	0.143
Sire-adjusted correlation	–0.04					
Sire-adjusted Poly-3 rate	12.4%	4.6%	8.1%	4.6%	0.0%	4.6%
	Vehicle Control	0.160**N**	0.306**N**	0.162**N**	0.023**N***	0.150**N**
		AZT-H	0.327	0.499**N**	0.171**N**	0.496**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.310**N**	0.074**N**	0.311**N**
				NVP-H	0.133**N**	0.496**N**
					AZT/3TC-H	0.136

TABLE G5
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Malignant Neoplasms						
Overall rate	7/25 (28.0%)	6/24 (25.0%)	5/25 (20.0%)	5/26 (19.2%)	8/25 (32.0%)	4/24 (16.7%)
Adjusted rate	7/25.0 (28.0%)	6/22.4 (26.8%)	5/24.2 (20.7%)	5/25.0 (20.0%)	8/25.0 (32.0%)	4/23.0 (17.4%)
Terminal rate	2/20 (10.0%)	2/18 (11.1%)	4/23 (17.4%)	2/22 (9.1%)	4/21 (19.0%)	3/22 (13.6%)
First incidence (days)	176	168	250	177	99	307
	Vehicle Control	0.590**N**	0.397**N**	0.372**N**	0.500	0.300**N**
		AZT-H	0.444**N**	0.419**N**	0.471	0.344**N**
Poly-3 test			**3TC-H**	0.614**N**	0.285	0.532**N**
				NVP-H	0.262	0.554**N**
					AZT/3TC-H	0.203**N**
Litter-adjusted correlation	–0.32					
Litter-adjusted Poly-3 rate	31.1%	23.0%	19.9%	20.6%	28.9%	19.4%
	Vehicle Control	0.229**N**	0.127**N**	0.136**N**	0.416**N**	0.115**N**
		AZT-H	0.395**N**	0.415**N**	0.316	0.376**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.476	0.213	0.479**N**
				NVP-H	0.226	0.454**N**
					AZT/3TC-H	0.199**N**
Sire-adjusted correlation	–0.02					
Sire-adjusted Poly-3 rate	27.7%	26.4%	20.9%	19.9%	31.8%	18.0%
	Vehicle Control	0.456**N**	0.237**N**	0.205**N**	0.360	0.165**N**
		AZT-H	0.311**N**	0.260**N**	0.336	0.236**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.461**N**	0.193	0.383**N**
				NVP-H	0.132	0.426**N**
					AZT/3TC-H	0.078**N**
All Organs: Benign Neoplasms						
Overall rate	1/25 (4.0%)	8/24 (33.3%)	5/25 (20.0%)	5/26 (19.2%)	9/25 (36.0%)	10/24 (41.7%)
Adjusted rate	1/22.9 (4.4%)	8/19.9 (40.2%)	5/23.6 (21.2%)	5/22.9 (21.9%)	9/22.2 (40.5%)	10/22.9 (43.8%)
Terminal rate	0/20 (0.0%)	8/18 (44.4%)	5/23 (21.7%)	5/22 (22.7%)	9/21 (42.9%)	10/22 (45.5%)
First incidence (days)	313	316 (T)	318 (T)	320 (T)	316 (T)	316 (T)
	Vehicle Control	0.004**	0.099	0.091	0.003**	0.001**
		AZT-H	0.150**N**	0.166**N**	0.615	0.530
Poly-3 test			**3TC-H**	0.615	0.135	0.089
				NVP-H	0.150	0.102
					AZT/3TC-H	0.532
Litter-adjusted correlation	–0.05					
Litter-adjusted Poly-3 rate	4.5%	39.8%	21.1%	22.0%	40.6%	43.2%
	Vehicle Control	<0.001***	0.029*	0.049*	<0.001***	<0.001***
		AZT-H	0.066**N**	0.098**N**	0.478	0.409
Litter-adjusted Poly-3 test			**3TC-H**	0.471	0.057	0.044*
				NVP-H	0.087	0.069
					AZT/3TC-H	0.429
Sire-adjusted correlation	–0.07					
Sire-adjusted Poly-3 rate	4.0%	43.6%	19.8%	19.6%	36.4%	40.7%
	Vehicle Control	<0.001***	0.041*	0.071	<0.001***	0.001**
		AZT-H	0.041**N***	0.038**N***	0.293**N**	0.431**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.493**N**	0.096	0.054
				NVP-H	0.124	0.093
					AZT/3TC-H	0.344

TABLE G5
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Benign or Malignant Neoplasms						
Overall rate	7/25 (28.0%)	12/24 (50.0%)	9/25 (36.0%)	9/26 (34.6%)	17/25 (68.0%)	14/24 (58.3%)
Adjusted rate	7/25.0 (28.0%)	12/22.4 (53.6%)	9/24.2 (37.2%)	9/25.0 (36.0%)	17/25.0 (68.0%)	14/23.0 (60.8%)
Terminal rate	2/20 (10.0%)	8/18 (44.4%)	8/23 (34.8%)	6/22 (27.3%)	13/21 (61.9%)	13/22 (59.1%)
First incidence (days)	176	168	250	177	99	307
	Vehicle Control	0.065	0.352	0.383	0.003**	0.019*
		AZT-H	0.206**N**	0.179**N**	0.238	0.424
Poly-3 test			**3TC-H**	0.580**N**	0.027*	0.090
				NVP-H	0.020*	0.074
					AZT/3TC-H	0.415**N**
Litter-adjusted correlation	–0.51					
Litter-adjusted Poly-3 rate	33.4%	46.4%	36.5%	37.2%	65.8%	56.5%
	Vehicle Control	0.121	0.374	0.367	<0.001***	0.027*
		AZT-H	0.194**N**	0.240**N**	0.043*	0.228
Litter-adjusted Poly-3 test			**3TC-H**	0.474	0.001**	0.053
				NVP-H	0.006**	0.081
					AZT/3TC-H	0.226**N**
Sire-adjusted correlation	–0.11					
Sire-adjusted Poly-3 rate	24.6%	61.1%	39.1%	29.2%	59.8%	67.7%
	Vehicle Control	0.004**	0.054	0.332	<0.001***	0.001**
		AZT-H	0.067**N**	0.006**N****	0.454**N**	0.352
Sire-adjusted Poly-3 test			**3TC-H**	0.160**N**	0.024*	0.016*
				NVP-H	0.004**	0.003**
					AZT/3TC-H	0.256

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the group stated to the left on the same row. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

TABLE G6
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
All Organs : Malignant Lymphoma				
Overall rate[b]	2/25 (8.0%)	2/25 (8.0%)	4/22 (18.2%)	4/25 (16.0%)
Adjusted rate[c]	2/25.0 (8.0%)	2/24.4 (8.2%)	4/22.0 (18.2%)	4/24.5 (16.3%)
Terminal rate[d]	2/25 (8.0%)	0/22 (0.0%)	1/19 (5.3%)	2/21 (9.5%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test[e]	0.156	0.687	0.274	0.324
Litter-adjusted correlation	–0.24			
Litter-adjusted Poly-3 rate	9.0%	8.6%	18.6%	16.8%
Litter-adjusted Poly-3 test	0.117	0.483**N**	0.147	0.189
Sire-adjusted correlation	–0.11			
Sire-adjusted Poly-3 rate	7.9%	8.1%	17.8%	14.3%
Sire-adjusted Poly-3 test	0.153	0.488	0.136	0.233
All Organs : Benign Neoplasms				
Overall rate	1/25 (4.0%)	0/25 (0.0%)	2/22 (9.1%)	4/25 (16.0%)
Adjusted rate	1/25.0 (4.0%)	0/23.4 (0.0%)	2/19.7 (10.2%)	4/24.1 (16.6%)
Terminal rate	1/25 (4.0%)	0/22 (0.0%)	2/19 (10.5%)	3/21 (14.3%)
First incidence (days)	316 (T)	—[f]	318 (T)	275
Poly-3 test	0.036*	0.513**N**	0.417	0.162
Litter-adjusted correlation	0.14			
Litter-adjusted Poly-3 rate	3.8%	0.0%	10.5%	16.3%
Litter-adjusted Poly-3 test	0.051	0.147**N**	0.194	0.094
Sire-adjusted correlation	0.02			
Sire-adjusted Poly-3 rate	3.9%	0.0%	10.5%	16.8%
Sire-adjusted Poly-3 test	0.055	0.153**N**	0.192	0.098
All Organs : Malignant Neoplasms				
Overall rate	3/25 (12.0%)	3/25 (12.0%)	6/22 (27.3%)	6/25 (24.0%)
Adjusted rate	3/25.0 (12.0%)	3/25.0 (12.0%)	6/22.0 (27.3%)	6/25.0 (24.0%)
Terminal rate	3/25 (12.0%)	0/22 (0.0%)	3/19 (15.8%)	2/21 (9.5%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test	0.092	0.665	0.170	0.233
Litter-adjusted correlation	–0.08			
Litter-adjusted Poly-3 rate	12.4%	12.2%	27.5%	24.4%
Litter-adjusted Poly-3 test	0.070	0.489**N**	0.095	0.137
Sire-adjusted correlation	–0.05			
Sire-adjusted Poly-3 rate	11.9%	13.2%	26.8%	24.2%
Sire-adjusted Poly-3 test	0.075	0.446	0.090	0.138

TABLE G6
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
All Organs: Benign or Malignant Neoplasms				
Overall rate	4/25 (16.0%)	3/25 (12.0%)	7/22 (31.8%)	9/25 (36.0%)
Adjusted rate	4/25.0 (16.0%)	3/25.0 (12.0%)	7/22.0 (31.8%)	9/25.0 (36.0%)
Terminal rate	4/25 (16.0%)	0/22 (0.0%)	4/19 (21.1%)	5/21 (23.8%)
First incidence (days)	316 (T)	186	101	247
Poly-3 test	0.023*	0.500**N**	0.176	0.097
Litter-adjusted correlation	0.25			
Litter-adjusted Poly-3 rate	14.4%	11.4%	31.1%	34.8%
Litter-adjusted Poly-3 test	0.023 *	0.379**N**	0.111	0.056
Sire-adjusted correlation	0.08			
Sire-adjusted Poly-3 rate	15.2%	10.8%	33.0%	36.1%
Sire-adjusted Poly-3 test	0.008**	0.347**N**	0.091	0.032*

(T) Terminal kill
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg; AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Bone: Osteosarcoma						
Overall rate[b]	0/25 (0.0%)	0/25 (0.0%)	2/25 (8.0%)	0/25 (0.0%)	0/24 (0.0%)	0/25 (0.0%)
Adjusted rate[c]	0/25.0 (0.0%)	0/23.8 (0.0%)	2/23.5 (8.5%)	0/23.2 (0.0%)	0/20.7 (0.0%)	0/23.7 (0.0%)
Terminal rate[d]	0/25 (0.0%)	0/20 (0.0%)	1/20 (5.0%)	0/20 (0.0%)	0/19 (0.0%)	0/21 (0.0%)
First incidence (days)	—[f]	—	297	—	—	—
	Vehicle Control	—[g]	0.221	—	—	—
		AZT-H	0.232	—	—	—
Poly-3 test[e]			**3TC-H**	0.237**N**	0.263**N**	0.232**N**
				NVP-H	—	—
					AZT/3TC-H	—
Litter-adjusted correlation	–0.01					
Litter-adjusted Poly-3 rate	0.0%	0.0%	8.5%	0.0%	0.0%	0.0%
	Vehicle Control	—	0.067	—	—	—
		AZT-H	0.067	—	—	—
Litter-adjusted Poly-3 test			**3TC-H**	0.067**N**	0.067**N**	0.067**N**
				NVP-H	—	—
					AZT/3TC-H	—
Sire-adjusted correlation	0.00					
Sire-adjusted Poly-3 rate	0.0%	0.0%	8.5%	0.0%	0.0%	0.0%
	Vehicle Control	—	0.067	—	—	—
		AZT-H	0.067	—	—	—
Sire-adjusted Poly-3 test			**3TC-H**	0.067**N**	0.067**N**	0.067**N**
				NVP-H	—	—
					AZT/3TC-H	—
Harderian Gland: Adenoma						
Overall rate	0/25 (0.0%)	1/24 (4.2%)	0/25 (0.0%)	2/25 (8.0%)	0/23 (0.0%)	1/24 (4.2%)
Adjusted rate	0/25.0 (0.0%)	1/23.1 (4.3%)	0/23.2 (0.0%)	2/23.2 (8.6%)	0/20.7 (0.0%)	1/23.3 (4.3%)
Terminal rate	0/25 (0.0%)	1/20 (5.0%)	0/20 (0.0%)	2/20 (10.0%)	0/19 (0.0%)	1/21 (4.8%)
First incidence (days)	—	320 (T)	—	320 (T)	—	318 (T)
	Vehicle Control	0.484	—	0.218	—	0.486
		AZT-H	0.499**N**	0.501	0.522**N**	0.759**N**
Poly-3 test			**3TC-H**	0.234	—	0.501
				NVP-H	0.260**N**	0.498**N**
					AZT/3TC-H	0.523
Litter-adjusted correlation	0.01					
Litter-adjusted Poly-3 rate	0.0%	4.4%	0.0%	8.7%	0.0%	4.3%
	Vehicle Control	0.155	—	0.075	—	0.150
		AZT-H	0.156**N**	0.279	0.156**N**	0.495**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.075	—	0.150
				NVP-H	0.075**N**	0.272**N**
					AZT/3TC-H	0.150
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	0.0%	4.3%	0.0%	8.6%	0.0%	4.3%
	Vehicle Control	0.156	—	0.076	—	0.149
		AZT-H	0.157**N**	0.281	0.157**N**	0.500**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.076	—	0.150
				NVP-H	0.076**N**	0.287**N**
					AZT/3TC-H	0.150

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Mammary Gland: Adenocarcinoma						
Overall rate	1/25 (4.0%)	0/25 (0.0%)	3/25 (12.0%)	0/23 (0.0%)	2/23 (8.7%)	0/25 (0.0%)
Adjusted rate	1/25.0 (4.0%)	0/23.8 (0.0%)	3/23.6 (12.7%)	0/21.8 (0.0%)	2/21.1 (9.5%)	0/23.7 (0.0%)
Terminal rate	1/25 (4.0%)	0/20 (0.0%)	2/20 (10.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	319 (T)	—	277	—	280	—
Poly-3 test	**Vehicle Control**	0.510**N**	0.282	0.527**N**	0.440	0.510**N**
		AZT-H	0.112	—	0.208	—
			3TC-H	0.128**N**	0.553**N**	0.112**N**
				NVP-H	0.226	—
					AZT/3TC-H	0.208**N**
Litter-adjusted correlation	–0.05					
Litter-adjusted Poly-3 rate	4.1%	0.0%	12.6%	0.0%	9.5%	0.0%
Litter-adjusted Poly-3 test	**Vehicle Control**	0.146**N**	0.134	0.146**N**	0.231	0.146**N**
		AZT-H	0.028*	—	0.063	—
			3TC-H	0.028**N***	0.365**N**	0.028**N***
				NVP-H	0.063	—
					AZT/3TC-H	0.063**N**
Sire-adjusted correlation	—[g]					
Sire-adjusted Poly-3 rate	—	—	—	—	—	—
Sire-adjusted Poly-3 test	**Vehicle Control**	—	—	—	—	—
		AZT-H	—	—	—	—
			3TC-H	—	—	—
				NVP-H	—	—
					AZT/3TC-H	—
Mammary Gland: Adenoacanthoma or Adenocarcinoma						
Overall rate	1/25 (4.0%)	1/25 (4.0%)	4/25 (16.0%)	0/23 (0.0%)	2/23 (8.7%)	1/25 (4.0%)
Adjusted rate	1/25.0 (4.0%)	1/23.9 (4.2%)	4/24.0 (16.6%)	0/21.8 (0.0%)	2/21.1 (9.5%)	1/23.9 (4.2%)
Terminal rate	1/25 (4.0%)	0/20 (0.0%)	2/20 (10.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	319 (T)	313	271	—	280	302
Poly-3 test	**Vehicle Control**	0.750	0.161	0.527**N**	0.440	0.751
		AZT-H	0.174	0.518**N**	0.456	0.760**N**
			3TC-H	0.066**N**	0.397**N**	0.173**N**
				NVP-H	0.226	0.519
					AZT/3TC-H	0.455**N**
Litter-adjusted correlation	0.10					
Litter-adjusted Poly-3 rate	3.9%	4.0%	16.7%	0.0%	9.6%	4.4%
Litter-adjusted Poly-3 test	**Vehicle Control**	0.489	0.095	0.147**N**	0.215	0.458
		AZT-H	0.099	0.146**N**	0.223	0.469
			3TC-H	0.033**N***	0.260**N**	0.112**N**
				NVP-H	0.064	0.156
					AZT/3TC-H	0.251**N**
Sire-adjusted correlation	0.10					
Sire-adjusted Poly-3 rate	4.4%	4.3%	17.0%	0.1%	10.4%	3.5%
Sire-adjusted Poly-3 test	**Vehicle Control**	0.496**N**	0.092	0.126**N**	0.116	0.442**N**
		AZT-H	0.090	0.131**N**	0.184	0.449**N**
			3TC-H	0.027**N***	0.156**N**	0.087**N**
				NVP-H	0.041*	0.211
					AZT/3TC-H	0.186**N**

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Malignant Lymphoma						
Overall rate	2/25 (8.0%)	2/25 (8.0%)	1/25 (4.0%)	5/25 (20.0%)	4/24 (16.7%)	4/25 (16.0%)
Adjusted rate	2/25.0 (8.0%)	2/24.4 (8.2%)	1/23.7 (4.2%)	5/23.7 (21.1%)	4/23.6 (16.9%)	4/24.5 (16.3%)
Terminal rate	2/25 (8.0%)	0/20 (0.0%)	0/20 (0.0%)	4/20 (20.0%)	0/19 (0.0%)	2/21 (9.5%)
First incidence (days)	316 (T)	277	258	251	102	247
	Vehicle Control	0.686	0.518**N**	0.186	0.307	0.324
		AZT-H	0.509**N**	0.196	0.319	0.336
Poly-3 test			**3TC-H**	0.092	0.171	0.182
				NVP-H	0.502**N**	0.479**N**
					AZT/3TC-H	0.628**N**
Litter-adjusted correlation	0.09					
Litter-adjusted Poly-3 rate	7.7%	7.8%	4.5%	21.3%	16.5%	16.2%
	Vehicle Control	0.491	0.314**N**	0.106	0.191	0.156
		AZT-H	0.308**N**	0.109	0.196	0.161
Litter-adjusted Poly-3 test			**3TC-H**	0.058	0.112	0.075
				NVP-H	0.358**N**	0.334**N**
					AZT/3TC-H	0.489**N**
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	7.9%	8.2%	4.3%	21.0%	17.1%	16.4%
	Vehicle Control	0.488	0.289**N**	0.087	0.167	0.154
		AZT-H	0.289**N**	0.141	0.189	0.183
Sire-adjusted Poly-3 test			**3TC-H**	0.061	0.084	0.064
				NVP-H	0.321**N**	0.356**N**
					AZT/3TC-H	0.476**N**
All Organs: Benign Neoplasms						
Overall rate	1/25 (4.0%)	1/25 (4.0%)	0/25 (0.0%)	3/25 (12.0%)	0/24 (0.0%)	4/25 (16.0%)
Adjusted rate	1/25.0 (4.0%)	1/23.8 (4.2%)	0/23.2 (0.0%)	3/23.2 (13.0%)	0/20.7 (0.0%)	4/24.1 (16.6%)
Terminal rate	1/25 (4.0%)	1/20 (5.0%)	0/20 (0.0%)	3/20 (15.0%)	0/19 (0.0%)	3/21 (14.3%)
First incidence (days)	316 (T)	320 (T)	—	320 (T)	—	275
	Vehicle Control	0.749	0.515**N**	0.275	0.537**N**	0.162
		AZT-H	0.504**N**	0.293	0.527**N**	0.177
Poly-3 test			**3TC-H**	0.112	—	0.059
				NVP-H	0.134**N**	0.523
					AZT/3TC-H	0.075
Litter-adjusted correlation	0.13					
Litter-adjusted Poly-3 rate	3.8%	4.5%	0.0%	13.5%	0.0%	16.4%
	Vehicle Control	0.454	0.147**N**	0.114	0.147**N**	0.094
		AZT-H	0.155**N**	0.141	0.155**N**	0.114
Litter-adjusted Poly-3 test			**3TC-H**	0.031*	—	0.032*
				NVP-H	0.031**N***	0.403
					AZT/3TC-H	0.032*
Sire-adjusted correlation	–0.02					
Sire-adjusted Poly-3 rate	4.1%	4.1%	0.0%	12.9%	0.0%	16.7%
	Vehicle Control	0.500	0.141**N**	0.140	0.142**N**	0.106
		AZT-H	0.159**N**	0.141	0.161**N**	0.104
Sire-adjusted Poly-3 test			**3TC-H**	0.030*	—	0.032*
				NVP-H	0.031**N***	0.382
					AZT/3TC-H	0.033*

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Malignant Neoplasms						
Overall rate	3/25 (12.0%)	4/25 (16.0%)	8/25 (32.0%)	9/25 (36.0%)	8/24 (33.3%)	6/25 (24.0%)
Adjusted rate	3/25.0 (12.0%)	4/24.7 (16.2%)	8/25.0 (32.0%)	9/24.9 (36.2%)	8/24.0 (33.3%)	6/25.0 (24.0%)
Terminal rate	3/25 (12.0%)	0/20 (0.0%)	3/20 (15.0%)	5/20 (25.0%)	3/19 (15.8%)	2/21 (9.5%)
First incidence (days)	316 (T)	277	258	251	102	247
	Vehicle Control	0.493	0.084	0.044	0.071	0.233
		AZT-H	0.167	0.100	0.146	0.373
Poly-3 test			**3TC-H**	0.495	0.580	0.378**N**
				NVP-H	0.536**N**	0.267**N**
					AZT/3TC-H	0.344**N**
Litter-adjusted correlation	0.08					
Litter-adjusted Poly-3 rate	11.6%	15.9%	32.6%	36.0%	32.5%	23.6%
	Vehicle Control	0.311	0.039*	0.021*	0.034*	0.127
		AZT-H	0.088	0.053	0.082	0.242
Litter-adjusted Poly-3 test			**3TC-H**	0.408	0.498**N**	0.258**N**
				NVP-H	0.403**N**	0.186**N**
					AZT/3TC-H	0.254**N**
Sire-adjusted correlation	0.02					
Sire-adjusted Poly-3 rate	12.0%	16.5%	31.7%	36.3%	33.2%	23.7%
	Vehicle Control	0.282	0.037*	0.018*	0.018*	0.142
		AZT-H	0.082	0.069	0.057	0.278
Sire-adjusted Poly-3 test			**3TC-H**	0.378	0.444	0.299**N**
				NVP-H	0.405**N**	0.170**N**
					AZT/3TC-H	0.234**N**

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
All Organs: Benign or Malignant Neoplasms						
Overall rate	4/25 (16.0%)	6/25 (24.0%)	8/25 (32.0%)	12/25 (48.0%)	8/24 (33.3%)	9/25 (36.0%)
Adjusted rate	4/25.0 (16.0%)	6/25.0 (24.0%)	8/25.0 (32.0%)	12/24.9 (48.2%)	8/24.0 (33.3%)	9/25.0 (36.0%)
Terminal rate	4/25 (16.0%)	1/20 (5.0%)	3/20 (15.0%)	8/20 (40.0%)	3/19 (15.8%)	5/21 (23.8%)
First incidence (days)	316 (T)	277	258	251	102	247
	Vehicle Control	0.364	0.161	0.013*	0.141	0.097
		AZT-H	0.378	0.066	0.344	0.271
Poly-3 test			**3TC-H**	0.191	0.580	0.500
				NVP-H	0.223**N**	0.281**N**
					AZT/3TC-H	0.541
Litter-adjusted correlation	0.23					
Litter-adjusted Poly-3 rate	14.5%	23.9%	33.5%	48.8%	30.9%	34.9%
	Vehicle Control	0.200	0.073	0.004**	0.094	0.056
		AZT-H	0.233	0.029*	0.288	0.198
Litter-adjusted Poly-3 test			**3TC-H**	0.151	0.428**N**	0.462
				NVP-H	0.104**N**	0.170**N**
					AZT/3TC-H	0.388
Sire-adjusted correlation	0.06					
Sire-adjusted Poly-3 rate	15.6%	25.2%	30.7%	48.9%	32.6%	35.0%
	Vehicle Control	0.184	0.114	0.004**	0.079	**0**.048*
		AZT-H	0.320	0.021*	0.259	0.225
Sire-adjusted Poly-3 test			**3TC-H**	0.120	0.431	0.396
				NVP-H	0.091**N**	0.166**N**
					AZT/3TC-H	0.430

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically.

