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

NTP Genetically Modified Model Report on the

Toxicology and Carcinogenicity Studies of 3′-Azido-3′-Deoxythymidine (CASRN 30516-87-1) in Genetically Modified C3B6.129F1-*Trp53*^{tm1Brd} N12 Haploinsufficient Mice (*In Utero* and Postnatal Gavage Study)

NTP GMM 14

OCTOBER 2013

NTP REPORT

ON THE

TOXICOLOGY AND CARCINOGENICITY

STUDIES OF

3′-AZIDO-3′-DEOXYTHYMIDINE
(CAS NO. 30516-87-1)

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

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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 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.

National Center for Toxicological Research, Food and Drug Administration

Conducted studies, evaluated and interpreted results and pathology findings, and reported findings

J.E.A. Leakey, Ph.D., Study Scientist
W.T. Allaben, Ph.D., Co-Study Scientist
J.K. Dunnick, Ph.D., Co-Study Scientist
National Toxicology Program
F.W. Lee, Ph.D., Co-Study Scientist
S.M. Lewis, Ph.D., Co-Study Scientist
P.C. Howard, Ph.D.
C.C. Weis, B.S.

Provided microbiological support

D.D. Paine, B.S.

Conducted chemical analysis of the purity of the test chemical

S.M. Billedeau, M.S.
B.R. Brown, B.S.
J.P Freeman, Ph.D.
J. Moody, B.S.
L.K. Schoenbachler, Ph.D.
P.H. Siitonen, B.S.

Conducted quality assurance audits

S.J. Culp, Ph.D.
J.M. Fowler, B.S.
R.D. Smith, B.S.

Provided statistical analysis

S. Appana, M.S.
S.J. Baek, Ph.D.
R.P. Felton, M.S.
B.T. Thorn, M.S.

Bionetics Corporation

Prepared animal feed and cared for mice

C.J. Cain
J.W. Carson, B.S.
A. Matson, B.S.

Z-Tech Corporation

Provided software systems development and data entry

K.A. Carroll
S.H. Green

Toxicologic Pathology Associates

Evaluated pathology findings

P.W. Mellick, D.V.M., Ph.D.
G.R. Olson, D.V.M., Ph.D.
A.R. Warbritton
L.P. Wiley, B.S.

Experimental Pathology Laboratories, Inc.

Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
J.F. Hardisty, D.V.M.
R.A. Miller, D.V.M., Ph.D.

NTP Pathology Working Group

Evaluated slides and contributed to pathology report on mice (March 28 and April 17, 2008)

J.F. Hardisty, D.V.M., Coordinator
Experimental Pathology Laboratories, Inc.
J.M. Cullen, V.M.D., Ph.D.
North Carolina State University
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
P.W. Mellick, D.V.M., Ph.D.
Toxicologic Pathology Associates
R.A. Miller, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
G.R. Olson, D.V.M., Ph.D.
Toxicologic Pathology Associates

NIEHS/FDA Interagency Agreement Project Officers

P.C. Howard, Ph.D.
National Center for Toxicological Research
W.T. Allaben, Ph.D.
National Center for Toxicological Research
N.J. Walker, Ph.D.
National Institute of Environmental Health Sciences
J.R. Bucher, Ph.D.
National Institute of Environmental Health Sciences

Biotechnical Services, Inc.

Prepared Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
E.S. Rathman, M.S.
D.C. Serbus, Ph.D.

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SUMMARY

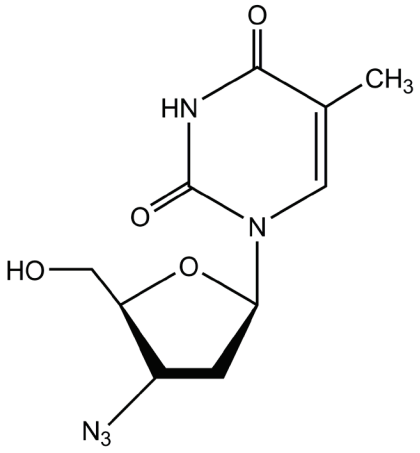
Background
3′Azido-3′-deoxythymidine (AZT) is the most widely used chemotherapeutic agent for the treatment of people with acquired immune deficiency syndrome (AIDS) or positive for human immunodeficiency virus (HIV). AZT treatment is also given to prevent transmission of HIV from pregnant mothers to children before or during birth. We tested the effects of AZT on the offspring of female mice (genetically modified to be sensitive to cancer induction) where the mothers were given the drug during pregnancy and the pups were given the drug following birth.

Method
We exposed groups of haploinsufficient C3B6.129F1-Trp53^{tm1Brd} N12 mice by depositing solutions of AZT in a methylcellulose solvent directly into the animals' stomachs through a tube five times per week for 30 or 45 weeks 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. Tissues from 34 organs were examined for every animal.

Results
Exposure to AZT caused increases in the rates of liver cancer in the male pups after 45 weeks. In addition, there were occurrences of a few malignant lymphomas in both male and female pups exposed to AZT in the 30-week studies.