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the group stated to the left on the same row. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

TABLE G8
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Adrenal Cortex: Hypertrophy				
Overall rate[b]	0/25 (0.0%)	0/25 (0.0%)	1/23 (4.3%)	2/23 (8.7%)
Adjusted rate[c]	0/22.8 (0.0%)	0/24.8 (0.0%)	1/22.9 (4.4%)	2/22.8 (8.8%)
Terminal rate[d]	0/20 (0.0%)	0/24 (0.0%)	1/22 (4.5%)	2/22 (9.1%)
First incidence (days)	—[g]	—	318 (T)	318 (T)
Average severity	—[g]	—	2.0	1.5
Poly-3 test[e]	0.055	—[h]	0.501	0.235
Litter-adjusted correlation	–0.01			
Litter-adjusted Poly-3 rate	0.0%	0.0%	4.4%	8.8%
Litter-adjusted Poly-3 test	0.043*	—	0.158	0.064
Sire-adjusted correlation	0.01			
Sire-adjusted Poly-3 rate	0.0%	0.0%	4.5%	8.9%
Sire-adjusted Poly-3 test	0.045*	—	0.153	0.064
JT/SW test[f]	0.031*	0.500	0.118	0.053
Bone Marrow: Hyperplasia				
Overall rate	3/23 (13.0%)	2/25 (8.0%)	4/23 (17.4%)	0/23 (0.0%)
Adjusted rate	3/21.9 (13.7%)	2/24.8 (8.1%)	4/23.0 (17.4%)	0/22.8 (0.0%)
Terminal rate	2/20 (10.0%)	2/24 (8.3%)	3/22 (13.6%)	0/22 (0.0%)
First incidence (days)	313	317 (T)	318	—
Average severity	3.3	3.5	3.0	—
Poly-3 test	0.167**N**	0.443**N**	0.528	0.105**N**
Litter-adjusted correlation	–0.15			
Litter-adjusted Poly-3 rate	14.4%	7.8%	17.3%	0.0%
Litter-adjusted Poly-3 test	0.069**N**	0.225**N**	0.384	0.019**N***
Sire-adjusted correlation	–0.08			
Sire-adjusted Poly-3 rate	14.2%	7.1%	17.3%	0.0%
Sire-adjusted Poly-3 test	0.068**N**	0.205**N**	0.380	0.014**N***
JT/SW test	0.137**N**	0.286**N**	0.548**N**	0.082**N**
Kidney: Hydronephrosis				
Overall rate	1/25 (4.0%)	13/25 (52.0%)	5/23 (21.7%)	12/23 (52.2%)
Adjusted rate	1/22.8 (4.4%)	13/24.8 (52.5%)	5/22.9 (21.8%)	12/23.0 (52.2%)
Terminal rate	1/20 (5.0%)	13/24 (54.2%)	5/22 (22.7%)	11/22 (50.0%)
First incidence (days)	320 (T)	315 (T)	318 (T)	307
Average severity	2.0	1.2	1.2	2.0
Poly-3 test	0.006**	<0.001***	0.093	<0.001***
Litter-adjusted correlation	0.30			
Litter-adjusted Poly-3 rate	4.9%	52.9%	21.8%	52.4%
Litter-adjusted Poly-3 test	0.003**	<0.001***	0.033*	<0.001***
Sire-adjusted correlation	0.27			
Sire-adjusted Poly-3 rate	7.3%	57.3%	17.7%	53.3%
Sire-adjusted Poly-3 test	0.001**	<0.001***	0.129	<0.001***
JT/SW test	0.002**	<0.001***	0.007**	<0.001***

TABLE G8
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Liver: Basophilic Focus				
Overall rate	0/25 (0.0%)	0/25 (0.0%)	2/23 (8.7%)	2/23 (8.7%)
Adjusted rate	0/22.8 (0.0%)	0/24.8 (0.0%)	2/22.9 (8.7%)	2/22.8 (8.8%)
Terminal rate	0/20 (0.0%)	0/24 (0.0%)	2/22 (9.1%)	2/22 (9.1%)
First incidence (days)	—	—	320 (T)	321 (T)
Average severity	—	—	5.0	5.0
Poly-3 test	0.052	—	0.236	0.235
Litter-adjusted correlation	–0.12			
Litter-adjusted Poly-3 rate	0.0%	0.0%	9.0%	9.4%
Litter-adjusted Poly-3 test	0.021*	—	0.057	0.052
Sire-adjusted correlation	–0.05			
Sire-adjusted Poly-3 rate	0.0%	0.0%	9.1%	9.0%
Sire-adjusted Poly-3 test	0.017*	—	0.051	0.055
JT/SW test	0.029*	0.500	0.039*	0.083
Liver: Cytoplasmic Vacuolization				
Overall rate	19/25 (76.0%)	21/25 (84.0%)	19/23 (82.6%)	20/23 (87.0%)
Adjusted rate	19/23.9 (79.5%)	21/24.8 (84.8%)	19/23.0 (82.6%)	20/22.8 (87.6%)
Terminal rate	17/20 (85.0%)	21/24 (87.5%)	18/22 (81.8%)	20/22 (90.9%)
First incidence (days)	207	315 (T)	316 (T)	316 (T)
Average severity	1.9	2.1	2.4	2.8
Poly-3 test	0.305	0.455	0.540	0.361
Litter-adjusted correlation	0.20			
Litter-adjusted Poly-3 rate	80.1%	84.2%	83.3%	88.2%
Litter-adjusted Poly-3 test	0.247	0.359	0.397	0.226
Sire-adjusted correlation	0.19			
Sire-adjusted Poly-3 rate	80.8%	86.7%	84.3%	90.2%
Sire-adjusted Poly-3 test	0.147	0.253	0.391	0.115
JT/SW test	<0.001***	0.112	0.047*	<0.001***
Liver: Inflammation				
Overall rate	0/25 (0.0%)	1/25 (4.0%)	2/23 (8.7%)	1/23 (4.3%)
Adjusted rate	0/22.8 (0.0%)	1/24.8 (4.0%)	2/23.0 (8.7%)	1/22.8 (4.4%)
Terminal rate	0/20 (0.0%)	1/24 (4.2%)	1/22 (4.5%)	1/22 (4.5%)
First incidence (days)	—	325 (T)	318	320 (T)
Average severity	—	1.0	1.5	2.0
Poly-3 test	0.253	0.517	0.237	0.500
Litter-adjusted correlation	–0.03			
Litter-adjusted Poly-3 rate	0.0%	4.1%	8.6%	4.3%
Litter-adjusted Poly-3 test	0.115	0.147	0.067	0.156
Sire-adjusted correlation	–0.05			
Sire-adjusted Poly-3 rate	0.0%	4.0%	9.5%	4.0%
Sire-adjusted Poly-3 test	0.111	0.149	0.048*	0.171
JT/SW test	0.151	0.159	0.076	0.164

TABLE G8
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Nose: Hyaline Droplet				
Overall rate	0/25 (0.0%)	3/25 (12.0%)	2/23 (8.7%)	0/24 (0.0%)
Adjusted rate	0/22.8 (0.0%)	3/25.0 (12.0%)	2/22.9 (8.7%)	0/22.9 (0.0%)
Terminal rate	0/20 (0.0%)	2/24 (8.3%)	2/22 (9.1%)	0/22 (0.0%)
First incidence (days)	—	297	320 (T)	—
Average severity	—	1.3	1.5	—
Poly-3 test	0.495**N**	0.131	0.236	—
Litter-adjusted correlation	–0.13			
Litter-adjusted Poly-3 rate	0.0%	12.5%	9.0%	0.0%
Litter-adjusted Poly-3 test	0.338**N**	0.020*	0.057	—
Sire-adjusted correlation	–0.05			
Sire-adjusted Poly-3 rate	0.0%	11.9%	9.2%	0.0%
Sire-adjusted Poly-3 test	0.376**N**	0.024*	0.049*	—
JT/SW test	0.456**N**	0.961**N**	0.937**N**	0.618**N**
Pancreatic Islets: Hyperplasia				
Overall rate	13/24 (54.2%)	9/25 (36.0%)	13/23 (56.5%)	6/23 (26.1%)
Adjusted rate	13/23.3 (55.9%)	9/25.0 (36.0%)	13/23.0 (56.5%)	6/22.8 (26.3%)
Terminal rate	10/20 (50.0%)	8/24 (33.3%)	12/22 (54.5%)	6/22 (27.3%)
First incidence (days)	279	297	318 (T)	316 (T)
Average severity	1.5	1.4	1.3	1.8
Poly-3 test	0.085**N**	0.136**N**	0.598	0.037**N***
Litter-adjusted correlation	0.08			
Litter-adjusted Poly-3 rate	55.2%	36.1%	56.2%	26.5%
Litter-adjusted Poly-3 test	0.065**N**	0.106**N**	0.469	0.018**N***
Sire-adjusted correlation	–0.04			
Sire-adjusted Poly-3 rate	55.8%	35.9%	58.1%	25.9%
Sire-adjusted Poly-3 test	0.042**N***	0.138**N**	0.432	0.013**N***
JT/SW test	0.108**N**	0.111**N**	0.291**N**	0.057**N**

TABLE G8
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Thymus: Atrophy				
Overall rate	2/24 (8.3%)	1/25 (4.0%)	0/20 (0.0%)	1/20 (5.0%)
Adjusted rate	2/22.0 (9.1%)	1/24.8 (4.0%)	0/19.9 (0.0%)	1/20.0 (5.0%)
Terminal rate	1/20 (5.0%)	1/24 (4.2%)	0/19 (0.0%)	1/20 (5.0%)
First incidence (days)	310	315 (T)	—	326 (T)
Average severity	3.5	4.0	—	3.0
Poly-3 test	0.297**N**	0.459**N**	0.257**N**	0.534**N**
Litter-adjusted correlation	0.00			
Litter-adjusted Poly-3 rate	9.1%	4.1%	0.0%	5.0%
Litter-adjusted Poly-3 test	0.244**N**	0.240**N**	0.062**N**	0.298**N**
Sire-adjusted correlation	–0.05			
Sire-adjusted Poly-3 rate	9.7%	4.1%	0.0%	4.8%
Sire-adjusted Poly-3 test	0.212**N**	0.221**N**	0.048**N***	0.264**N**
JT/SW test	0.212**N**	0.274**N**	0.109**N**	0.224**N**

(T) Terminal kill
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg; AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of lesion-bearing animals/number of animals examined microscopically
[c] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Beneath the vehicle control incidence is the P value associated with the Jonckheere/Terpstra monotonic trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group by William's modified Shirley's test. A negative trend or lower incidence in a dose group is indicated by **N**.
[g] Not applicable, no lesions in animal group
[h] Value of statistic cannot be computed.

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Adrenal Cortex: Subcapsular Hyperplasia						
Overall rate[b]	13/25 (52.0%)	11/23 (47.8%)	13/25 (52.0%)	7/25 (28.0%)	11/23 (47.8%)	12/23 (52.2%)
Adjusted rate[c]	13/23.3 (55.8%)	11/21.3 (51.6%)	13/24.2 (53.8%)	7/22.4 (31.2%)	11/20.4 (54.0%)	12/22.8 (52.6%)
Terminal incidence[d]	11/20 (55.0%)	9/18 (50.0%)	12/23 (52.2%)	7/22 (31.8%)	11/20 (55.0%)	12/22 (54.5%)
First incidence (days)	279	176	250	320 (T)	316 (T)	318 (T)
Average severity	1.0	1.0	1.0	1.0	1.1	1.1
	Vehicle Control	0.507**N**	0.560**N**	0.081**N**	0.572**N**	0.529**N**
		AZT-H	0.558	0.142**N**	0.560	0.592
Poly-3 test[e]			**3TC-H**	0.102**N**	0.611	0.581**N**
				NVP-H	0.113	0.123
					AZT/3TC-H	0.582**N**
Litter-adjusted correlation	0.08					
Litter-adjusted Poly-3 rate	56.4%	50.9%	54.0%	31.2%	54.5%	52.6%
	Vehicle Control	0.371**N**	0.438**N**	0.044**N***	0.454**N**	0.404**N**
		AZT-H	0.421	0.085**N**	0.411	0.456
Litter-adjusted Poly-3 test			**3TC-H**	0.042**N***	0.485	0.462**N**
				NVP-H	0.047*	0.051
					AZT/3TC-H	0.449**N**
Sire-adjusted correlation	0.04					
Sire-adjusted Poly-3 rate	56.8%	52.6%	54.4%	31.6%	55.1%	52.5%
	Vehicle Control	0.393**N**	0.425**N**	0.025**N***	0.461**N**	0.380**N**
		AZT-H	0.452	0.090**N**	0.436	0.499**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.041**N***	0.482	0.445**N**
				NVP-H	0.039*	0.043*
					AZT/3TC-H	0.426**N**
Bone Marrow: Hyperplasia						
Overall rate	3/23 (13.0%)	3/24 (12.5%)	1/24 (4.2%)	2/25 (8.0%)	2/24 (8.3%)	0/23 (0.0%)
Adjusted rate	3/21.9 (13.7%)	3/22.2 (13.5%)	1/23.5 (4.3%)	2/24.2 (8.3%)	2/21.4 (9.4%)	0/22.8 (0.0%)
Terminal incidence	2/20 (10.0%)	0/18 (0.0%)	1/23 (4.3%)	0/22 (0.0%)	2/21 (9.5%)	0/22 (0.0%)
First incidence (days)	313	42	320 (T)	39	319 (T)	—[f]
Average severity	3.3	3.3	3.0	3.0	2.0	—[f]
	Vehicle Control	0.659**N**	0.277**N**	0.454**N**	0.511**N**	0.105**N**
		AZT-H	0.283**N**	0.461**N**	0.518**N**	0.108**N**
Poly-3 test			**3TC-H**	0.510	0.468	0.505**N**
				NVP-H	0.651	0.248**N**
					AZT/3TC-H	0.220**N**
Litter-adjusted correlation	–0.07					
Litter-adjusted Poly-3 rate	14.0%	13.0%	4.4%	8.2%	9.3%	0.0%
	Vehicle Control	0.462**N**	0.114**N**	0.253**N**	0.302**N**	0.020**N***
		AZT-H	0.151**N**	0.297**N**	0.345**N**	0.037**N***
Litter-adjusted Poly-3 test			**3TC-H**	0.289	0.255	0.145**N**
				NVP-H	0.449	0.067**N**
					AZT/3TC-H	0.065**N**
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	13.7%	13.3%	4.3%	8.3%	9.3%	0.0%
	Vehicle Control	0.483**N**	0.122**N**	0.257**N**	0.313**N**	0.016**N***
		AZT-H	0.075**N**	0.292**N**	0.333**N**	0.038**N***
Sire-adjusted Poly-3 test			**3TC-H**	0.283	0.249	0.145**N**
				NVP-H	0.450	0.066**N**
					AZT/3TC-H	0.046**N***

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Kidney: Hydronephrosis						
Overall rate	1/25 (4.0%)	8/23 (34.8%)	7/25 (28.0%)	5/25 (20.0%)	9/24 (37.5%)	12/23 (52.2%)
Adjusted rate	1/22.8 (4.4%)	8/19.9 (40.2%)	7/24.5 (28.6%)	5/23.2 (21.6%)	9/21.4 (42.1%)	12/23.0 (52.2%)
Terminal incidence	1/20 (5.0%)	8/18 (44.4%)	6/23 (26.1%)	4/22 (18.2%)	9/21 (42.9%)	11/22 (50.0%)
First incidence (days)	320 (T)	316 (T)	182	215	317 (T)	307
Average severity	2.0	1.8	1.4	1.6	1.4	2.0
	Vehicle Control	0.004**	0.029*	0.096	0.002**	<0.001***
		AZT-H	0.314N	0.160N	0.576	0.319
Poly-3 test			**3TC-H**	0.412N	0.262	0.085
				NVP-H	0.124	0.028*
					AZT/3TC-H	0.358
Litter-adjusted correlation	0.07					
Litter-adjusted Poly-3 rate	4.5%	40.0%	28.6%	21.1%	43.3%	52.2%
	Vehicle Control	<0.001***	0.003**	0.050*	<0.001***	<0.001***
		AZT-H	0.190N	0.085N	0.415	0.216
Litter-adjusted Poly-3 test			**3TC-H**	0.264N	0.144	0.045*
				NVP-H	0.065	0.017*
					AZT/3TC-H	0.293
Sire-adjusted correlation	0.08					
Sire-adjusted Poly-3 rate	5.0%	40.1%	27.8%	21.6%	44.4%	52.2%
	Vehicle Control	<0.001***	0.006**	0.076	<0.001***	<0.001***
		AZT-H	0.163N	0.095N	0.386	0.208
Sire-adjusted Poly-3 test			**3TC-H**	0.306N	0.097	0.038*
				NVP-H	0.065	0.013*
					AZT/3TC-H	0.275
Kidney: Cellular Infiltration						
Overall rate	8/25 (32.0%)	2/23 (8.7%)	10/25 (40.0%)	6/25 (24.0%)	2/24 (8.3%)	7/23 (30.4%)
Adjusted rate	8/22.8 (35.1%)	2/19.9 (10.1%)	10/23.6 (42.3%)	6/22.4 (26.7%)	2/21.4 (9.4%)	7/22.8 (30.7%)
Terminal incidence	8/20 (40.0%)	2/18 (11.1%)	10/23 (43.5%)	6/22 (27.3%)	2/21 (9.5%)	7/22 (31.8%)
First incidence (days)	317 (T)	316 (T)	318 (T)	319 (T)	320 (T)	316 (T)
Average severity	1.1	1.0	1.1	1.0	1.0	1.7
	Vehicle Control	0.054N	0.420	0.388N	0.042N*	0.498N
		AZT-H	0.017*	0.161	0.671N	0.100
Poly-3 test			**3TC-H**	0.212N	0.012N*	0.303N
				NVP-H	0.135N	0.514
					AZT/3TC-H	0.081
Litter-adjusted correlation	–0.14					
Litter-adjusted Poly-3 rate	33.5%	10.0%	42.1%	26.7%	8.4%	30.6%
	Vehicle Control	0.025N*	0.268	0.299N	0.016N*	0.414N
		AZT-H	0.003**	0.052	0.430N	0.027*
Litter-adjusted Poly-3 test			**3TC-H**	0.108N	0.001N**	0.186N
				NVP-H	0.033N*	0.369
					AZT/3TC-H	0.017*
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	35.2%	10.3%	42.2%	26.7%	9.2%	30.7%
	Vehicle Control	0.014N*	0.315	0.254N	0.012N*	0.371N
		AZT-H	0.007**	0.039*	0.440N	0.028*
Sire-adjusted Poly-3 test			**3TC-H**	0.111N	0.003N**	0.186N
				NVP-H	0.047N*	0.339
					AZT/3TC-H	0.021*

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Liver: Basophilic Focus						
Overall rate	0/25 (0.0%)	0/23 (0.0%)	1/25 (4.0%)	1/25 (4.0%)	1/25 (4.0%)	2/23 (8.7%)
Adjusted rate	0/22.8 (0.0%)	0/19.9 (0.0%)	1/23.6 (4.2%)	1/22.4 (4.5%)	1/23.0 (4.4%)	2/22.8 (8.8%)
Terminal incidence	0/20 (0.0%)	0/18 (0.0%)	1/23 (4.3%)	1/22 (4.5%)	0/21 (0.0%)	2/22 (9.1%)
First incidence (days)	—	—	319 (T)	320 (T)	203	321 (T)
	Vehicle Control	—[g]	0.507	0.497	0.502	0.235
		AZT-H	0.534	0.524	0.529	0.267
Poly-3 test			**3TC-H**	0.749	0.754	0.488
				NVP-H	0.756**N**	0.506
					AZT/3TC-H	0.498
Litter-adjusted correlation	–0.08					
Litter-adjusted Poly-3 rate	0.0%	0.0%	4.4%	4.6%	4.1%	9.2%
	Vehicle Control	—	0.146	0.145	0.157	0.053
		AZT-H	0.146	0.145	0.158	0.053
Litter-adjusted Poly-3 test			**3TC-H**	0.484	0.485**N**	0.245
				NVP-H	0.470**N**	0.259
					AZT/3TC-H	0.235
Sire-adjusted correlation	–0.03					
Sire-adjusted Poly-3 rate	0.0%	0.0%	4.1%	4.5%	4.5%	9.3%
	Vehicle Control	—	0.155	0.143	0.143	0.043*
		AZT-H	0.155	0.144	0.143	0.044*
Sire-adjusted Poly-3 test			**3TC-H**	0.471	0.473	0.238
				NVP-H	0.498**N**	0.249
					AZT/3TC-H	0.252
Liver: Cytoplasmic Vacuolization						
Overall rate	19/25 (76.0%)	18/23 (78.3%)	20/25 (80.0%)	20/25 (80.0%)	21/25 (84.0%)	20/23 (87.0%)
Adjusted rate	19/23.9 (79.5%)	18/19.9 (90.5%)	20/23.6 (84.7%)	20/22.4 (89.1%)	21/22.2 (94.5%)	20/22.8 (87.6%)
Terminal incidence	17/20 (85.0%)	18/18 (100.0%)	20/23 (87.0%)	20/22 (90.9%)	21/21 (100.0%)	20/22 (90.9%)
First incidence (days)	207	316 (T)	318 (T)	319 (T)	316 (T)	316 (T)
Average severity	1.9	2.5	2.4	2.3	2.3	2.8
	Vehicle Control	0.265	0.465	0.306	0.127	0.361
		AZT-H	0.448**N**	0.659**N**	0.546	0.581**N**
Poly-3 test			**3TC-H**	0.496	0.256	0.555
				NVP-H	0.449	0.622**N**
					AZT/3TC-H	0.378**N**
Litter-adjusted correlation	–0.09					
Litter-adjusted Poly-3 rate	79.2%	90.8%	84.0%	88.7%	94.7%	87.3%
	Vehicle Control	0.134	0.334	0.193	0.061	0.238
		AZT-H	0.193**N**	0.390**N**	0.259	0.328**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.289	0.069	0.358
				NVP-H	0.190	0.432**N**
					AZT/3TC-H	0.156**N**
Sire-adjusted correlation	–0.03					
Sire-adjusted Poly-3 rate	79.0%	90.7%	84.3%	88.8%	94.5%	87.2%
	Vehicle Control	0.131	0.334	0.172	0.051	0.174
		AZT-H	0.165**N**	0.396**N**	0.278	0.335**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.301	0.087	0.374
				NVP-H	0.198	0.428**N**
					AZT/3TC-H	0.179**N**

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Lymph Node, Mesenteric: Hyperplasia						
Overall rate	9/24 (37.5%)	5/23 (21.7%)	12/25 (48.0%)	11/25 (44.0%)	11/24 (45.8%)	9/23 (39.1%)
Adjusted rate	9/22.3 (40.4%)	5/20.7 (24.1%)	12/23.6 (50.8%)	11/22.4 (49.0%)	11/22.1 (49.7%)	9/22.8 (39.4%)
Terminal incidence	8/20 (40.0%)	4/18 (22.2%)	12/23 (52.2%)	11/22 (50.0%)	10/21 (47.6%)	9/22 (40.9%)
First incidence (days)	279	176	318 (T)	319 (T)	203	316 (T)
Average severity	2.0	1.6	1.7	2.4	1.9	1.6
	Vehicle Control	0.208**N**	0.342	0.392	0.376	0.592**N**
		AZT-H	0.061	0.081	0.075	0.226
Poly-3 test			**3TC-H**	0.568**N**	0.586**N**	0.316**N**
				NVP-H	0.599	0.365**N**
					AZT/3TC-H	0.349**N**
Litter-adjusted correlation	–0.14					
Litter-adjusted Poly-3 rate	41.4%	23.5%	49.7%	49.1%	50.1%	38.8%
	Vehicle Control	0.084**N**	0.272	0.274	0.265	0.431**N**
		AZT-H	0.023*	0.019*	0.024*	0.144
Litter-adjusted Poly-3 test			**3TC-H**	0.482**N**	0.488	0.234**N**
				NVP-H	0.470	0.235**N**
					AZT/3TC-H	0.229**N**
Sire-adjusted correlation	0.00					
Sire-adjusted Poly-3 rate	40.4%	24.1%	50.8%	49.0%	49.7%	39.4%
	Vehicle Control	0.108**N**	0.234	0.262	0.267	0.468**N**
		AZT-H	0.032*	0.020*	0.030*	0.182
Sire-adjusted Poly-3 test			**3TC-H**	0.442**N**	0.469**N**	0.215**N**
				NVP-H	0.475	0.230**N**
					AZT/3TC-H	0.227**N**
Lymph Node, Mandibular: Hyperplasia						
Overall rate	2/25 (8.0%)	1/23 (4.3%)	2/25 (8.0%)	2/25 (8.0%)	2/24 (8.3%)	5/23 (21.7%)
Adjusted rate	2/22.8 (8.8%)	1/19.9 (5.0%)	2/24.2 (8.3%)	2/22.4 (8.9%)	2/21.4 (9.4%)	5/22.8 (21.9%)
Terminal incidence	2/20 (10.0%)	1/18 (5.6%)	1/23 (4.3%)	2/22 (9.1%)	2/21 (9.5%)	5/22 (22.7%)
First incidence (days)	315 (T)	319 (T)	250	319 (T)	321 (T)	316 (T)
Average severity	1.5	1.0	1.0	1.0	2.0	2.2
	Vehicle Control	0.548**N**	0.674**N**	0.691	0.673	0.207
		AZT-H	0.568	0.542	0.525	0.126
Poly-3 test			**3TC-H**	0.669	0.651	0.184
				NVP-H	0.679	0.214
					AZT/3TC-H	0.235
Litter-adjusted correlation	–0.10					
Litter-adjusted Poly-3 rate	8.8%	5.3%	8.1%	8.3%	9.7%	21.8%
	Vehicle Control	0.321**N**	0.466**N**	0.476**N**	0.458	0.095
		AZT-H	0.349	0.346	0.286	0.041*
Litter-adjusted Poly-3 test			**3TC-H**	0.491	0.424	0.080
				NVP-H	0.435	0.088
					AZT/3TC-H	0.114
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	8.7%	5.0%	8.2%	9.0%	9.3%	21.7%
	Vehicle Control	0.312**N**	0.473**N**	0.489	0.472	0.101
		AZT-H	0.332	0.208	0.290	0.049*
Sire-adjusted Poly-3 test			**3TC-H**	0.460	0.442	0.080
				NVP-H	0.486	0.135
					AZT/3TC-H	0.079

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Lymph Node (All Sites): Hyperplasia						
Overall rate	10/25 (40.0%)	6/23 (26.1%)	13/25 (52.0%)	14/25 (56.0%)	11/24 (45.8%)	11/23 (47.8%)
Adjusted rate	10/23.3 (42.9%)	6/20.7 (28.9%)	13/24.2 (53.8%)	14/22.4 (62.4%)	11/22.1 (49.7%)	11/22.8 (48.2%)
Terminal incidence	8/20 (40.0%)	5/18 (27.8%)	12/23 (52.2%)	14/22 (63.6%)	10/21 (47.6%)	11/22 (50.0%)
First incidence (days)	279	176	250	319 (T)	203	316 (T)
Average severity	1.9	1.5	1.6	2.1	1.9	1.9
	Vehicle Control	0.259**N**	0.325	0.152	0.437	0.475
		AZT-H	0.081	**0.024***	0.139	0.161
Poly-3 test			**3TC-H**	0.385	0.506**N**	0.464**N**
				NVP-H	0.292**N**	0.255**N**
					AZT/3TC-H	0.576**N**
Litter-adjusted correlation	–0.16					
Litter-adjusted Poly-3 rate	44.0%	28.5%	52.7%	61.0%	50.2%	47.5%
	Vehicle Control	0.134**N**	0.272	0.110	0.338	0.410
		AZT-H	0.029*	0.004**	0.053	0.088
Litter-adjusted Poly-3 test			**3TC-H**	0.253	0.429**N**	0.360**N**
				NVP-H	0.209**N**	0.166**N**
					AZT/3TC-H	0.428**N**
Sire-adjusted correlation	0.01					
Sire-adjusted Poly-3 rate	42.9%	28.8%	53.8%	62.4%	49.6%	48.1%
	Vehicle Control	0.131**N**	0.235	0.064	0.341	0.366
		AZT-H	0.031*	0.004**	0.083	0.100
Sire-adjusted Poly-3 test			**3TC-H**	0.225	0.375**N**	0.326**N**
				NVP-H	0.159**N**	0.106**N**
					AZT/3TC-H	0.457**N**
Nose: Hyaline Droplet						
Overall rate	0/25 (0.0%)	0/24 (0.0%)	1/25 (4.0%)	0/25 (0.0%)	2/25 (8.0%)	0/24 (0.0%)
Adjusted rate	0/22.8 (0.0%)	0/19.9 (0.0%)	1/23.6 (4.2%)	0/22.4 (0.0%)	2/22.2 (9.0%)	0/22.9 (0.0%)
Terminal incidence	0/20 (0.0%)	0/18 (0.0%)	1/23 (4.3%)	0/22 (0.0%)	2/21 (9.5%)	0/22 (0.0%)
First incidence (days)	—	—	319 (T)	—	321 (T)	—
Average severity	—	—	2.0	—	1.5	—
	Vehicle Control	—	0.507	—	0.229	—
		AZT-H	0.534	—	0.260	—
Poly-3 test			**3TC-H**	0.510**N**	0.478	0.507**N**
				NVP-H	0.232	—
					AZT/3TC-H	0.228**N**
Litter-adjusted correlation	–0.06					
Litter-adjusted Poly-3 rate	0.0%	0.0%	4.3%	0.0%	9.2%	0.0%
	Vehicle Control	—	0.146	—	0.055	—
		AZT-H	0.146	—	0.055	—
Litter-adjusted Poly-3 test			**3TC-H**	0.146**N**	0.244	0.146**N**
				NVP-H	0.055	—
					AZT/3TC-H	0.055**N**
Sire-adjusted correlation	0.03					
Sire-adjusted Poly-3 rate	0.0%	0.0%	4.1%	0.0%	8.8%	0.0%
	Vehicle Control	—	0.150	—	0.047*	—
		AZT-H	0.150	—	0.047*	—
Sire-adjusted Poly-3 test			**3TC-H**	0.150**N**	0.130	0.150**N**
				NVP-H	0.047*	—
					AZT/3TC-H	0.047**N***

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Preputial Gland: Acinus Degeneration						
Overall rate	3/25 (12.0%)	2/24 (8.3%)	3/25 (12.0%)	3/25 (12.0%)	1/24 (4.2%)	5/23 (21.7%)
Adjusted rate	3/22.9 (13.1%)	2/20.2 (9.9%)	3/23.6 (12.7%)	3/22.4 (13.4%)	1/21.4 (4.7%)	5/23.0 (21.7%)
Terminal incidence	2/20 (10.0%)	1/18 (5.6%)	3/23 (13.0%)	3/22 (13.6%)	1/21 (4.8%)	4/22 (18.2%)
First incidence (days)	310	285	319 (T)	319 (T)	318 (T)	307
Average severity	3.7	3.0	3.0	2.0	3.0	2.4
	Vehicle Control	0.558**N**	0.652**N**	0.657	0.328**N**	0.352
		AZT-H	0.572	0.548	0.480**N**	0.263
Poly-3 test			**3TC-H**	0.642	0.339**N**	0.336
				NVP-H	0.320**N**	0.365
					AZT/3TC-H	0.109
Litter-adjusted correlation	–0.13					
Litter-adjusted Poly-3 rate	13.7%	9.2%	12.7%	13.4%	4.3%	21.6%
	Vehicle Control	0.313**N**	0.456**N**	0.486**N**	0.117**N**	0.224
		AZT-H	0.351	0.327	0.261**N**	0.113
Litter-adjusted Poly-3 test			**3TC-H**	0.471	0.138**N**	0.193
				NVP-H	0.127**N**	0.216
					AZT/3TC-H	0.028*
Sire-adjusted correlation	–0.04					
Sire-adjusted Poly-3 rate	12.8%	9.3%	12.1%	13.9%	4.6%	22.8%
	Vehicle Control	0.355**N**	0.469**N**	0.455	0.156**N**	0.180
		AZT-H	0.386	0.319	0.282**N**	0.109
Sire-adjusted Poly-3 test			**3TC-H**	0.427	0.177**N**	0.187
				NVP-H	0.065**N**	0.195
					AZT/3TC-H	0.010**
Preputial Gland: Duct Ectasia						
Overall rate	3/25 (12.0%)	2/24 (8.3%)	2/25 (8.0%)	4/25 (16.0%)	1/24 (4.2%)	5/23 (21.7%)
Adjusted rate	3/22.9 (13.1%)	2/20.2 (9.9%)	2/23.6 (8.5%)	4/22.4 (17.8%)	1/21.4 (4.7%)	5/23.0 (21.7%)
Terminal incidence	2/20 (10.0%)	1/18 (5.6%)	2/23 (8.7%)	4/22 (18.2%)	1/21 (4.8%)	4/22 (18.2%)
First incidence (days)	310	285	319 (T)	319 (T)	318 (T)	307
Average severity	3.3	3.0	3.5	2.0	2.0	2.4
	Vehicle Control	0.558**N**	0.486**N**	0.488	0.328**N**	0.352
		AZT-H	0.639**N**	0.383	0.480**N**	0.263
Poly-3 test			**3TC-H**	0.309	0.535**N**	0.196
				NVP-H	0.186**N**	0.516
					AZT/3TC-H	0.109
Litter-adjusted correlation	–0.16					
Litter-adjusted Poly-3 rate	13.9%	9.0%	8.9%	18.0%	4.1%	21.6%
	Vehicle Control	0.298**N**	0.286**N**	0.337	0.109**N**	0.230
		AZT-H	0.496**N**	0.173	0.260**N**	0.108
Litter-adjusted Poly-3 test			**3TC-H**	0.160	0.247**N**	0.097
				NVP-H	0.049**N***	0.372
					AZT/3TC-H	0.027*
Sire-adjusted correlation	–0.05					
Sire-adjusted Poly-3 rate	12.7%	9.2%	8.0%	18.2%	4.6%	23.1%
	Vehicle Control	0.353**N**	0.286**N**	0.295	0.159**N**	0.172
		AZT-H	0.446**N**	0.188	0.287**N**	0.104
Sire-adjusted Poly-3 test			**3TC-H**	0.134	0.323**N**	0.090
				NVP-H	0.024**N***	0.326
					AZT/3TC-H	0.008**

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Salivary Glands: Cellular Infiltration						
Overall rate	18/25 (72.0%)	12/23 (52.2%)	13/25 (52.0%)	18/25 (72.0%)	11/24 (45.8%)	16/23 (69.6%)
Adjusted rate	18/23.2 (77.7%)	12/19.9 (60.3%)	13/23.6 (55.0%)	18/22.4 (80.2%)	11/21.4 (51.5%)	16/22.8 (70.1%)
Terminal incidence	17/20 (85.0%)	12/18 (66.7%)	13/23 (56.5%)	18/22 (81.8%)	11/21 (52.4%)	16/22 (72.7%)
First incidence (days)	279	318 (T)	318 (T)	319 (T)	316 (T)	316 (T)
Average severity	1.2	1.1	1.2	1.2	1.2	1.4
	Vehicle Control	0.174**N**	0.085**N**	0.565	0.058**N**	0.399**N**
		AZT-H	0.482**N**	0.131	0.397**N**	0.364
Poly-3 test			**3TC-H**	0.060	0.523**N**	0.225
				NVP-H	0.040**N***	0.329**N**
					AZT/3TC-H	0.168
Litter-adjusted correlation	–0.01					
Litter-adjusted Poly-3 rate	77.6%	60.2%	54.9%	80.2%	51.4%	70.1%
	Vehicle Control	0.084**N**	0.036**N***	0.406	0.027**N***	0.282**N**
		AZT-H	0.352**N**	0.057	0.278**N**	0.250
Litter-adjusted Poly-3 test			**3TC-H**	0.022*	0.409**N**	0.148
				NVP-H	0.017**N***	0.219**N**
					AZT/3TC-H	0.113
Sire-adjusted correlation	0.15					
Sire-adjusted Poly-3 rate	76.8%	66.5%	58.6%	82.3%	53.1%	76.6%
	Vehicle Control	0.199**N**	0.048**N***	0.341	0.027**N***	0.494**N**
		AZT-H	0.265**N**	0.112	0.174**N**	0.139
Sire-adjusted Poly-3 test			**3TC-H**	0.033*	0.333**N**	0.074
				NVP-H	0.024**N***	0.337**N**
					AZT/3TC-H	0.054
Spleen: Hyperplasia						
Overall rate	12/25 (48.0%)	14/23 (60.9%)	16/24 (66.7%)	18/25 (72.0%)	14/24 (58.3%)	12/23 (52.2%)
Adjusted rate	12/24.0 (49.9%)	14/19.2 (72.8%)	16/23.5 (68.2%)	18/22.4 (80.2%)	14/22.1 (63.2%)	12/22.8 (52.6%)
Terminal incidence	9/20 (45.0%)	14/18 (77.8%)	16/23 (69.6%)	18/22 (81.8%)	13/21 (61.9%)	12/22 (54.5%)
First incidence (days)	207	316 (T)	318 (T)	319 (T)	203	316 (T)
Average severity	2.4	2.3	2.1	2.3	2.5	2.4
	Vehicle Control	0.109	0.161	0.027*	0.271	0.544
		AZT-H	0.504**N**	0.421	0.373**N**	0.150**N**
Poly-3 test			**3TC-H**	0.277	0.483**N**	0.215**N**
				NVP-H	0.175**N**	0.044**N***
					AZT/3TC-H	0.338**N**
Litter-adjusted correlation	–0.03					
Litter-adjusted Poly-3 rate	49.9%	72.9%	68.1%	80.4%	63.3%	52.8%
	Vehicle Control	0.052	0.104	0.013*	0.187	0.423
		AZT-H	0.358**N**	0.269	0.241**N**	0.065**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.165	0.365**N**	0.131**N**
				NVP-H	0.097**N**	0.015**N***
					AZT/3TC-H	0.232**N**
Sire-adjusted correlation	0.21					
Sire-adjusted Poly-3 rate	54.1%	73.1%	73.4%	77.7%	62.8%	50.5%
	Vehicle Control	**0.037***	0.069	0.016*	0.194	0.403**N**
		AZT-H	0.488	0.369	0.121**N**	0.032**N***
Sire-adjusted Poly-3 test			**3TC-H**	0.373	0.160**N**	0.070**N**
				NVP-H	0.111**N**	0.038**N***
					AZT/3TC-H	0.193**N**

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Thymus: Hyperplasia						
Overall rate	2/24 (8.3%)	0/22 (0.0%)	0/24 (0.0%)	1/23 (4.3%)	3/22 (13.6%)	1/20 (5.0%)
Adjusted rate	2/22.6 (8.8%)	0/19.2 (0.0%)	0/23.2 (0.0%)	1/22.3 (4.5%)	3/20.4 (14.7%)	1/20.0 (5.0%)
Terminal incidence	1/20 (5.0%)	0/18 (0.0%)	0/23 (0.0%)	1/22 (4.5%)	3/20 (15.0%)	1/20 (5.0%)
First incidence (days)	207	—	—	322 (T)	319 (T)	320 (T)
Average severity	2.5	—	—	2.0	2.0	1.0
	Vehicle Control	0.272**N**	0.229**N**	0.506**N**	0.450	0.544**N**
		AZT-H	—	0.529	0.122	0.508
Poly-3 test			**3TC-H**	0.492	0.090	0.471
				NVP-H	0.269	0.736
					AZT/3TC-H	0.307**N**
Litter-adjusted correlation	–0.07					
Litter-adjusted Poly-3 rate	8.9%	0.0%	0.0%	4.6%	14.7%	4.9%
	Vehicle Control	0.063**N**	0.063**N**	0.277**N**	0.267	0.298**N**
		AZT-H	—	0.145	0.023*	0.156
Litter-adjusted Poly-3 test			**3TC-H**	0.145	0.023*	0.155
				NVP-H	0.119	0.483
					AZT/3TC-H	0.132**N**
Sire-adjusted correlation	0.02					
Sire-adjusted Poly-3 rate	8.8%	0.0%	0.0%	4.4%	14.6%	5.0%
	Vehicle Control	0.065**N**	0.065**N**	0.273**N**	0.310	0.306**N**
		AZT-H	—	0.152	0.054	0.158
Sire-adjusted Poly-3 test			**3TC-H**	0.150	0.054	0.158
				NVP-H	0.123	0.462
					AZT/3TC-H	0.175**N**

TABLE G9
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Urinary Bladder: Cellular Infiltration						
Overall rate	2/25 (8.0%)	0/23 (0.0%)	0/25 (0.0%)	3/25 (12.0%)	1/24 (4.2%)	1/23 (4.3%)
Adjusted rate	2/22.8 (8.8%)	0/19.9 (0.0%)	0/23.6 (0.0%)	3/23.2 (13.0%)	1/21.4 (4.7%)	1/22.8 (4.4%)
Terminal incidence	2/20 (10.0%)	0/18 (0.0%)	0/23 (0.0%)	2/22 (9.1%)	1/21 (4.8%)	1/22 (4.5%)
First incidence (days)	320 (T)			215	321 (T)	325 (T)
Average severity	1.0	—	—	3.0	1.0	1.0
	Vehicle Control	0.266**N**	0.226**N**	0.508	0.522**N**	0.499**N**
		AZT-H	—	0.142	0.514	0.527
Poly-3 test			**3TC-H**	0.110	0.480	0.493
				NVP-H	0.332**N**	0.307**N**
					AZT/3TC-H	0.746**N**
Litter-adjusted correlation	–0.06					
Litter-adjusted Poly-3 rate	8.5%	0.0%	0.0%	13.0%	4.8%	4.6%
	Vehicle Control	0.074**N**	0.074**N**	0.309	0.306**N**	0.292**N**
		AZT-H	—	0.025*	0.144	0.144
Litter-adjusted Poly-3 test			**3TC-H**	0.025*	0.144	0.144
				NVP-H	0.154**N**	0.143**N**
					AZT/3TC-H	0.485**N**
Sire-adjusted correlation	–0.05					
Sire-adjusted Poly-3 rate	8.7%	0.1%	0.0%	12.7%	4.9%	4.8%
	Vehicle Control	0.061**N**	0.059**N**	0.328	0.302**N**	0.290**N**
		AZT-H	—	0.028*	0.137	0.130
Sire-adjusted Poly-3 test			**3TC-H**	0.027*	0.133	0.126
				NVP-H	0.183**N**	0.163**N**
					AZT/3TC-H	0.496**N**

(T) Terminal kill

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Number of lesion-bearing animals/number of animals with tissue examined microscopically

[c] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the group stated to the left on the same row. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

TABLE G10
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Kidney: Hydronephrosis				
Overall rate[b]	0/25 (0.0%)	0/25 (0.0%)	0/22 (0.0%)	3/25 (12.0%)
Adjusted rate[c]	0/25.0 (0.0%)	0/23.4 (0.0%)	0/19.7 (0.0%)	3/24.5 (12.2%)
Terminal rate[d]	0/25 (0.0%)	0/22 (0.0%)	0/19 (0.0%)	1/21 (4.8%)
First incidence (days)	—[g]	—	—	247
Average severity	—[g]	—	—	3.0
Poly-3 test[e]	0.015*	—[h]	—	0.110
Litter-adjusted correlation	–0.04			
Litter-adjusted Poly-3 rate	0.0%	0.0%	0.0%	12.3%
Litter-adjusted Poly-3 test	0.026*	—	—	0.026*
Sire-adjusted correlation	–0.01			
Sire-adjusted Poly-3 rate	0.0%	0.0%	0.0%	12.3%
Sire-adjusted Poly-3 test	0.026*	—	—	0.026*
JT/SW test[f]	0.010*	0.500	0.583	0.008**
Liver: Centrilobular Degeneration				
Overall rate	0/25 (0.0%)	0/25 (0.0%)	0/22 (0.0%)	3/25 (12.0%)
Adjusted rate	0/25.0 (0.0%)	0/23.4 (0.0%)	0/19.7 (0.0%)	3/23.8 (12.6%)
Terminal incidence (days)	0/25 (0.0%)	0/22 (0.0%)	0/19 (0.0%)	2/21 (9.5%)
First incidence (days)	—	—	—	312
Average severity	—	—	—	2.7
Poly-3 test	0.015*	—	—	0.105
Litter-adjusted correlation	–0.06			
Litter-adjusted Poly-3 rate	0.0%	0.0%	0.0%	12.7%
Litter-adjusted Poly-3 test	0.024*	—	—	0.024*
Sire-adjusted correlation	–0.01			
Sire-adjusted Poly-3 rate	0.0%	0.0%	0.0%	12.6%
Sire-adjusted Poly-3 test	0.024*	—	—	0.024*
JT/SW test	0.010*	0.500	0.583	0.008**
Liver: Cellular Infiltration				
Overall rate	1/25 (4.0%)	2/25 (8.0%)	4/22 (18.2%)	4/25 (16.0%)
Adjusted rate	1/25.0 (4.0%)	2/23.4 (8.6%)	4/19.7 (20.3%)	4/23.7 (16.9%)
Terminal incidence (days)	1/25 (4.0%)	2/22 (9.1%)	4/19 (21.1%)	4/21 (19.0%)
First incidence (days)	318 (T)	319 (T)	318 (T)	318 (T)
Average severity	1.0	1.0	1.3	1.3
Poly-3 test	0.063	0.476	0.106	0.157
Litter-adjusted correlation	0.10			
Litter-adjusted Poly-3 rate	4.2%	8.4%	20.5%	17.2%
Litter-adjusted Poly-3 test	0.039*	0.269	0.081	0.068
Sire-adjusted correlation	0.01			
Sire-adjusted Poly-3 rate	4.0%	8.2%	20.5%	16.8%
Sire-adjusted Poly-3 test	0.015*	0.279	0.076	0.066
JT/SW test	0.051	0.278	0.057	0.089

TABLE G10
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Liver: Cytoplasmic Vacuolization				
Overall rate	14/25 (56.0%)	17/25 (68.0%)	13/22 (59.1%)	15/25 (60.0%)
Adjusted rate	14/25.0 (56.0%)	17/23.4 (72.8%)	13/19.7 (66.0%)	15/23.8 (62.9%)
Terminal incidence (days)	14/25 (56.0%)	17/22 (77.3%)	13/19 (68.4%)	14/21 (66.7%)
First incidence (days)	316 (T)	315 (T)	318 (T)	312
Average severity	1.1	1.2	1.3	1.7
Poly-3 test	0.395	0.179	0.357	0.422
Litter-adjusted correlation	0.17			
Litter-adjusted Poly-3 rate	56.5%	72.9%	66.5%	63.6%
Litter-adjusted Poly-3 test	0.369	0.138	0.242	0.305
Sire-adjusted correlation	0.13			
Sire-adjusted Poly-3 rate	51.8%	74.7%	60.2%	62.9%
Sire-adjusted Poly-3 test	0.360	0.074	0.291	0.252
JT/SW test	0.113	0.109	0.201	0.108
Lung: Infiltration Cellular				
Overall rate	0/25 (0.0%)	1/25 (4.0%)	0/22 (0.0%)	3/25 (12.0%)
Adjusted rate	0/25.0 (0.0%)	1/23.4 (4.3%)	0/19.7 (0.0%)	3/23.7 (12.6%)
Terminal incidence (days)	0/25 (0.0%)	1/22 (4.5%)	0/19 (0.0%)	3/21 (14.3%)
First incidence (days)	—	318 (T)	—	320 (T)
Average severity	—	1.0	—	1.7
Poly-3 test	0.047*	0.486	—	0.104
Litter-adjusted correlation	0.23			
Litter-adjusted Poly-3 rate	0.0%	4.1%	0.0%	11.6%
Litter-adjusted Poly-3 test	0.100	0.147	—	0.071
Sire-adjusted correlation	0.03			
Sire-adjusted Poly-3 rate	0.0%	4.0%	0.1%	12.7%
Sire-adjusted Poly-3 test	0.096	0.165	—	0.067
JT/SW test	0.033*	0.159	0.321	0.019*
Nose: Hyaline Droplet				
Overall rate	1/25 (4.0%)	4/25 (16.0%)	1/22 (4.5%)	6/25 (24.0%)
Adjusted rate	1/25.0 (4.0%)	4/23.4 (17.1%)	1/19.7 (5.1%)	6/23.7 (25.3%)
Terminal incidence (days)	1/25 (4.0%)	4/22 (18.2%)	1/19 (5.3%)	6/21 (28.6%)
First incidence (days)	320 (T)	318 (T)	318 (T)	318 (T)
Average severity	1.0	1.8	1.0	1.8
Poly-3 test	0.046*	0.152	0.705	0.040*
Litter-adjusted correlation	0.31			
Litter-adjusted Poly-3 rate	4.6%	15.9%	6.6%	26.1%
Litter-adjusted Poly-3 test	0.058	0.120	0.401	0.027*
Sire-adjusted correlation	–0.03			
Sire-adjusted Poly-3 rate	4.1%	17.1%	5.0%	25.0%
Sire-adjusted Poly-3 test	0.074	0.091	0.443	0.030*
JT/SW test	0.040*	0.076	0.241	0.015*

TABLE G10
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Pancreas: Cellular Infiltration				
Overall rate	2/25 (8.0%)	3/25 (12.0%)	5/21 (23.8%)	2/24 (8.3%)
Terminal incidence (days)	2/25 (8.0%)	3/22 (13.6%)	5/19 (26.3%)	1/21 (4.8%)
First incidence (days)	316 (T)	318 (T)	318 (T)	302
Average severity	1.0	1.3	1.0	2.0
Adjusted rate	2/25.0 (8.0%)	3/23.4 (12.8%)	5/19.7 (25.4%)	2/23.5 (8.5%)
Poly-3 test	0.393	0.469	0.119	0.673
Litter-adjusted correlation	–0.25			
Litter-adjusted Poly-3 rate	9.0%	13.6%	25.9%	8.3%
Litter-adjusted Poly-3 test	0.346	0.295	0.045*	0.467**N**
Sire-adjusted correlation	–0.11			
Sire-adjusted Poly-3 rate	8.6%	13.9%	21.5%	7.8%
Sire-adjusted Poly-3 test	0.420	0.260	0.091	0.460**N**
JT/SW test	0.308	0.307	0.078	0.262
Salivary Glands: Cellular Infiltration				
Overall rate	22/25 (88.0%)	18/25 (72.0%)	12/22 (54.5%)	20/25 (80.0%)
Adjusted rate	22/25.0 (88.0%)	18/25.0 (72.0%)	12/19.7 (61.0%)	20/23.7 (84.3%)
Terminal incidence (days)	22/25 (88.0%)	15/22 (68.2%)	12/19 (63.2%)	20/21 (95.2%)
First incidence (days)	316 (T)	186	318 (T)	317 (T)
Average severity	1.4	1.5	1.3	1.4
Poly-3 test	0.328**N**	0.144**N**	0.035**N***	0.516**N**
Litter-adjusted correlation	–0.12			
Litter-adjusted Poly-3 rate	88.3%	72.3%	59.9%	84.4%
Litter-adjusted Poly-3 test	0.225**N**	0.053**N**	0.004**N****	0.347**N**
Sire-adjusted correlation	–0.06			
Sire-adjusted Poly-3 rate	88.9%	70.7%	60.0%	84.0%
Sire-adjusted Poly-3 test	0.229**N**	0.009**N****	0.002**N****	0.317**N**
JT/SW test	0.175**N**	0.173**N**	0.012**N***	0.098**N**

TABLE G10
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Spleen: Hematopoietic Cell Proliferation				
Overall rate	2/25 (8.0%)	2/25 (8.0%)	8/22 (36.4%)	7/25 (28.0%)
Adjusted rate	2/25.0 (8.0%)	2/23.9 (8.4%)	8/22.0 (36.4%)	7/23.8 (29.4%)
Terminal incidence (days)	2/25 (8.0%)	1/22 (4.5%)	5/19 (26.3%)	6/21 (28.6%)
First incidence (days)	316 (T)	249	101	312
Average severity	2.5	2.5	2.0	2.6
Poly-3 test	0.007**	0.679	0.018*	0.056
Litter-adjusted correlation	–0.18			
Litter-adjusted Poly-3 rate	8.7%	8.7%	37.0%	30.4%
Litter-adjusted Poly-3 test	0.003**	0.499	0.004**	0.023*
Sire-adjusted correlation	–0.04			
Sire-adjusted Poly-3 rate	7.9%	9.1%	36.3%	29.5%
Sire-adjusted Poly-3 test	0.003**	0.442	0.004**	0.018*
JT/SW test	0.007**	0.500	0.008**	0.024*

(T) Terminal kill
[a] AZT/3TC/NVP-L = 80/50/56 mg/kg; AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg
[b] Number of lesion-bearing animals/number of animals examined microscopically
[c] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Beneath the vehicle control incidence is the P value associated with the Jonckheere/Terpstra monotonic trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group by William's modified Shirley's test. A negative trend or lower incidence in a dose group is indicated by **N**.
[g] Not applicable, no lesions in animal group
[h] Value of statistic cannot be computed.

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Brain, Cerebrum: Mineralization						
Overall rate[b]	2/25 (8.0%)	0/24 (0.0%)	0/25 (0.0%)	3/24 (12.5%)	0/23 (0.0%)	0/25 (0.0%)
Adjusted rate[c]	2/25.0 (8.0%)	0/23.1 (0.0%)	0/23.2 (0.0%)	3/22.7 (13.2%)	0/20.7 (0.0%)	0/23.7 (0.0%)
Terminal incidence[d]	2/25 (8.0%)	0/20 (0.0%)	0/20 (0.0%)	3/20 (15.0%)	0/19 (0.0%)	0/21 (0.0%)
First incidence (days)	318 (T)	—[f]	—	319 (T)	—	—
Average severity	1.0	—[f]	—	1.0	—	—
	Vehicle Control	0.253**N**	0.252**N**	0.455	0.279**N**	0.247**N**
		AZT-H	—[g]	0.110	—	—
Poly-3 test[e]			**3TC-H**	0.109	—	—
				NVP-H	0.130**N**	0.105**N**
					AZT/3TC-H	—
Litter-adjusted correlation	0.02					
Litter-adjusted Poly-3 rate	8.1%	0.0%	0.0%	13.3%	0.0%	0.0%
	Vehicle Control	0.075**N**	0.075**N**	0.282	0.075**N**	0.075**N**
		AZT-H	—	0.031*	—	—
Litter-adjusted Poly-3 test			**3TC-H**	0.031*	—	—
				NVP-H	0.031**N***	0.031**N***
					AZT/3TC-H	—
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	8.0%	0.0%	0.0%	13.2%	0.0%	0.0%
	Vehicle Control	0.076**N**	0.076**N**	0.289	0.076**N**	0.076**N**
		AZT-H	—	0.031*	—	—
Sire-adjusted Poly-3 test			**3TC-H**	0.031*	—	—
				NVP-H	0.031**N***	0.031**N***
					AZT/3TC-H	—
Clitoral Gland: Acinus Degeneration						
Overall rate	9/24 (37.5%)	12/24 (50.0%)	6/23 (26.1%)	5/21 (23.8%)	6/21 (28.6%)	11/22 (50.0%)
Adjusted rate	9/24.0 (37.5%)	12/23.4 (51.3%)	6/21.7 (27.7%)	5/20.6 (24.3%)	6/19.2 (31.3%)	11/22.0 (50.0%)
Terminal incidence	9/24 (37.5%)	10/20 (50.0%)	6/20 (30.0%)	3/18 (16.7%)	5/17 (29.4%)	9/20 (45.0%)
First incidence (days)	316 (T)	303	318 (T)	257	264	275
Average severity	2.9	3.2	3.0	3.4	3.2	2.7
	Vehicle Control	0.255	0.349**N**	0.270**N**	0.460**N**	0.292
		AZT-H	0.091**N**	0.060**N**	0.158**N**	0.581**N**
Poly-3 test			**3TC-H**	0.541**N**	0.534	0.112
				NVP-H	0.446	0.076
					AZT/3TC-H	0.186
Litter-adjusted correlation	0.03					
Litter-adjusted Poly-3 rate	37.7%	51.3%	27.6%	24.5%	31.2%	50.0%
	Vehicle Control	0.194	0.235**N**	0.165**N**	0.305**N**	0.185
		AZT-H	0.069**N**	0.043**N***	0.089**N**	0.467**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.411**N**	0.393	0.055
				NVP-H	0.299	0.030*
					AZT/3TC-H	0.071
Sire-adjusted correlation	0.01					
Sire-adjusted Poly-3 rate	37.5%	51.2%	27.6%	24.2%	31.3%	49.8%
	Vehicle Control	0.169	0.265**N**	0.188**N**	0.322**N**	0.179
		AZT-H	0.094**N**	0.023**N***	0.052**N**	0.464**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.414**N**	0.385	0.074
				NVP-H	0.259	0.015*
					AZT/3TC-H	0.075

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Harderian Gland: Cellular Infiltration						
Overall rate	2/25 (8.0%)	0/24 (0.0%)	0/25 (0.0%)	0/25 (0.0%)	1/23 (4.3%)	0/24 (0.0%)
Adjusted rate	2/25.0 (8.0%)	0/23.1 (0.0%)	0/23.2 (0.0%)	0/23.2 (0.0%)	1/20.7 (4.8%)	0/23.3 (0.0%)
Terminal incidence	2/25 (8.0%)	0/20 (0.0%)	0/20 (0.0%)	0/20 (0.0%)	1/19 (5.3%)	0/21 (0.0%)
First incidence (days)	316 (T)	—	—	—	320 (T)	—
Average severity	1.0	—	—	—	1.0	—
	Vehicle Control	0.253**N**	0.252**N**	0.253**N**	0.566**N**	0.251**N**
		AZT-H	—	—	0.478	—
Poly-3 test			**3TC-H**	—	0.477	—
				NVP-H	0.478	—
					AZT/3TC-H	0.476**N**
Litter-adjusted correlation	–0.03					
Litter-adjusted Poly-3 rate	8.1%	0.0%	0.0%	0.0%	4.8%	0.0%
	Vehicle Control	0.057**N**	0.057**N**	0.057**N**	0.314**N**	0.057**N**
		AZT-H	—	—	0.156	—
Litter-adjusted Poly-3 test			**3TC-H**	—	0.156	—
				NVP-H	0.156	—
					AZT/3TC-H	0.156**N**
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	8.1%	0.0%	0.0%	0.0%	4.8%	0.0%
	Vehicle Control	0.048**N***	0.048**N***	0.048**N***	0.322**N**	0.048**N***
		AZT-H	—	—	0.157	—
Sire-adjusted Poly-3 test			**3TC-H**	—	0.157	—
				NVP-H	0.156	—
					AZT/3TC-H	0.157**N**
Kidney: Protein Casts						
Overall rate	14/25 (56.0%)	14/24 (58.3%)	8/24 (33.3%)	10/24 (41.7%)	12/23 (52.2%)	15/25 (60.0%)
Adjusted rate	14/25.0 (56.0%)	14/23.3 (60.0%)	8/22.5 (35.6%)	10/22.8 (43.9%)	12/20.7 (58.0%)	15/23.7 (63.2%)
Terminal incidence	14/25 (56.0%)	13/20 (65.0%)	8/20 (40.0%)	9/20 (45.0%)	12/19 (63.2%)	15/21 (71.4%)
First incidence (days)	316 (T)	297	318 (T)	257	316 (T)	317 (T)
Average severity	1.1	1.0	1.0	1.0	1.0	1.2
	Vehicle Control	0.504	0.131**N**	0.294**N**	0.565	0.413
		AZT-H	0.083**N**	0.210**N**	0.567**N**	0.530
Poly-3 test			**3TC-H**	0.396	0.117	0.051
				NVP-H	0.266	0.148
					AZT/3TC-H	0.480
Litter-adjusted correlation	–0.08					
Litter-adjusted Poly-3 rate	56.7%	60.0%	35.8%	43.8%	57.6%	63.3%
	Vehicle Control	0.393	0.077**N**	0.185**N**	0.473	0.302
		AZT-H	0.041**N***	0.118**N**	0.425**N**	0.389
Litter-adjusted Poly-3 test			**3TC-H**	0.308	0.074	0.029*
				NVP-H	0.175	0.086
					AZT/3TC-H	0.332
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	56.1%	59.9%	35.4%	44.1%	58.3%	63.4%
	Vehicle Control	0.362	0.078**N**	0.218**N**	0.438	0.264
		AZT-H	0.042**N***	0.117**N**	0.449**N**	0.389
Sire-adjusted Poly-3 test			**3TC-H**	0.308	0.065	0.036*
				NVP-H	0.145	0.094
					AZT/3TC-H	0.354

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Kidney: Cellular Infiltration						
Overall rate	11/25 (44.0%)	10/24 (41.7%)	11/24 (45.8%)	15/24 (62.5%)	11/23 (47.8%)	11/25 (44.0%)
Adjusted rate	11/25.0 (44.0%)	10/23.1 (43.3%)	11/22.8 (48.2%)	15/22.3 (67.3%)	11/21.1 (52.2%)	11/23.7 (46.4%)
Terminal incidence	11/25 (44.0%)	10/20 (50.0%)	10/20 (50.0%)	15/20 (75.0%)	10/19 (52.6%)	11/21 (52.4%)
First incidence (days)	316 (T)	316 (T)	277	318 (T)	280	318 (T)
Average severity	1.4	1.2	1.0	1.1	1.3	1.2
	Vehicle Control	0.594**N**	0.501	0.090	0.399	0.548
		AZT-H	0.487	0.086	0.386	0.533
Poly-3 test			**3TC-H**	0.153	0.513	0.567**N**
				NVP-H	0.237**N**	0.122**N**
					AZT/3TC-H	0.463**N**
Litter-adjusted correlation	–0.16					
Litter-adjusted Poly-3 rate	44.6%	41.8%	47.7%	66.3%	51.3%	45.6%
	Vehicle Control	0.416**N**	0.411	0.030*	0.309	0.468
		AZT-H	0.345	0.025*	0.254	0.392
Litter-adjusted Poly-3 test			**3TC-H**	0.084	0.406	0.444**N**
				NVP-H	0.127**N**	0.051**N**
					AZT/3TC-H	0.346**N**
Sire-adjusted correlation	0.04					
Sire-adjusted Poly-3 rate	44.2%	43.7%	49.7%	67.1%	51.9%	45.5%
	Vehicle Control	0.483**N**	0.354	0.035*	0.308	0.455
		AZT-H	0.334	0.016*	0.281	0.440
Sire-adjusted Poly-3 test			**3TC-H**	0.114	0.437	0.391**N**
				NVP-H	0.109**N**	0.035**N***
					AZT/3TC-H	0.330**N**
Kidney: Hydronephrosis						
Overall rate	0/25 (0.0%)	0/24 (0.0%)	0/24 (0.0%)	0/24 (0.0%)	0/23 (0.0%)	3/25 (12.0%)
Adjusted rate	0/25.0 (0.0%)	0/23.1 (0.0%)	0/22.5 (0.0%)	0/22.3 (0.0%)	0/20.7 (0.0%)	3/24.5 (12.2%)
Terminal incidence	0/25 (0.0%)	0/20 (0.0%)	0/20 (0.0%)	0/20 (0.0%)	0/19 (0.0%)	1/21 (4.8%)
First incidence (days)	—	—	—	—	—	247
Average severity	—	—	—	—	—	3.0
	Vehicle Control	—	—	—	—	0.110
		AZT-H	—	—	—	0.124
Poly-3 test			**3TC-H**	—	—	0.130
				NVP-H	—	0.131
					AZT/3TC-H	0.146
Litter-adjusted correlation	–0.03					
Litter-adjusted Poly-3 rate	0.0%	0.0%	0.0%	0.0%	0.0%	12.3%
	Vehicle Control	—	—	—	—	0.026*
		AZT-H	—	—	—	0.026*
Litter-adjusted Poly-3 test			**3TC-H**	—	—	0.026*
				NVP-H	—	0.026*
					AZT/3TC-H	0.026*
Sire-adjusted correlation	0.00					
Sire-adjusted Poly-3 rate	0.0%	0.0%	0.0%	0.0%	0.0%	12.3%
	Vehicle Control	—	—	—	—	0.026*
		AZT-H	—	—	—	0.026*
Sire-adjusted Poly-3 test			**3TC-H**	—	—	0.026*
				NVP-H	—	0.026*
					AZT/3TC-H	0.026*

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Liver: Centrilobular Degeneration						
Overall rate	0/25 (0.0%)	0/25 (0.0%)	0/23 (0.0%)	1/24 (4.2%)	0/23 (0.0%)	3/25 (12.0%)
Adjusted rate	0/25.0 (0.0%)	0/23.8 (0.0%)	0/21.7 (0.0%)	1/22.3 (4.5%)	0/20.7 (0.0%)	3/23.8 (12.6%)
Terminal incidence	0/25 (0.0%)	0/20 (0.0%)	0/20 (0.0%)	1/20 (5.0%)	0/19 (0.0%)	2/21 (9.5%)
First incidence (days)	—	—	—	320 (T)	—	312
Average severity	—	—	—	2.0	—	2.7
	Vehicle Control	—	—	0.477	—	0.105
		AZT-H	—	0.487	—	0.114
Poly-3 test			**3TC-H**	0.505	—	0.131
				NVP-H	0.515**N**	0.327
					AZT/3TC-H	0.140
Litter-adjusted correlation	–0.06					
Litter-adjusted Poly-3 rate	0.0%	0.0%	0.0%	4.6%	0.0%	12.8%
	Vehicle Control	—	—	0.145	—	0.024*
		AZT-H	—	0.145	—	0.024*
Litter-adjusted Poly-3 test			**3TC-H**	0.145	—	0.024*
				NVP-H	0.145**N**	0.146
					AZT/3TC-H	0.024*
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	0.0%	0.0%	0.0%	4.5%	0.0%	12.6%
	Vehicle Control	—	—	0.145	—	0.025*
		AZT-H	—	0.145	—	0.025*
Sire-adjusted Poly-3 test			**3TC-H**	0.145	—	0.025*
				NVP-H	0.145**N**	0.147
					AZT/3TC-H	0.025*
Liver: Cytoplasmic Vacuolization						
Overall rate	14/25 (56.0%)	13/25 (52.0%)	14/23 (60.9%)	11/24 (45.8%)	13/23 (56.5%)	15/25 (60.0%)
Adjusted rate	14/25.0 (56.0%)	13/24.1 (54.0%)	14/22.1 (63.3%)	11/22.7 (48.4%)	13/21.1 (61.7%)	15/23.8 (62.9%)
Terminal incidence	14/25 (56.0%)	11/20 (55.0%)	13/20 (65.0%)	10/20 (50.0%)	12/19 (63.2%)	14/21 (66.7%)
First incidence (days)	316 (T)	303	271	268	280	312
Average severity	1.1	1.4	1.3	1.5	1.8	1.7
	Vehicle-Control	0.559**N**	0.417	0.409**N**	0.464	0.422
		AZT-H	0.367	0.464**N**	0.413	0.371
Poly-3 test			**3TC-H**	0.240**N**	0.582**N**	0.610**N**
				NVP-H	0.280	0.240
					AZT/3TC-H	0.590
Litter-adjusted correlation	-0.13					
Litter-adjusted Poly-3 rate	55.5%	54.0%	63.1%	48.2%	60.9%	62.4%
	Vehicle-Control	0.458**N**	0.296	0.296**N**	0.348	0.310
		AZT-H	0.261	0.338**N**	0.309	0.274
Litter-adjusted Poly-3 test			**3TC-H**	0.124**N**	0.433**N**	0.480**N**
				NVP-H	0.155	0.132
					AZT/3TC-H	0.453
Sire-adjusted correlation	-0.05					
Sire-adjusted Poly-3 rate	54.7%	54.5%	62.8%	47.3%	58.1%	60.2%
	Vehicle-Control	0.493**N**	0.296	0.305**N**	0.405	0.369
		AZT-H	0.291	0.305**N**	0.410	0.335
Sire-adjusted Poly-3 test			**3TC-H**	0.127**N**	0.322**N**	0.426**N**
				NVP-H	0.211	0.154
					AZT/3TC-H	0.441

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Liver: Tension Lipidosis						
Overall rate	2/25 (8.0%)	0/25 (0.0%)	2/23 (8.7%)	3/24 (12.5%)	2/23 (8.7%)	3/25 (12.0%)
Adjusted rate	2/25.0 (8.0%)	0/23.8 (0.0%)	2/21.7 (9.2%)	3/22.3 (13.5%)	2/20.7 (9.7%)	3/23.8 (12.6%)
Terminal incidence	2/25 (8.0%)	0/20 (0.0%)	2/20 (10.0%)	3/20 (15.0%)	2/19 (10.5%)	2/21 (9.5%)
First incidence (days)	320 (T)	—	320 (T)	320 (T)	320 (T)	312
Average severity	1.0	—	1.0	1.7	1.5	2.0
	Vehicle Control	0.247**N**	0.643	0.447	0.625	0.478
		AZT-H	0.214	0.101	0.204	0.114
Poly-3 test			**3TC-H**	0.512	0.679	0.544
				NVP-H	0.534**N**	0.634**N**
					AZT/3TC-H	0.565
Litter-adjusted correlation	–0.11					
Litter-adjusted Poly-3 rate	8.0%	0.0%	9.4%	13.4%	9.6%	12.5%
	Vehicle Control	0.066**N**	0.426	0.263	0.420	0.294
		AZT-H	0.056	0.024*	0.063	0.028*
Litter-adjusted Poly-3 test			**3TC-H**	0.329	0.492	0.363
				NVP-H	0.340**N**	0.462**N**
					AZT/3TC-H	0.374
Sire-adjusted correlation	0.03					
Sire-adjusted Poly-3 rate	8.0%	0.0%	9.5%	13.3%	9.6%	12.3%
	Vehicle Control	0.072**N**	0.425	0.302	0.428	0.326
		AZT-H	0.047*	0.025*	0.062	0.031*
Sire-adjusted Poly-3 test			**3TC-H**	0.327	0.491	0.379
				NVP-H	0.319**N**	0.453**N**
					AZT/3TC-H	0.358
Lymph Node, Mandibular: Hyperplasia						
Overall rate	3/25 (12.0%)	0/25 (0.0%)	3/24 (12.5%)	4/24 (16.7%)	2/23 (8.7%)	1/25 (4.0%)
Adjusted rate	3/25.0 (12.0%)	0/23.8 (0.0%)	3/23.4 (12.8%)	4/22.3 (18.0%)	2/20.7 (9.7%)	1/23.7 (4.2%)
Terminal incidence	3/25 (12.0%)	0/20 (0.0%)	1/20 (5.0%)	4/20 (20.0%)	2/19 (10.5%)	1/21 (4.8%)
First incidence (days)	316 (T)	—	258	318 (T)	316 (T)	320 (T)
Average severity	2.3	—	1.7	1.5	1.5	2.0
	Vehicle Control	0.123**N**	0.634	0.436	0.586**N**	0.322**N**
		AZT-H	0.110	0.046*	0.204	0.500
Poly-3 test			**3TC-H**	0.473	0.556**N**	0.297**N**
				NVP-H	0.367**N**	0.152**N**
					AZT/3TC-H	0.452**N**
Litter-adjusted correlation	–0.11					
Litter-adjusted Poly-3 rate	12.2%	0.0%	12.7%	17.5%	9.6%	4.4%
	Vehicle Control	0.025**N***	0.476	0.292	0.386**N**	0.149**N**
		AZT-H	0.026*	0.011*	0.063	0.145
Litter-adjusted Poly-3 test			**3TC-H**	0.315	0.366**N**	0.142**N**
				NVP-H	0.211**N**	0.065**N**
					AZT/3TC-H	0.245**N**
Sire-adjusted correlation	–0.02					
Sire-adjusted Poly-3 rate	12.2%	0.0%	12.6%	18.0%	9.4%	4.2%
	Vehicle Control	0.026**N***	0.480	0.247	0.352**N**	0.155**N**
		AZT-H	0.027*	0.010**	0.069	0.145
Sire-adjusted Poly-3 test			**3TC-H**	0.306	0.357**N**	0.137**N**
				NVP-H	0.202**N**	0.068**N**
					AZT/3TC-H	0.262**N**

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Nose: Hyaline Droplet						
Overall rate	1/25 (4.0%)	3/25 (12.0%)	4/25 (16.0%)	1/24 (4.2%)	6/24 (25.0%)	6/25 (24.0%)
Adjusted rate	1/25.0 (4.0%)	3/23.8 (12.6%)	4/23.2 (17.2%)	1/22.3 (4.5%)	6/20.7 (28.9%)	6/23.7 (25.3%)
Terminal incidence	1/25 (4.0%)	3/20 (15.0%)	4/20 (20.0%)	1/20 (5.0%)	6/19 (31.6%)	6/21 (28.6%)
First incidence (days)	320 (T)	320 (T)	320 (T)	318 (T)	320 (T)	318 (T)
Average severity	1.0	1.3	1.5	1.0	1.8	1.8
	Vehicle Control	0.284	0.150	0.734	0.024*	0.040*
		AZT-H	0.487	0.326**N**	0.165	0.230
Poly-3 test			**3TC-H**	0.184**N**	0.288	0.376
				NVP-H	0.035*	0.056
					AZT/3TC-H	0.526**N**
Litter-adjusted correlation	0.17					
Litter-adjusted Poly-3 rate	4.4%	12.9%	17.2%	4.3%	27.7%	25.8%
	Vehicle Control	0.145	0.063	0.496**N**	0.030*	0.026*
		AZT-H	0.334	0.140**N**	0.137	0.148
Litter-adjusted Poly-3 test			**3TC-H**	0.059**N**	0.221	0.246
				NVP-H	0.029*	0.025*
					AZT/3TC-H	0.449**N**
Sire-adjusted correlation	–0.06					
Sire-adjusted Poly-3 rate	4.7%	12.9%	15.5%	3.8%	26.1%	25.8%
	Vehicle Control	0.143	0.088	0.440**N**	0.049*	0.030*
		AZT-H	0.399	0.127**N**	0.173	0.144
Sire-adjusted Poly-3 test			**3TC-H**	0.076**N**	0.230	0.220
				NVP-H	0.044*	0.025*
					AZT/3TC-H	0.491**N**
Ovary: Cyst						
Overall rate	1/25 (4.0%)	3/24 (12.5%)	1/25 (4.0%)	2/24 (8.3%)	3/23 (13.0%)	0/25 (0.0%)
Adjusted rate	1/25.0 (4.0%)	3/23.1 (13.0%)	1/23.2 (4.3%)	2/22.3 (9.0%)	3/21.7 (13.8%)	0/23.7 (0.0%)
Terminal incidence	1/25 (4.0%)	3/20 (15.0%)	1/20 (5.0%)	2/20 (10.0%)	2/19 (10.5%)	0/21 (0.0%)
First incidence (days)	321 (T)	318 (T)	320 (T)	320 (T)	102	—
Average severity	2.0	2.3	2.0	2.0	2.0	—
	Vehicle Control	0.274	0.744	0.460	0.252	0.510**N**
		AZT-H	0.300**N**	0.517**N**	0.636	0.108**N**
Poly-3 test			**3TC-H**	0.485	0.276	0.496**N**
				NVP-H	0.487	0.220**N**
					AZT/3TC-H	0.097**N**
Litter-adjusted correlation	–0.11					
Litter-adjusted Poly-3 rate	3.8%	13.2%	4.4%	8.8%	14.3%	0.0%
	Vehicle Control	0.108	0.453	0.234	0.088	0.157**N**
		AZT-H	0.132**N**	0.308**N**	0.453	0.024**N***
Litter-adjusted Poly-3 test			**3TC-H**	0.272	0.109	0.146**N**
				NVP-H	0.268	0.066**N**
					AZT/3TC-H	0.018**N***
Sire-adjusted correlation	—					
Sire-adjusted Poly-3 rate	—	—	—	—	—	—
	Vehicle Control	—	—	—	—	—
		AZT-H	—	—	—	—
Sire-adjusted Poly-3 test			**3TC-H**	—	—	—
				NVP-H	—	—
					AZT/3TC-H	—

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Spleen: Hematopoietic Cell Proliferation						
Overall rate	2/25 (8.0%)	8/25 (32.0%)	7/24 (29.2%)	6/24 (25.0%)	6/23 (26.1%)	7/25 (28.0%)
Adjusted rate	2/25.0 (8.0%)	8/24.6 (32.5%)	7/23.5 (29.8%)	6/22.3 (26.9%)	6/21.1 (28.5%)	7/23.8 (29.4%)
Terminal incidence	2/25 (8.0%)	4/20 (20.0%)	4/20 (20.0%)	6/20 (30.0%)	5/19 (26.3%)	6/21 (28.6%)
First incidence (days)	316 (T)	284	271	318 (T)	280	312
Average severity	2.5	2.8	3.1	2.5	2.5	2.6
	Vehicle Control	0.032*	0.053	0.087	0.073	0.056
		AZT-H	0.543**N**	0.462**N**	0.512**N**	0.529**N**
Poly-3 test			**3TC-H**	0.545**N**	0.591**N**	0.611**N**
				NVP-H	0.588	0.557
					AZT/3TC-H	0.603
Litter-adjusted correlation	0.15					
Litter-adjusted Poly-3 rate	7.5%	32.4%	30.6%	26.7%	28.0%	28.6%
	Vehicle Control	0.012*	0.024*	0.041*	0.036*	0.021*
		AZT-H	0.452**N**	0.344**N**	0.381**N**	0.391**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.395**N**	0.431**N**	0.443**N**
				NVP-H	0.464	0.445
					AZT/3TC-H	0.483
Sire-adjusted correlation	–0.01					
Sire-adjusted Poly-3 rate	8.0%	32.2%	29.6%	27.1%	28.3%	29.6%
	Vehicle Control	0.023*	0.032*	0.048*	0.013*	0.017*
		AZT-H	0.418**N**	0.368**N**	0.394**N**	0.433**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.432**N**	0.465**N**	0.499
				NVP-H	0.470	0.424
					AZT/3TC-H	0.465
Thymus: Hyperplasia						
Overall rate	6/25 (24.0%)	2/21 (9.5%)	2/22 (9.1%)	8/22 (36.4%)	6/24 (25.0%)	5/24 (20.8%)
Adjusted rate	6/25.0 (24.0%)	2/20.4 (9.8%)	2/21.1 (9.5%)	8/20.7 (38.6%)	6/20.7 (28.9%)	5/22.8 (21.9%)
Terminal incidence	6/25 (24.0%)	2/19 (10.5%)	2/20 (10.0%)	8/19 (42.1%)	6/19 (31.6%)	5/21 (23.8%)
First incidence (days)	316 (T)	319 (T)	321 (T)	318 (T)	316 (T)	318 (T)
Average severity	2.2	2.0	2.5	2.0	2.0	2.2
	Vehicle Control	0.197**N**	0.184**N**	0.230	0.485	0.566**N**
		AZT-H	0.684**N**	0.032*	0.122	0.255
Poly-3 test			**3TC-H**	0.027*	0.112	0.240
				NVP-H	0.371**N**	0.191**N**
					AZT/3TC-H	0.428**N**
Litter-adjusted correlation	–0.26					
Litter-adjusted Poly-3 rate	24.8%	8.6%	8.6%	39.0%	29.3%	23.2%
	Vehicle Control	0.046**N***	0.043**N***	0.125	0.341	0.441**N**
		AZT-H	0.499**N**	0.005**	0.022*	0.063
Litter-adjusted Poly-3 test			**3TC-H**	0.004**	0.020*	0.060
				NVP-H	0.226**N**	0.101**N**
					AZT/3TC-H	0.291**N**
Sire-adjusted correlation	0.03					
Sire-adjusted Poly-3 rate	24.1%	9.6%	10.4%	38.7%	29.9%	21.6%
	Vehicle Control	0.077**N**	0.068**N**	0.130	0.273	0.397**N**
		AZT-H	0.463	0.004**	0.027*	0.043*
Sire-adjusted Poly-3 test			**3TC-H**	0.008**	0.031*	0.110
				NVP-H	0.255**N**	0.043**N***
					AZT/3TC-H	0.235**N**

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Thyroid Gland: Ectopic Thymus						
Overall rate	3/23 (13.0%)	0/24 (0.0%)	1/25 (4.0%)	0/23 (0.0%)	1/22 (4.5%)	2/25 (8.0%)
Adjusted rate	3/23.0 (13.0%)	0/22.8 (0.0%)	1/23.2 (4.3%)	0/21.8 (0.0%)	1/20.7 (4.8%)	2/24.1 (8.3%)
Terminal incidence	3/23 (13.0%)	0/19 (0.0%)	1/20 (5.0%)	0/20 (0.0%)	1/19 (5.3%)	1/21 (4.8%)
First incidence (days)	316 (T)	—	320 (T)	—	320 (T)	275
	Vehicle Control	0.115**N**	0.298**N**	0.123**N**	0.342**N**	0.478**N**
		AZT-H	0.504	—	0.481	0.248
Poly-3 test			**3TC-H**	0.513**N**	0.735	0.513
				NVP-H	0.489	0.258
					AZT/3TC-H	0.554
Litter-adjusted correlation	–0.11					
Litter-adjusted Poly-3 rate	13.7%	0.0%	4.4%	0.0%	4.5%	8.7%
	Vehicle Control	0.017**N***	0.116**N**	0.017**N***	0.123**N**	0.277**N**
		AZT-H	0.146	—	0.157	0.056
Litter-adjusted Poly-3 test			**3TC-H**	0.146**N**	0.494	0.269
				NVP-H	0.157	0.056
					AZT/3TC-H	0.279
Sire-adjusted correlation	0.01					
Sire-adjusted Poly-3 rate	13.1%	0.0%	4.3%	0.0%	4.8%	8.3%
	Vehicle Control	0.020**N***	0.129**N**	0.020**N***	0.151**N**	0.293**N**
		AZT-H	0.146	—	0.160	0.057
Sire-adjusted Poly-3 test			**3TC-H**	0.146**N**	0.471	0.153
				NVP-H	0.160	0.056
					AZT/3TC-H	0.314
Urinary Bladder: Cellular Infiltration						
Overall rate	8/25 (32.0%)	4/24 (16.7%)	8/24 (33.3%)	4/23 (17.4%)	8/23 (34.8%)	3/25 (12.0%)
Adjusted rate	8/25.0 (32.0%)	4/23.1 (17.3%)	8/23.0 (34.8%)	4/21.8 (18.3%)	8/21.1 (38.0%)	3/23.7 (12.6%)
Terminal incidence	8/25 (32.0%)	4/20 (20.0%)	7/20 (35.0%)	4/20 (20.0%)	7/19 (36.8%)	3/21 (14.3%)
First incidence (days)	316 (T)	318 (T)	258	320 (T)	280	318 (T)
Average severity	1.1	1.3	1.1	1.0	1.0	1.0
	Vehicle Control	0.201**N**	0.538	0.234**N**	0.455	0.100**N**
		AZT-H	0.153	0.616	0.113	0.485**N**
Poly-3 test			**3TC-H**	0.181**N**	0.538	0.071**N**
				NVP-H	0.136	0.453**N**
					AZT/3TC-H	0.049**N***
Litter-adjusted correlation	0.07					
Litter-adjusted Poly-3 rate	32.1%	17.7%	35.2%	18.2%	38.1%	12.9%
	Vehicle Control	0.108**N**	0.418	0.107**N**	0.333	0.038**N***
		AZT-H	0.114	0.480	0.068	0.327**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.116**N**	0.429	0.055**N**
				NVP-H	0.068	0.299**N**
					AZT/3TC-H	0.027**N***
Sire-adjusted correlation	0.07					
Sire-adjusted Poly-3 rate	33.7%	18.1%	36.0%	18.6%	40.3%	12.9%
	Vehicle Control	0.072**N**	0.430	0.064**N**	0.305	0.025**N***
		AZT-H	0.093	0.480	0.048*	0.240**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.118**N**	0.401	0.036**N***
				NVP-H	0.069	0.288**N**
					AZT/3TC-H	0.012**N***

TABLE G11
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Uterus: Endometrium Hyperplasia						
Overall rate	22/25 (88.0%)	19/25 (76.0%)	19/25 (76.0%)	19/23 (82.6%)	17/23 (73.9%)	17/25 (68.0%)
Adjusted rate	22/25.0 (88.0%)	19/24.3 (78.2%)	19/23.7 (80.3%)	19/22.1 (86.1%)	17/21.5 (79.0%)	17/24.1 (70.5%)
Terminal incidence	22/25 (88.0%)	16/20 (80.0%)	18/20 (90.0%)	18/20 (90.0%)	15/19 (78.9%)	16/21 (76.2%)
First incidence (days)	316 (T)	297	271	297	264	275
Average severity	1.8	1.4	1.8	2.1	1.4	1.9
	Vehicle Control	0.293**N**	0.362**N**	0.599**N**	0.332**N**	0.116**N**
		AZT-H	0.572	0.371	0.618	0.385**N**
Poly-3 test			**3TC-H**	0.445	0.605**N**	0.319**N**
				NVP-H	0.409**N**	0.167**N**
					AZT/3TC-H	0.374**N**
Litter-adjusted correlation	–0.05					
Litter-adjusted Poly-3 rate	88.1%	78.4%	80.8%	85.9%	79.3%	70.5%
	Vehicle Control	0.167**N**	0.234**N**	0.397**N**	0.205**N**	0.054**N**
		AZT-H	0.411	0.223	0.469	0.254**N**
Litter-adjusted Poly-3 test			**3TC-H**	0.304	0.446**N**	0.191**N**
				NVP-H	0.265**N**	0.076**N**
					AZT/3TC-H	0.240**N**
Sire-adjusted correlation	0.01					
Sire-adjusted Poly-3 rate	87.7%	77.9%	80.1%	85.6%	78.6%	70.4%
	Vehicle Control	0.149**N**	0.246**N**	0.404**N**	0.214**N**	0.050**N***
		AZT-H	0.405	0.215	0.471	0.266**N**
Sire-adjusted Poly-3 test			**3TC-H**	0.282	0.451**N**	0.224**N**
				NVP-H	0.255**N**	0.078**N**
					AZT/3TC-H	0.225**N**

(T) Terminal kill
[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP
[b] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[c] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the group stated to the left on the same row. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

APPENDIX H
LITTER SUCCESS AND SURVIVAL

TABLE H1
Litter Parameters and Pup Survival to 28 Days for Mouse Pups in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/NVP-L	AZT/3TC/NVP-M	AZT/3TC/NVP-H
Total dams pregnant[b]	30	26	24	37
Dams that did not litter	2	2	1	2
Total litters	28	24	23	35
Total pups born	204	180	156	208
Average per litter	7.29	7.5	6.78	5.94
Number of males born	105	85	71	93
Sex ratio of live pups (female:male)	1:1.18	1:1.10	1:0.90	1:0.89
Pups born dead (%)	10 (4.9)	18 (10.0)	6 (3.8)	11 (5.3)
Percent survival PND 1 - PND 10[c]	82.74%	78.70%	90.58%	81.03%
Survival analysis[d]	NS	NS	NS	NS
Percent survival PND 11 - PND 28[e]	96.54%	98.02%	81.52%	48.82%
Survival analysis[f]	P<0.001	NS	P=0.006	P<0.001
Pups assigned to 45-week study	50	50	50	50
Litters used for 45-week study	24	16	16	24

[a] AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

[b] Plug positive dams were randomly assigned to dose groups on gestational day (GD) 0 (day plug was observed), and their weight gain was monitored to confirm pregnancy. Dams that did not gain sufficient weight to indicate pregnancy by GD 11 were returned to the breeding pool and are not included in this table.

[c] Excludes pups that were culled on postnatal day (PND) 1, and includes both males and females

[d] Overall survival within litters to PND 10. Beneath the vehicle control group is the P value associated with a linear trend test. Beneath the dosed groups are the P values corresponding to pairwise comparisons by Dunnett's test. NS=not significant

[e] Excludes pups that died or were culled before PND 11, and includes both males and females

[f] Beneath the vehicle control group is the P value associated with a linear trend test. Beneath the dosed groups are the P values corresponding to pairwise comparisons by Dunnett-Hsu adjusted comparisons among groups in log-rank analysis between the vehicle controls and that dosed group.

TABLE H2
Litter Parameters and Pup Survival to 28 Days for Mouse Pups in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/ 3TC-H	AZT/ 3TC/ NVP-H
Total dams pregnant[b]	30	29	28	26	28	37
Dams that did not litter	2	0	3	3	1	2
Total litters	28	29	25	23	27	35
Total pups born	204	206	147	155	184	208
Average per litter	7.29	7.10	5.88	6.74	6.81	5.94
Number of males born	105	100	64	74	91	93
Sex ratio of live pups (female:male)	1:1.18	1:0.99	1:0.84	1:0.94	1:1.12	1:0.89
Pups born dead (%)	10 (4.9)	5 (2.4)	7 (4.8)	2 (1.3)	12 (6.5)	11 (5.3)
Percent survival PND 1 - PND 10[c]	82.74%	83.10%	86.11%	86.23%	94.00%	81.03%
Survival analysis [vs. vehicle control][d]		NS	NS	NS	NS	NS
Survival analysis [vs. AZT/3TC/NVP-H][d]	NS	NS	NS	NS	NS	
Percent survival PND 11 - PND 28[e]	96.54%	80.60%	99.09%	99.24%	71.47%	48.82%
Survival analysis [vs. vehicle control][f]		P=0.037	NS	NS	P<0.001	P<0.001
Survival analysis [vs. AZT/3TC/NVP-H][f]	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	
Pups assigned to 45-week study	50	50	50	51	50	50
Litters used for study	24	22	17	19	20	24

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] Plug positive dams were randomly assigned to dose groups on gestational day (GD) 0 (day plug was observed), and their weight gain was monitored to confirm pregnancy. Dams that did not gain sufficient weight to indicate pregnancy by GD 11 were returned to the breeding pool and are not included in this table.

[c] Excludes pups that were culled on postnatal day (PND) 1, and includes both males and females

[d] Overall survival within litters to PND 10. P values represent pairwise comparisons with a Tukey's test. NS=not significant

[e] Excludes pups that died or were culled before PND 11, and includes both males and females

[f] P values represent Tukey-Kramer adjusted comparisons among groups in log-ranked analysis. NS=not significant

TABLE H3
Effect of Dose on Survival from Postnatal Day 11 to PND 28 in Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison[a]

	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Hazard Ratio[b]	100%	42.9%	545.1%	2207.7%
P Value[c]	<0.001***	0.908	0.006**	<0.001***

[a] Includes mice that were culled from the study at PND 28; AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.
[b] The Hazard Ratio represents the treatment group hazard relative to vehicle controls. The ratio for the vehicle control group is always 100%.
[c] The P values presented are from the Dunnett-Hsu adjusted comparisons among groups in the log-rank analysis.

TABLE H4
Effect of Dose on Survival from Postnatal Day 11 to PND 28 in Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison[a]

Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/3TC-H	AZT/ 3TC/ NVP-H
Vehicle Control	0.037*	1.000	1.000	<0.001***	<0.001***
569.9%	**AZT-H**	0.031*	0.037*	0.287	<0.001***
13.4%	2.3%	**3TC-H**	1.000	<0.001***	<0.001***
38.7%	6.8%	289.5%	**NVP-H**	<0.001***	<0.001***
965.7%	169.4%	7,224.1%	2,495.3%	**AZT/3TC-H**	<0.001***
2,178.2%	382.2%	16,294.2%	5,628.1%	225.6%	**AZT/3TC/NVP-H**

[a] Includes mice that were culled from the study at PND 28; P values are two-sided Tukey-Kramer comparisons among groups in the log-rank analysis; comparisons are between column dose group listed above the value and the row dose group listed to the left of it. Percentages represent the hazard ratio of the column dose group listed above the value compared to the row dose group listed to the right of it. AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

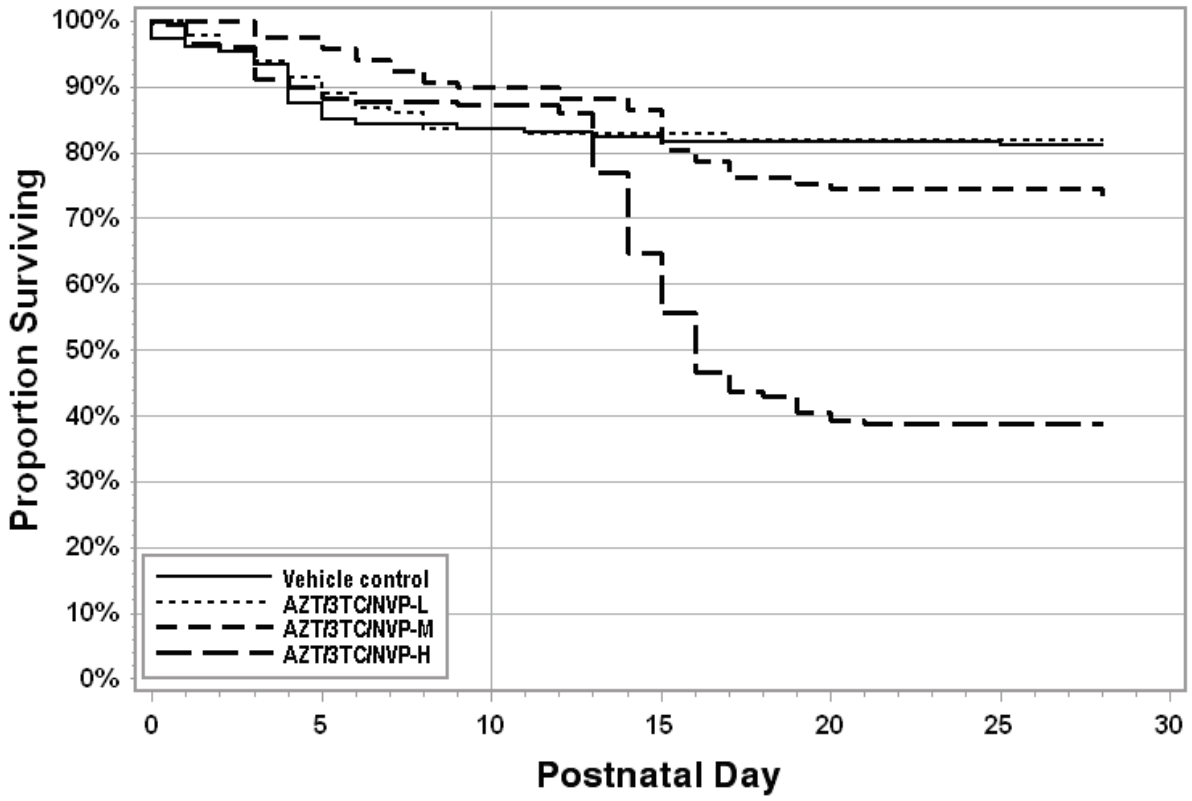

FIGURE H1
Pup Survival from Postnatal Day 1 to PND 28 for Mouse Pups in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison
Line at PND 10 marks the change from the neonatal to the adult dose

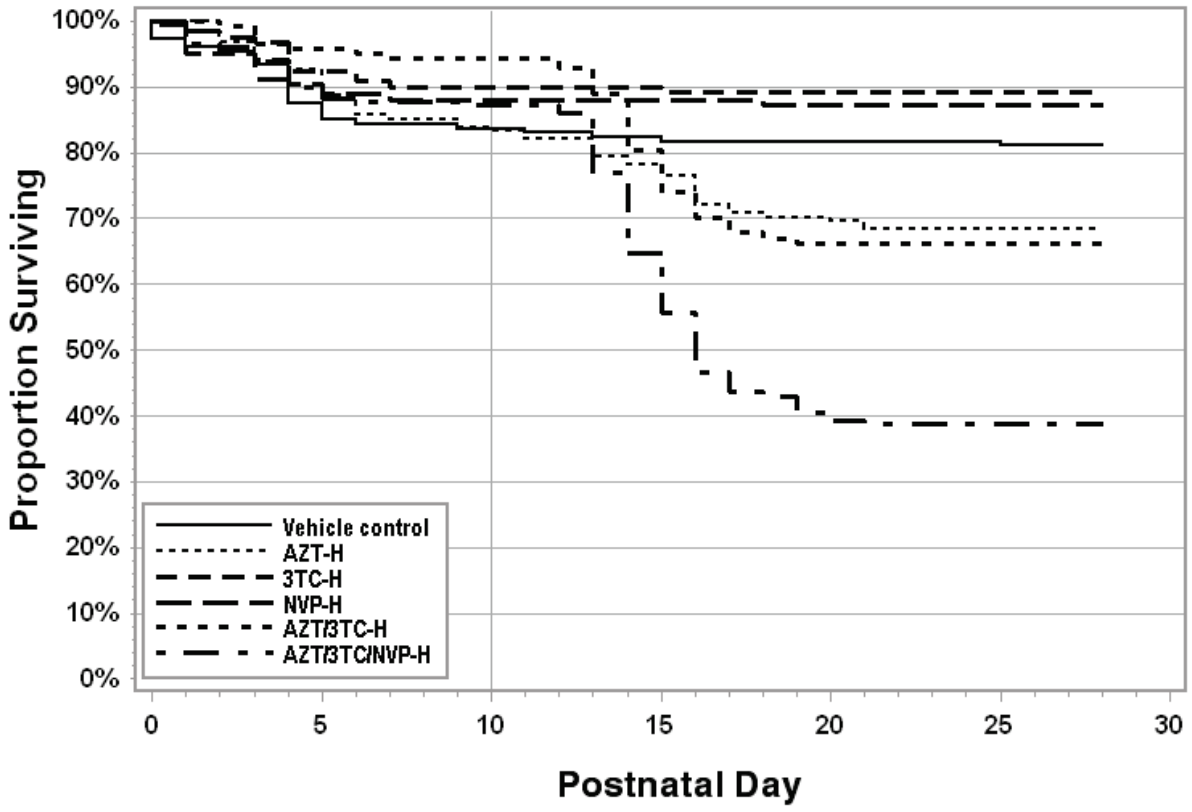

FIGURE H2
Pup Survival from Postnatal Day 1 to PND 28 for Mouse Pups in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison
Line at PND 10 marks the change from the neonatal to the adult dose

TABLE H5
Effects of Dose on Gestational Weight Gain in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison (Comparisons Adjusted for Litter Size)[a,b]

Gestational Day	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
2	0.1 ± 0.2 0.365	0.2 ± 0.2 0.451	0.2 ± 0.2 0.460	0.2 ± 0.2 0.351
3	0.5 ± 0.2 0.362**N**	0.5 ± 0.3 0.418**N**	0.4 ± 0.2 0.323**N**	0.4 ± 0.2 0.385**N**
4	0.9 ± 0.2 0.389**N**	0.9 ± 0.3 0.479**N**	0.9 ± 0.3 0.482	0.8 ± 0.2 0.367**N**
5	1.0 ± 0.3 0.459**N**	1.1 ± 0.3 0.471	1.0 ± 0.3 0.432**N**	1.0 ± 0.2 0.490**N**
6	1.3 ± 0.3 0.415**N**	1.4 ± 0.3 0.372	1.2 ± 0.3 0.411**N**	1.3 ± 0.2 0.486**N**
7	1.5 ± 0.3 0.293	1.7 ± 0.3 0.359	1.5 ± 0.3 0.445**N**	1.9 ± 0.3 0.222
8	1.8 ± 0.3 0.195	2.1 ± 0.3 0.228	1.8 ± 0.3 0.493	2.3 ± 0.3 0.116
9	2.2 ± 0.3 0.148	2.4 ± 0.3 0.339	2.3 ± 0.3 0.417	2.7 ± 0.3 0.114
10	3.0 ± 0.3 0.281	3.3 ± 0.3 0.252	3.1 ± 0.3 0.343	3.3 ± 0.3 0.235
11	3.9 ± 0.3 0.263	4.1 ± 0.3 0.282	3.9 ± 0.3 0.496	4.2 ± 0.3 0.187
12	4.9 ± 0.3 0.177	5.4 ± 0.3 0.113	5.0 ± 0.3 0.361	5.4 ± 0.2 0.095
13	6.8 ± 0.1 0.191**N**	6.6 ± 0.1 0.186**N**	6.6 ± 0.1 0.193**N**	6.6 ± 0.1 0.171**N**
14	8.0 ± 0.2 0.494	7.9 ± 0.2 0.339**N**	7.9 ± 0.2 0.481**N**	7.9 ± 0.2 0.456**N**
15	9.5 ± 0.2 0.230**N**	9.2 ± 0.2 0.157**N**	9.4 ± 0.2 0.419**N**	9.2 ± 0.2 0.140**N**
16	11.1 ± 0.2 0.061**N**	10.9 ± 0.2 0.251**N**	11.0 ± 0.2 0.395**N**	10.6 ± 0.2 0.034**N***

TABLE H5
Effects of Dose on Gestational Weight Gain in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison (Comparisons Adjusted for Litter Size)

Gestational Day	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
17	12.9 ± 0.2	12.6 ± 0.3	12.9 ± 0.3	12.4 ± 0.2
	0.094**N**	0.195**N**	0.468	0.039**N***
18	14.8 ± 0.3	14.6 ± 0.3	14.8 ± 0.3	14.0 ± 0.3
	0.033**N***	0.255**N**	0.416**N**	0.015**N***
19	16.7 ± 0.3	16.1 ± 0.4	16.7 ± 0.4	16.0 ± 0.3
	0.152**N**	0.139**N**	0.495**N**	0.067**N**

[a] The first line for a gestational day represents cumulative weight gain ± standard error, expressed in grams. AZT/3TC/NVP-L = 80/50/56 mg/kg AZT/3TC/NVP; M = 160/100/112 mg/kg; H = 240/150/168 mg/kg.

[b] Beneath the vehicle control weight is the P value associated with the trend test. Beneath the dosed group weights are P values representing pairwise comparisons to the vehicle control group by Dunnett's test with no multiple comparison adjustment. A negative trend or lower weight in a dosed group is indicated by **N**.

TABLE H6
Effects of Dose on Gestational Weight Gain in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison (Comparisons Adjusted for Litter Size)[a,b]

Gestational Day	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/3TC-H	AZT/ 3TC/ NVP-H
2	0.1 ± 0.2	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
	Vehicle control	0.413	0.202	0.421	0.410	0.408
		AZT-H	0.262	0.497**N**	0.495	0.495
			3TC-H	0.270**N**	0.271**N**	0.257**N**
				NVP-H	0.492	0.492
					AZT/3TC-H	0.499**N**
3	0.6 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.4 ± 0.2
	Vehicle control	0.350**N**	0.411	0.435	0.426	0.300**N**
		AZT-H	0.276	0.298	0.286	0.445**N**
			3TC-H	0.478**N**	0.484**N**	0.223**N**
				NVP-H	0.493	0.249**N**
					AZT/3TC-H	0.238**N**
4	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	0.8 ± 0.2
	Vehicle control	0.499	0.390	0.361	0.205	0.293**N**
		AZT-H	0.389	0.361	0.203	0.289**N**
			3TC-H	0.470	0.303	0.199**N**
				NVP-H	0.332	0.185**N**
					AZT/3TC-H	0.082**N**
5	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	1.0 ± 0.2
	Vehicle control	0.459	0.293	0.423	0.145	0.427**N**
		AZT-H	0.325	0.460	0.168	0.385**N**
			3TC-H	0.369**N**	0.316	0.222**N**
				NVP-H	0.208	0.351**N**
					AZT/3TC-H	0.101**N**
6	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.7 ± 0.2	1.3 ± 0.2
	Vehicle control	0.323	0.402	0.370	0.086	0.479**N**
		AZT-H	0.425**N**	0.461**N**	0.177	0.300**N**
			3TC-H	0.467	0.143	0.376**N**
				NVP-H	0.166	0.346**N**
					AZT/3TC-H	0.072**N**
7	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.3	1.7 ± 0.3	2.2 ± 0.2	1.8 ± 0.2
	Vehicle control	0.248	0.468	0.386	0.036*	0.264
		AZT-H	0.284**N**	0.363**N**	0.126	0.476**N**
			3TC-H	0.419	0.049*	0.293
				NVP-H	0.077	0.380
					AZT/3TC-H	0.108**N**
8	1.8 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.3	2.5 ± 0.2	2.2 ± 0.2
	Vehicle control	0.151	0.178	0.108	0.013*	0.100
		AZT-H	0.477**N**	0.394	0.111	0.406
			3TC-H	0.377	0.110	0.384
				NVP-H	0.185	0.480**N**
					AZT/3TC-H	0.153**N**

TABLE H6
Effects of Dose on Gestational Weight Gain in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison (Comparisons Adjusted for Litter Size)

Gestational Day	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/3TC-H	AZT/ 3TC/ NVP-H
9	2.2 ± 0.3	2.6 ± 0.2	2.7 ± 0.3	2.8 ± 0.3	2.9 ± 0.3	2.7 ± 0.2
	Vehicle control	0.173	0.117	0.054	0.030*	0.079
		AZT-H	0.381	0.233	0.165	0.322
			3TC-H	0.340	0.262	0.447
				NVP-H	0.416	0.377**N**
					AZT/3TC-H	0.291**N**
10	3.0 ± 0.2	3.2 ± 0.2	3.4 ± 0.3	3.4 ± 0.3	3.7 ± 0.3	3.2 ± 0.2
	Vehicle control	0.236	0.113	0.103	0.013*	0.206
		AZT-H	0.296	0.278	0.063	0.464
			3TC-H	0.477	0.173	0.317**N**
				NVP-H	0.192	0.300**N**
					AZT/3TC-H	0.069**N**
11	3.9 ± 0.2	4.1 ± 0.2	4.1 ± 0.3	4.5 ± 0.3	4.6 ± 0.3	4.2 ± 0.2
	Vehicle control	0.261	0.283	0.032*	0.023*	0.182
		AZT-H	0.488**N**	0.105	0.083	0.396
			3TC-H	0.108	0.088	0.385
				NVP-H	0.467	0.149**N**
					AZT/3TC-H	0.123**N**
12	4.9 ± 0.3	5.1 ± 0.2	5.3 ± 0.3	5.6 ± 0.3	5.5 ± 0.3	5.4 ± 0.2
	Vehicle control	0.232	0.096	0.017*	0.030*	0.055
		AZT-H	0.269	0.075	0.121	0.195
			3TC-H	0.213	0.304	0.423
				NVP-H	0.381**N**	0.253**N**
					AZT/3TC-H	0.361**N**
13	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	7.0 ± 0.1	6.8 ± 0.11
	Vehicle control	0.182**N**	0.116**N**	0.196**N**	0.258	0.221**N**
		AZT-H	0.371**N**	0.496	0.063	0.437
			3TC-H	0.371	0.036*	0.306
				NVP-H	0.072	0.443
					AZT/3TC-H	0.077**N**
14	8.1 ± 0.2	7.8 ± 0.2	8.0 ± 0.2	7.9 ± 0.2	8.2 ± 0.2	8.0 ± 0.2
	Vehicle control	0.087**N**	0.375**N**	0.247**N**	0.330	0.387**N**
		AZT-H	0.166	0.272	0.038*	0.137
			3TC-H	0.363**N**	0.231	0.479
				NVP-H	0.137	0.334
					AZT/3TC-H	0.231**N**
15	9.6 ± 0.2	9.2 ± 0.2	9.5 ± 0.2	9.5 ± 0.2	9.6 ± 0.2	9.3 ± 0.2
	Vehicle control	0.096**N**	0.453**N**	0.431**N**	0.413	0.168**N**
		AZT-H	0.131	0.143	0.067	0.356
			3TC-H	0.478**N**	0.372	0.204**N**
				NVP-H	0.351	0.227**N**
					AZT/3TC-H	0.118**N**

TABLE H6
Effects of Dose on Gestational Weight Gain in the 45-Week *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison (Comparisons Adjusted for Litter Size)

Gestational Day	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/3TC-H	AZT/ 3TC/ NVP-H
16	11.2 ± 0.4	10. 8 ± 0.4	11.1± 0.4	11.1 ± 0.4	12.0 ± 0.4	10.7 ± 0.3
	Vehicle control	0.201**N**	0.418**N**	0.459**N**	0.047*	0.145**N**
		AZT-H	0.278	0.244	0.006**	0.419**N**
			3TC-H	0.460	0.036*	0.204**N**
				NVP-H	0.045*	0.180**N**
					AZT/3TC-H	0.003**N****
17	13.0 ± 0.2	12.7 ± 0.2	12.9 ± 0.3	13.0 ± 0.3	13.3 ± 0.3	12.5 ± 0.2
	Vehicle control	0.186**N**	0.467**N**	0.423	0.153	0.063**N**
		AZT-H	0.224	0.150	0.029*	0.268**N**
			3TC-H	0.395	0.146	0.079**N**
				NVP-H	0.217	0.047**N***
					AZT/3TC-H	0.005**N****
18	14.9 ± 0.2	14.6 ± 0.2	14.8 ± 0.3	15.0 ± 0.3	15.2 ± 0.3	14.1 ± 0.2
	Vehicle control	0.198**N**	0.421**N**	0.392	0.221	0.008**N****
		AZT-H	0.273	0.141	0.056	0.061**N**
			3TC-H	0.324	0.177	0.015**N***
				NVP-H	0.323	0.005**N****
					AZT/3TC-H	<0.001**N*****
19	16.7 ± 0.3	16.4 ± 0.3	17.0 ± 0.3	16.9 ± 0.3	17.0 ± 0.3	16.0 ± 0.3
	Vehicle control	0.263**N**	0.198	0.335	0.233	0.047**N***
		AZT-H	0.074	0.154	0.090	0.151**N**
			3TC-H	0.340**N**	0.440**N**	0.005**N****
				NVP-H	0.393	0.020**N***
					AZT/3TC-H	0.008**N****

[a] AZT-H = 240 mg/kg AZT; 3TC-H = 150 mg/kg 3TC; NVP-H = 168 mg/kg NVP; AZT/3TC-H = 240/150 mg/kg AZT/3TC; AZT/3TC/NVP-H = 240/150/168 mg/kg AZT/3TC/NVP

[b] The first line of a gestational day represents mean body weight ± standard error expressed in grams. The remaining lines represent P values from the pairwise comparisons, here the comparison is between the group listed in the column header and the group listed to the left. A lower body weight in a dose group is indicated by **N**.

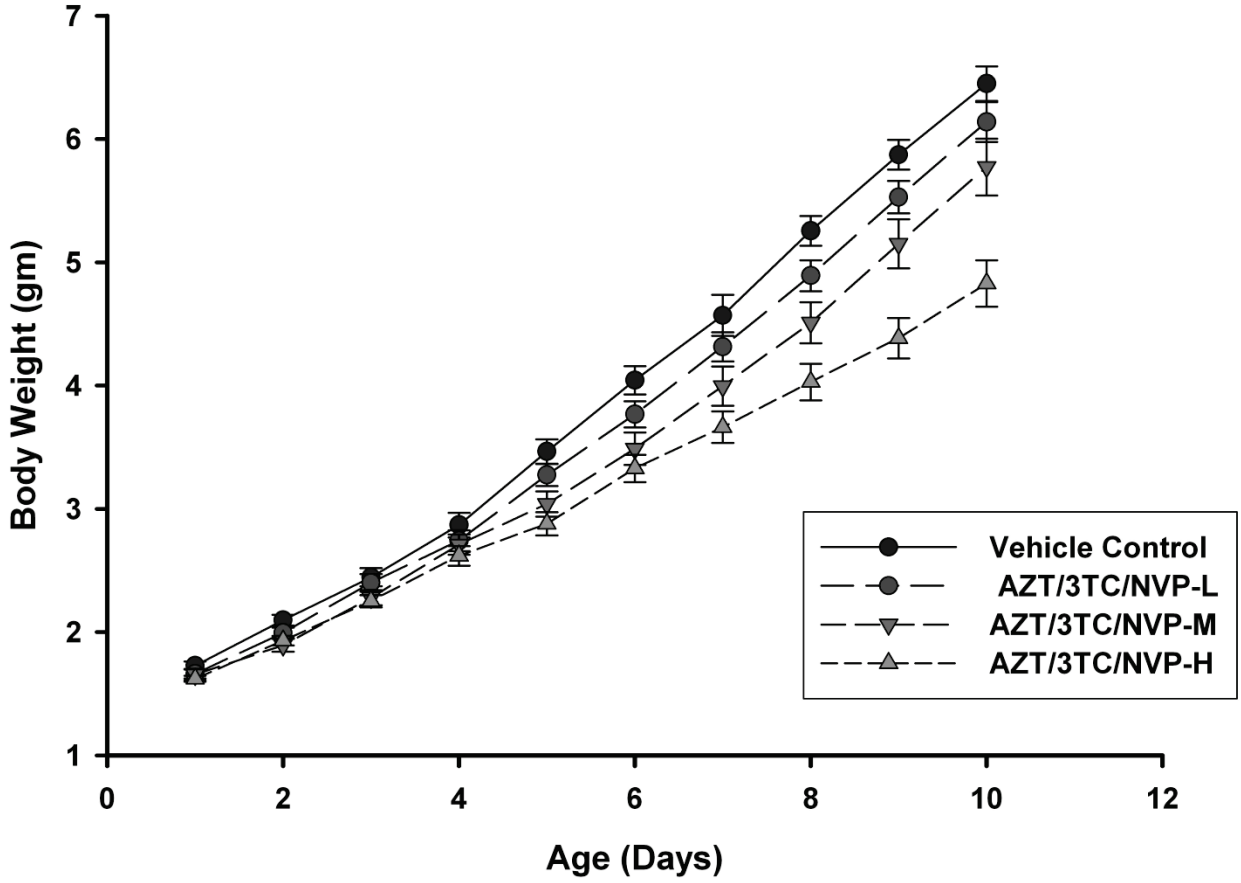

FIGURE H3
Pup Body Weights to Postnatal Day 10 for Mouse Pups in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: Combination Dose Comparison

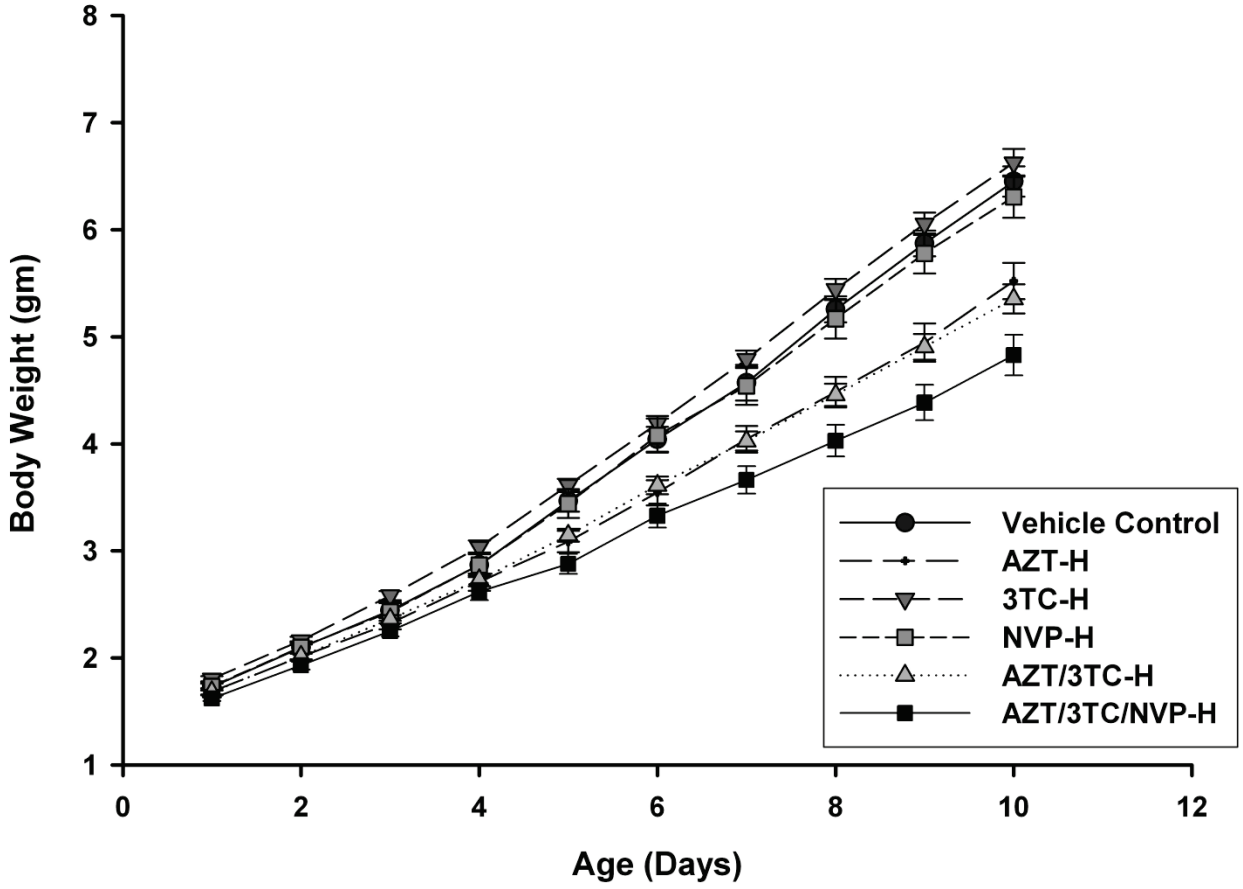

FIGURE H4
Pup Body Weights to Postnatal Day 10 for Mouse Pups in the *In Utero*/Postnatal Gavage Study of AZT, 3TC, and NVP: High Dose Comparison

APPENDIX I
TOXICOKINETIC STUDIES

TOXICOKINETIC STUDIES

INTRODUCTION

Toxicokinetic studies of AZT and 3TC were performed on B6C3F1/N mice as part of transplacental carcinogenicity studies of these Highly Active Antiretroviral Therapy (HAART) drugs (NTP, 2013); these studies are reported in detail with kinetic parameters in Williams *et al.* (2003). The current report describes evaluations of serum taken from heterozygous F1 $p53^{+/-}$ mice that were used in range-finding studies associated with the studies reported in GMM 16. Serum samples were assayed for AZT and 3TC concentrations to determine how the doses used in the study compared to human therapeutic exposure.

MATERIALS AND METHODS

When pups were killed, blood samples were collected from pups treated with AZT alone or AZT in combination with 3TC. The pups were killed either on postnatal day (PND) 10 or on PND 28 following the pre- and postnatal treatments indicated in Tables I1 and I2. The blood samples were left on ice for 30 minutes and then centrifuged to prepare serum, which was stored at –80° C until analysis.

Serum was analyzed for AZT and 3TC concentrations by the methods of Williams *et al.* (2003), which utilize solid phase extraction of the samples followed by liquid chromatography-mass spectrometry (LC-MS). Details of these analyses are given below. The LC-MS system could also resolve and quantitate the AZT metabolites 3′-amino-3′-deoxythymidine (AMT) and 3′-azido-3′-deoxy-5′-*O*-β-D-glucopyranuronosylthymidine (GAZT).

Materials: AZT and 3TC were obtained from Cipla Ltd. (Mumbai, India); $^{13}C_1{}^{15}N_1d_3$-AZT and $^{13}C_2{}^{15}N_3$-3TC internal standards were a gift to Dr. Dan Doerge (NCTR) from Burroughs-Wellcome Co. (Research Triangle Park, NC). AMT and GAZT standards were obtained from Sigma-Aldrich® Corp. (St. Louis, MO). All solvents were HPLC grade, and Milli-Q® water was used throughout.

Solid phase extraction: Solid phase extraction of the nucleoside analogues and their metabolites was carried out using the 96-well format under reduced pressure. Isolute ENV+® cartridges (50 mg, 1 cm^3, Jones Chromatography, Inc., Lakewood, CO) were used for the extraction. Activation of the cartridges was achieved with 2 × 1 mL washes of methanol, followed by 2 × 1 mL washes of 0.1% formic acid. Serum samples (10 µL) were spiked with the labeled internal standards [$^{13}C_1{}^{15}N_1d_3$ -AZT (100 ng) and $^{13}C_2{}^{15}N_3$ -3TC (either 100 ng for 3TC dosing studies or 10 ng for AZT dosing studies)] contained in 10 µL of water and diluted with 150 µL of 0.1% formic acid prior to extraction. The cartridges, containing sample, were then washed with 1 × 1 mL of water and 1 × 1 mL of water/methanol (90/10). Elution of the sample occurred with 3 × 400 µL washes of 10% ammonium acetate (100 mM, pH 4) in methanol. The eluate was reduced to dryness using a centrifugal vacuum concentrator and reconstituted into 200 µL of water.

Liquid chromatography: LC was performed using a Waters 2695 liquid handling system (Waters Assoc., Milford, MA). Chromatographic separation was achieved on a Polaris® C_{18} analytical column (150 mm × 2.0 mm, 3 µm particle size; ANSYS Technologies, Lake Forest, CA), equipped with a C_{18} SecurityGuard® column (4 mm × 2.0 mm; Phenomenex, Torrance, CA), at a flow rate of 200 µL/minute. The mobile phase consisted of 0.1% aqueous formic acid and acetonitrile. Initial conditions were set to 0% acetonitrile increasing to 31% over 4 minutes. This gradient was sufficient to separate the AMT peak from the unretained material at the beginning of the run. A fast gradient to 90% acetonitrile at 8.9 minutes was employed in order to elute 3TC, AZT, AMT, and GAZT in the shortest possible time. At 9 minutes, the gradient was programmed to initial conditions for column equilibration. Injection volumes ranged from 5 to 100 µL and all separations were performed at ambient temperature.

Mass spectrometry: The entire column effluent was directed into a Platform II single quadrupole mass spectrometer (Micromass UK Ltd., Manchester, UK) equipped with an electrospray interface for mass analysis. Because 3TC and AMT were ionized more effectively in positive ionization mode, while AZT and GAZT required negative ionization, the chromatographic run was split into three scan events. The first scan event was from 0 to 6 minutes and monitored positive ions in selected ion monitoring (SIM) mode for the protonated molecule of AMT and its

major product ion (*m*/*z* 116). The protonated molecular ion [$(M+H)^+$] for AMT (*m*/*z* 242) had optimal ionization at a cone-skimmer potential of 15 volts (V), while the fragment ion required 25 V. The second scan event was from 6.1 to 8.1 minutes and used positive SIM for the protonated molecules of 3TC, $^{13}C_2{}^{15}N_3$-3TC internal standard, and the major fragment ion. The $(M+H)^+$ ion for 3TC (*m*/*z* 230) and its internal standard (*m*/*z* 235) had optimal ionization at a cone-skimmer potential of 15 V, while the fragment ion of 3TC (*m*/*z* 112) required 25 V. In the third scan event, which was from 8.2 to 11 minutes, the deprotonated molecular ions [$(M-H)^-$] for AZT (*m*/*z* 266), GAZT (*m*/*z* 442), and $^{13}C_1{}^{15}N_1d_3$-AZT (*m*/*z* 271) were analyzed with a cone-skimmer potential of 25 V, while the AZT fragment ion (*m*/*z* 233) was analyzed at 40 V. Quality control procedures included concurrent analysis of spiked serum samples (0.1, 1, and 10 µg/mL), blank serum, and a mixture of labeled and unlabeled standards interspersed throughout each sample set. Full details of the assay validation are given in Williams *et al.* (2003).

The data were plotted in SigmaPlot® 9.01 (Systat Software Inc., Chicago, IL) to derive kinetic parameters. Apparent half-lives and C_{max} values were obtained from linear regression of plots of the natural log of serum concentrations against time-after-dose, assuming a t_{max} of 15 minutes, as reported for B6C3F1/N mice by Williams *et al.* (2003). Integrated area-under-the-curve (AUC) values for each dose group were calculated using a SigmaPlot® area transform of the serum concentration versus time-after-dose plots.

RESULTS AND DISCUSSION

Although both AMT and GAZT were present in serum from heterozygous F1 $p53^{+/-}$ mouse pups that were exposed to AZT, their concentrations were relatively low, and unmetabolized AZT was the predominant form present.

Serum AZT concentrations evaluated on PND 10 and PND 28 are shown in Tables I3 and I4, respectively, and serum AZT profiles are plotted in Figure I1. Because no significant sex differences were observed, corresponding values for male and female mice were combined. On PND 10, the apparent serum half-life for AZT ranged from 0.99 to 1.20 hours for the four doses of AZT alone (mean=1.08 ± 0.05 hours). By PND 28, the apparent half-life of serum AZT had decreased to between 0.75 and 0.88 hours for the four doses of AZT alone (mean=0.80 ± 0.03 hours); this decrease was significant (P=0.0025) when tested by a one-tailed Student's *t*-test.

Both AMT and GAZT were detected at levels that were close to the limit of detection for the assay, and so kinetic parameters could not be calculated with accuracy. Their concentrations and percentage of AZT concentration are shown in Table I5. Serum AMT concentrations increased as a percentage of AZT concentration with time-after-dose suggesting that it had a longer serum half-life than AZT and its concentration as a percentage of AZT concentration was significantly greater in 28-day-old mice than in 10-day-old mice. Serum GAZT concentrations were less than 2% of AZT concentrations for all doses and were not significantly different between mice evaluated on PND 10 and those evaluated on PND 28. Rodents are known to be much less efficient at glucuronidating AZT than humans and non-human primates (Nicolas *et al.*, 1995). It is probable that the decrease in AZT serum half-life that occurs between PND 10 and PND 28 results from a combination of increased oxidation to AMT and increased rates of renal clearance of AZT, since the latter is the major route of AZT elimination in mice (Ayers *et al.*, 1996).

Serum AZT and 3TC concentrations in heterozygous F1 $p53^{+/-}$ mice that were exposed to combination doses of AZT and 3TC are shown in Table I6 and Figure I2. The serum concentrations and kinetic parameters for AZT in these animals were similar to those in mice administered the same doses of AZT alone (Tables I3 and I4), suggesting that coadministration of 3TC does not influence the absorption, distribution, metabolism, and excretion of AZT. Serum 3TC concentrations were lower than those of AZT, but its apparent half-life values were similar to those of AZT.

Donnerer *et al.* (2008) reported that plasma AZT concentrations in a series of patients receiving AZT alone or in combination with other HAART drugs ranged from 0.07 to 2.59 µg/mL when measured 1 to 3 hours after dose (300 mg bid) ingestion. The highest concentration in this range (2.59 µg/mL or 9.7 µM), which was obtained approximately 1 hour postdosing, was used to estimate a maximum human dose profile (Figure I3) in conjunction with the reported plasma half-life of 1.2 hours in humans. This was compared to the corresponding serum concentration curves for dosed heterozygous F1 $p53^{+/-}$ mice as shown in Figure I1. The human dose curve exhibited a lower C_{max} and AUC than those of all the dosed mouse groups so it was directly compared with that of the PND 28 mouse group that received 40 mg AZT/kg once per day, because this dose group exhibited the lowest C_{max} and AUC

values of all the dosed mouse groups. The kinetic parameters from this dose group (Table I4) were used to create the hypothetical serum concentration curve for PND 28 mice receiving two daily doses of 40 mg/kg, 6.5 hours apart (Figure I3). The AZT/3TC/NVP-L dose group of the main 45-week study received an equivalent dose of AZT. As shown in Table I7, while the apparent C_{max} value of the 40 mg/kg twice daily dose group was more than twofold greater (212.4%) than the human C_{max}, the AUC value was only moderately greater (135.8%).

In conclusion, these toxicokinetic studies suggest that the doses of AZT used in the 45-week study would produce AUC values for serum concentrations of AZT that ranged from the maximum of the human therapeutic range for the AZT/3TC/NVP-L dose group up to approximately 25-fold greater than the maximum of the human therapeutic range for the AZT-H, AZT/3TC-H and AZT/3TC/NVP-H dose groups.

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TABLE I1
Daily Doses for Heterozygous F1 p53$^{+/-}$ Mice Evaluated on Postnatal Day 10[a]

Dose Group	Prenatal Treatment: Gestational Day 12 - 18 Maternal Gavage	Postnatal Treatment: Postnatal Day 1 - 10 Gavage
3	40 mg AZT/kg	20 mg AZT/kg
4	80 mg AZT/kg	40 mg AZT/kg
5	160 mg AZT/kg	80 mg AZT/kg
6	160 mg AZT/kg + 100 mg 3TC/kg	80 mg AZT/kg + 50 mg 3TC/kg
11	240 mg AZT/kg	120 mg AZT/kg

[a] Mice were dosed once per day and were evaluated 1, 2, 4, 6, 10 and 12 hours after the final dose.

TABLE I2
Daily Doses for Heterozygous F1 p53$^{+/-}$ Mice Evaluated on Postnatal Day 28[a]

Dose Group	Prenatal Treatment: Gestational Days 12 - 18 Maternal Gavage	Postnatal Treatment: Postnatal Day 1 - 10 Gavage	Postnatal Treatment: Postnatal Day 11 - 28 Gavage
3	40 mg AZT/kg	20 mg AZT/kg	40 mg AZT/kg
4	80 mg AZT/kg	40 mg AZT/kg	80 mg AZT/kg
5	160 mg AZT/kg	80 mg AZT/kg	160 mg AZT/kg
6	160 mg AZT/kg + 100 mg 3TC/kg	80 mg AZT/kg + 50 mg 3TC/kg	160 mg AZT/kg + 100 mg 3TC/kg
11	240 mg AZT/kg	120 mg AZT/kg	240 mg AZT/kg

[a] Mice were dosed once per day and were evaluated 1, 2, 4, 6, 10 and 12 hours after the final dose.

TABLE I3
Serum Concentrations of AZT and Kinetic Parameters for Heterozygous F1 p53$^{+/-}$ Mice Administered Once Daily Doses of AZT and Evaluated on Postnatal Day 10[a]

	AZT 20 mg/kg	AZT 40 mg/kg	AZT 80 mg/kg	AZT 120 mg/kg
Serum Concentration (μM)[b]				
Hours after final dose				
1	28.5 ± 1.5 (8)	52.5 ± 6.0 (10)	157 ± 7 (7)	619 ± 51 (9)
2	15.5 ± 1.2 (8)	27.6 ± 2.6 (7)	84.8 ± 9.7 (8)	206 ± 39 (10)
4	4.4 ± 1.7 (8)	9.2 ± 0.9 (7)	20.7 ± 3.4 (9)	73 ± 11.6 (10)
6	—[c]	—	5.5 ± 1.0 (8)	21.7 ± 5.7 (9)
10	—	—	1.8 ± 0.5 (6)	4.29 ± 1.5 (9)
24	—	—	0.2 ± 0.1 (7)	0.5 ± 0.3 (8)
Kinetic Parameter[d]				
AUC (μM•hour)	84.6	156.1	487.1	1,544.0
Apparent C_{max} (μM)	45.7	78.7	294.8	879.1
Apparent $t_{½}$ (hour)	1.11	1.20	0.99	1.01

[a] Dose groups are listed according to the final dose on PND 10. Serum concentration values are given as mean ± standard error (n).
[b] Each serum sample was prepared from trunk blood from a single mouse; serum concentrations in male and female mice were combined because the values were not significantly different within the dose groups.
[c] Not determined
[d] Kinetic calculations assume a t_{max} of 15 minutes (Williams *et al.,* 2003).

TABLE I4
Serum Concentrations of AZT and Kinetic Parameters for Heterozygous F1 p53$^{+/-}$ Mice Administered Once Daily Doses of AZT and Evaluated on Postnatal Day 28[a]

	AZT 40 mg/kg	AZT 80 mg/kg	AZT 160 mg/kg	AZT 240 mg/kg
Serum Concentration (μM)[b]				
Hours after final dose				
1	12.7 ± 1.7 (6)	33.8 ± 6.0 (10)	78.6 ± 6.4 (8)	293 ± 50 (10)
2	4.4 ± 1.5 (6)	15.6 ± 9.0 (10)	31.8 ± 7.8 (10)	161 ± 67 (10)
4	1.1 ± 0.4 (5)	2.5 ± 0.8 (10)	5.5 ± 2.0 (10)	30.5 ± 11.8 (8)
6	—[c]	—	1.9 ± 0.5 (9)	3.0 ± 1.3 (7)
10	—	—	0.6 ± 0.3 (6)	3.3 ± 1.4 (6)
24	—	—	0.5 ± 0.4 (6)	0.4 ± 0.4 (8)
Kinetic Parameter[d]				
AUC (μM•hour)	31.0	93.2	218	958
Apparent C_{max} (μM)	20.6	68	152	718
Apparent $t_{½}$ (hour)	0.88	0.79	0.78	0.75

[a] Dose groups are listed according to the final dose on PND 28. Serum concentration values are given as mean ± standard error (n).
[b] Each serum sample was prepared from trunk blood from a single mouse; serum concentrations in male and female mice were combined because the values were not significantly different within the dose groups.
[c] Not determined
[d] Kinetic calculations assume a t_{max} of 15 minutes (Williams *et al.,* 2003).

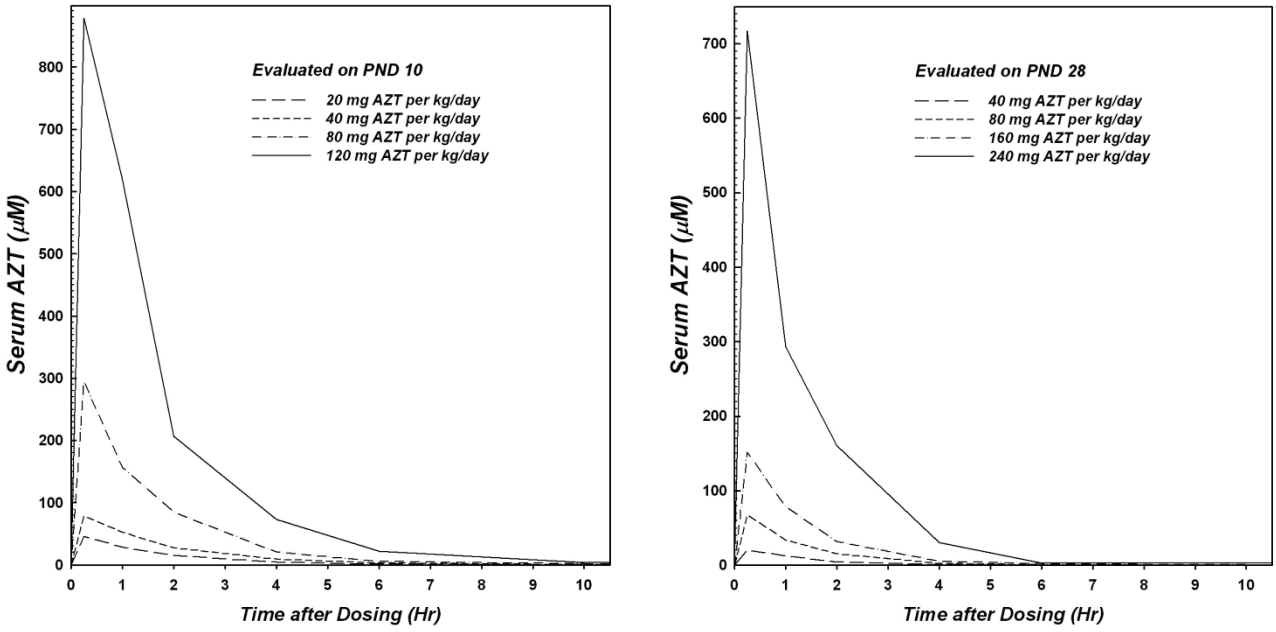

FIGURE I1
Serum Concentrations of AZT in Heterozygous F1 p53$^{+/-}$ Mice Administered Once Daily Doses of AZT and Evaluated on Postnatal Day 10 or 28

TABLE I5
Percent of AZT Dose Converted to AMT or GAZT in Heterozygous F1 p53$^{+/-}$ Mice Administered Once Daily Doses of AZT or AZT/3TC and Evaluated on Postnatal Day 10 or 28[a]

Dose[b]	AMT concentration as % of AZT concentration		GAZT concentration as % of AZT concentration	
	1 Hour[c]	**All**[d]	**1 Hour**	**All**
PND 10				
20 mg AZT/kg	0.49%	1.01%	0.50%	0.34%
40 mg AZT/kg	0.29%	0.83%	0.40%	0.21%
80 mg AZT/kg	0.27%	1.38%	0.73%	0.38%
80 mg AZT/kg + 50 mg 3TC/kg	0.33%	1.05%	0.64%	0.94%
120 mg AZT/kg	0.50%	2.91%	0.92%	0.22%
Overall mean ± standard error[e]	0.38% ± 0.05%	1.44% ± 0.038%	0.64% ± 0.09%	0.42% ± 0.13%
PND 28				
40 mg AZT/kg	1.04%	9.88%	0.00%	0.00%
80 mg AZT/kg	0.93%	4.89%	0.45%	1.42%
160 mg AZT/kg	0.72%	7.72%	0.13%	0.18%
160 mg AZT/kg + 100 mg 3TC/kg	1.13%	3.97%	0.72%	0.37%
240 mg AZT/kg	0.52%	26.61%	0.00%	0.07%
Overall mean ± standard error	0.87% ± 0.11%	10.61% ± 4.13%	0.26% ± 0.14%	0.41% ± 0.26%
P value[f]	**0.0097**	**0.0359**	0.060	0.490

[a] Percentages were calculated on the molar concentrations of AZT, AMT, and GAZT in serum.
[b] The dose groups are listed according to final dose on PND 10 or PND 28.
[c] One hour after the final dose for the dose group
[d] Mean for all six postdosing time points evaluated for the dose group
[e] Mean for all five dose groups on the indicated postnatal day
[f] Probability of significant difference between the overall mean PND 10 and PND 28 values by a one-tailed Student's *t*-test.

TABLE I6
Serum Concentrations of AZT and 3TC and Kinetic Parameters for Heterozygous F1 p53$^{+/-}$ Mice Administered Once Daily Doses of AZT/3TC and Evaluated on Postnatal Day 10 or 28[a]

	AZT/3TC 80/50 mg/kg PND 10		AZT/3TC 160/100 mg/kg PND 28	
	AZT	**3TC**	**AZT**	**3TC**
Serum Concentration (μM)[b]				
Hours after final dose				
1	154 ± 9.5 (8)	111 ± 4.7 (8)	78.9 ± 14.3 (9)	45.7 ± 9.2 (9)
2	98.6 ± 9.0 (9)	55.4 ± 4.6 (9)	38.2 ± 13.4 (6)	16.6 ± 4.2 (6)
4	20.6 ± 2.6 (9)	8.8 ± 1.0 (9)	6.1 ± 2.0 (6)	5.0 ± 1.5 (6)
Kinetic Parameter[c]				
AUC (μM•hour)	488	317	216	114
Apparent C_{max} (μM)	287	227	154	69.8
Apparent $t_{½}$ (hour)	1.01	0.81	0.81	0.96

[a] Dose groups are listed according to the final dose on PND 10 or PND 28. Serum concentration values are given as mean standard ± error (n).
[b] Each serum sample was prepared from trunk blood from a single mouse; serum concentrations in male and female mice were combined because the values were not significantly different within the dose groups.
[c] Kinetic calculations assume a t_{max} of 15 minutes (Williams *et al.,* 2003).

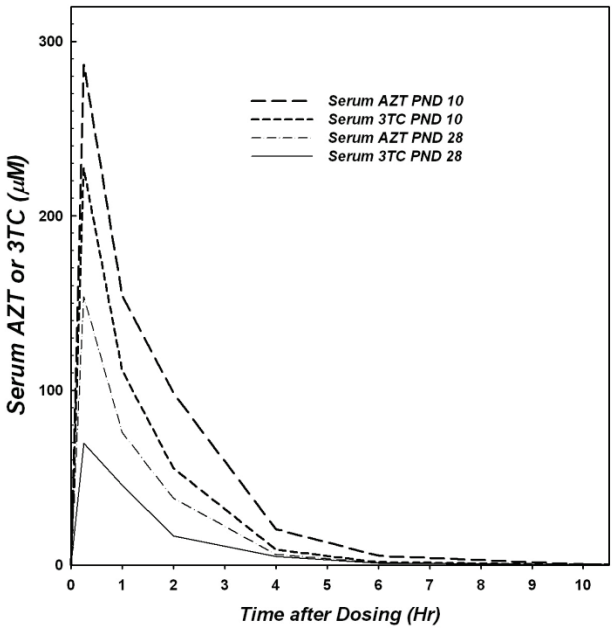

FIGURE I2
Serum Concentrations of AZT and 3TC in Heterozygous F1 p53$^{+/-}$ Mice Administered Once Daily Doses of AZT/3TC and Evaluated on Postnatal Day 10 or 28 (Dose concentrations are given in Tables I1 and I2)

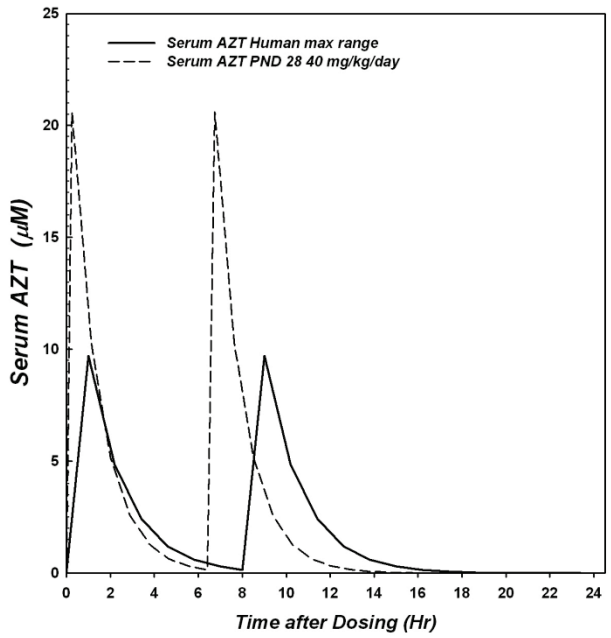

FIGURE I3
Simulated Serum Concentrations of AZT in Humans Administered 300 mg Twice Daily Therapeutic Doses of AZT Resulting in a C_{max} of 9.7 µM and in Heterozygous F1 $p53^{+/-}$ Mice Administered Twice Daily Doses of 40 mg/kg AZT on PND 28

TABLE I7
Simulated Kinetic Parameters for AZT in Humans and Heterozygous F1 $p53^{+/-}$ Mice Administered Twice Daily Doses of AZT

Kinetic Parameter	Humans; AZT 4 mg/kg Twice Daily[a]	Mice; AZT 40 mg/kg Twice Daily on PND 28
AUC (µM•hour)[b]	44.38	60.28 (135.8%)[e]
Apparent C_{max} (µM)[c]	9.7[d]	20.6 (212.4%)[e]
Apparent $t_{½}$ [t_{max}](hour)[c]	1.2 [1.0]	0.88 [0.25]

[a] Reported dose was 300 mg twice daily, corresponding to 4.3 mg/kg twice daily for a 70 kg person (Donnerer *et al.,* 2008)
[b] AUC values calculated from both doses shown in Figure I3.
[c] Human values are taken from Donnerer *et al.* (2008) and correspond to values observed in a patient exhibiting high AZT plasma concentrations following AZT therapy; mouse values are derived from the parameter estimates resulting from a single daily dose as shown in Table I4.
[d] 9.7 µM = 2.59 µg/mL
[e] Parenthetical value is percent of human value

APPENDIX J
HISTORICAL CONTROL INCIDENCES

TABLE J1
Historical Incidences of Neoplasms in Control Male Heterozygous F1 p53$^{+/-}$ Mice in the 30- and 45-Week NCTR Studies of AZT, the 45-Week NCTR Study of AZT/3TC/NVP, and the 40-Week NTP Study of Senna[a]

	AZT (45 Weeks)[b]	AZT (45-Week Stop Study)[c]	AZT/3TC /NVP (45 Weeks)[d]	Senna (40 Weeks)[e]	Overall
Bone					
Osteosarcoma[f]	0/27	0/24	1/25	0/25	1/101
Humerus, osteosarcoma	1/27	0/24	1/25	0/25	2/101
Femur, osteosarcoma	1/27	0/24	0/25	0/25	1/101
Tibia, osteosarcoma	0/27	0/24	1/25	0/25	1/101
Any location, osteosarcoma	2/27	0/24	3/25	0/25	5/101
Harderian gland					
Adenoma	1/27	1/24	0/25	0/25	2/101
Liver					
Hepatocellular adenoma	3/26	3/24	1/25	1/25	8/100
Lung					
Alveolar/bronchiolar adenoma	0/27	2/24	0/25	1/25	3/101
Mesentery					
Sarcoma	0/27	0/24	1/25	0/25	1/101
Pancreas					
Acinar cell, Carcinoma	0/27	1/23	0/24	0/25	1/99
Small intestine,					
Duodenum, leiomyosarcoma	0/26	0/24	1/23	0/25	1/98
Jejunum, adenocarcinoma	0/26	0/24	1/23	0/25	1/98
Tissue NOS,					
Sarcoma	1/26	0/24	0/25	0/25	1/100
Abdominal sarcoma	0/27	0/24	1/25	0/25	1/101
All organs					
Lymphoma, malignant	1/27	0/24	1/25	1/25	3/101

[a] Data as of November 18, 2011. The AZT and AZT/3TC/NVP studies involved transplacental dosing and may not be comparable to the senna study.

[b] Control F_1 mice received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by once-daily gavage from postnatal day (PND) 1 through 28 then 5 days/week until the end of study. F_0 dams received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by once-daily gavage from gestational days (GDs) 12 through 18. Mice were fed NIH-31 pelleted diet.

[c] Control F_1 mice received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by once-daily gavage from PNDs 1 through 8. F_0 dams received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by once-daily gavage from GDs 12 through 18. Mice were fed NIH-31 pelleted diet.

[d] Control F_1 mice received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by twice-daily gavage from PNDs 1 through 28. F_0 dams received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by twice-daily gavage from GDs 12 through 18. Mice were fed NIH-31 pelleted diet.

[e] Control mice were not dosed (feed exposure study) and were 6 to 8 weeks of age when the study was initiated so that their age at the end of the study was 45 to 48 weeks. Mice were fed NTP-2000 meal diet.

[f] Number of neoplasm-bearing animals/number of animals examined microscopically

TABLE J2
Historical Incidences of Neoplasms in Control Female Heterozygous F1 p53$^{+/-}$ Mice in the 30- and 45-Week NCTR Studies of AZT, the 45-Week NCTR Study of AZT/3TC/NVP, and the 40-Week NTP Study of Senna[a]

	AZT (45 Weeks)[b]	AZT (45-Week Stop Study)[c]	AZT/3TC /NVP (45 Weeks)[d]	Senna (40 Weeks)[e]	Overall
Adrenal medulla					
Pheochromocytoma, benign[f]	0/25	0/26	0/23	1/25	1/99
Bone					
Femur, osteosarcoma	1/26	0/26	0/25	0/25	1/102
Rib, osteosarcoma	0/26	0/26	0/25	1/25	1/102
Vertebra, osteosarcoma	0/26	0/26	0/25	1/25	1/102
Any location, osteosarcoma	1/26	0/26	0/25	2/25	3/102
Bone marrow					
Sarcoma	1/26	0/25	0/25	0/25	1/101
Brain					
Cerebrum, sarcoma	1/26	0/26	0/25	0/25	1/102
Large intestine					
Cecum polyp	0/25	0/25	0/25	0/25	0/100
Liver					
Sarcoma	1/26	0/25	0/25	0/25	1/101
Lung					
Alveolar/bronchiolar adenoma	0/26	0/26	1/25	0/25	1/102
Mediastium, sarcoma	1/26	0/26	0/25	0/25	1/102
Lymph node					
Mandibular, sarcoma	1/26	0/25	0/25	0/25	1/101
Mesenteric, sarcoma	1/26	0/25	0/25	0/23	1/99
Mammary gland					
Adenoacanthoma	0/25	0/26	0/25	0/25	0/101
Adenocarcinoma	0/25	0/26	1/25	0/25	1/101
Adenoma	0/25	1/26	0/25	0/25	1/101
Nose					
Sarcoma	1/26	0/26	0/25	0/25	1/102
Ovary					
Cystadenoma	0/26	0/26	0/25	1/25	1/102
Granuloma cell tumor, malignant	0/26	0/26	0/25	1/25	1/102
Luteoma	1/26	0/26	0/25	0/25	1/102
Teratoma malignant	0/26	1/26	0/25	0/25	1/102
Yolk sac carcinoma	0/26	1/26	0/25	0/25	1/102
Spleen					
Sarcoma	1/26	0/26	0/25	0/25	1/102
Uterus					
Polyp stromal	0/26	1/26	0/25	0/25	1/102
Endometrium, polyp stromal	0/26	0/26	0/25	0/25	0/102

TABLE J2
Historical Incidences of Neoplasms in Control Female Heterozygous F1 p53$^{+/-}$ Mice in the 30- and 45-Week NCTR Studies of AZT, the 45-Week NCTR Study of AZT/3TC/NVP, and the 40-Week NTP Study of Senna

	AZT (45 Weeks)	AZT (45-Week Stop Study)	AZT/3TC /NVP (45 Weeks)	Senna (40 Weeks)	Overall
All organs					
Lymphoma, malignant	0/26	0/26	2/25	1/25	3/102
Mesothelioma, malignant	1/26	0/26	0/25	0/25	1/102

[a] Data as of November 18, 2011. The AZT and AZT/3TC/NVP studies involved transplacental dosing and may not be comparable to the senna study.

[b] Control F_1 mice received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by once-daily gavage from postnatal day (PND) 1 through 28 then 5 days/week until the end of study. F_0 dams received 0.2% methylcellulose/0.1% Tween® 80 aqueous solution by once-daily gavage from gestational days (GDs) 12 through 18. Mice were fed NIH-31 pelleted diet.

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