Conclusions
We conclude that AZT caused liver cancers in male pups exposed to AZT before and following birth. Malignant lymphomas in male and female pups may have been related to AZT exposure 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: AZT; 3′-azido-2′,3′-dideoxythymidine; azidodeoxythymidine; azidothymidine; 3′-azidothymidine; 3′-deoxy-3′-azidothymidine; 3′-deoxy-(8CI) (9CI); BW A509U; Compound S; ZDV; zidovudine
Trade Name: Retrovir®

Antiviral therapy is essential for treatment and prevention of human immunodeficiency virus (HIV) disease in adults and children 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 3′-azido-3′-deoxythymidine (AZT) treatment, often used in combination with other antivirals, in preventing mother-to-child transmission of HIV. AZT is the most widely used and evaluated chemotherapeutic agent for the treatment of persons with acquired immune deficiency syndrome (AIDS).

Male and female heterozygous F1 $p53^{+/-}$ mice were exposed, by maternal gavage, to AZT *in utero* on gestation days (GD) 12 through 18, then administered AZT by gavage from postnatal day (PND) 1 through 30 weeks of age (30-week study), 45 weeks of age (45-week study), or PND 8 (45-week stop-study). Mice in the 0 mg/kg groups received only an aqueous solution containing 0.2% methylcellulose and 0.1% Tween® 80. Mice were dosed once daily until PND 28, then 5 days per week. Genetic toxicology studies were conducted in mouse peripheral blood erythrocytes.

30-WEEK STUDY

Pregnant dams were administered 0 or 240 mg AZT/kg body weight per day on GDs 12 through 18. Groups of 26 or 27 male and 26 or 27 female pups were administered 0 or 120 mg/kg by gavage on PNDs 1 through 10, then 0 or 240 mg/kg until the end of the study. Survival of 240/120/240 mg/kg males was significantly less than that of 0/0/0 mg/kg males. Mean body weights of dosed males and females were less than those of the 0/0/0 mg/kg groups, and absolute kidney weights of dosed males and females were significantly less than those of the 0/0/0 mg/kg groups. Mean cell volume and mean cell hemoglobin in dosed females and mean cell

volume in dosed males were significantly increased, suggesting moderately severe macrocytic anemia.

The incidence of malignant lymphoma was increased in male mice administered 240/120/240 mg/kg. The increase was significant when adjusted for litter correlations.

45-WEEK STUDY

Pregnant dams were administered 0, 80, 160, or 240 mg/kg on GDs 12 through 18. Corresponding groups of 27 male and 26 or 27 female pups were administered 0, 40, 80, or 120 mg/kg on PNDs 1 through 10, then 0, 80, 160, or 240 mg/kg until the end of the study. There was no effect of AZT administration on the survival of dosed mice. Mean body weights of dosed males and females were generally less than those of the 0/0/0 mg/kg groups. Absolute brain weights of males and females administered 240/120/240 mg/kg were significantly less than those of the 0/0/0 mg/kg groups. Mean cell volume and mean cell hemoglobin at 160 days were increased in 240/120/240 mg/kg males and females, suggesting moderately severe macrocytic anemia.

The incidences of hepatocellular adenoma occurred with a positive trend in males, and the incidence in the 240/120/240 mg/kg group was significantly increased. In females, there was a positive trend in the incidences of malignant lymphoma, and the increased incidence in the 240/120/240 mg/kg group was significant when adjusted for litter correlations.

45-WEEK STOP-STUDY

Pregnant dams were administered 0 or 240 mg/kg on GDs 12 through 18. Groups of 24 or 25 male and 26 female pups were administered 0 or 40 mg/kg on PNDs 1 through 8; pups were then maintained on study until 45 weeks of age without dosing. There was no effect of AZT administration on the survival of dosed mice. Mean body weights of dosed males were generally less than those of the 0/0 mg/kg group. Absolute, but not relative, brain weights of dosed males and females were significantly less than those of the 0/0 mg/kg groups.

The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were slightly increased in 240/40 mg/kg males.

GENETIC TOXICOLOGY

Micronucleated normochromatic erythrocyte and reticulocyte frequencies were generally significantly increased relative to the corresponding 0, 0/0, or 0/0/0 mg/kg group values in 1-day-old pups exposed to AZT *in utero* at 160 or 240 mg/kg, in 10-day-old pups administered 80/40, 160/80, or 240/120 mg/kg, in 28-day-old pups administered 80/40/80, 160/80/160, or 240/120/240 mg/kg, and in 30-week-old mice administered 240/120/240 mg/kg.

CONCLUSIONS

Under the conditions of these gavage studies, there was *clear evidence of carcinogenic activity** of AZT in male heterozygous F1 $p53^{+/-}$ mice based on the occurrence of hepatocellular neoplasms (predominantly adenomas) after 45 weeks of administration. The occurrence of malignant lymphoma may have been related to AZT administration for 30 weeks. There was *equivocal evidence of carcinogenic activity* of AZT in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of malignant lymphoma after 45 weeks of administration.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Peer Review Panel comments and the public discussion on this Report appears on page 12.

Summary of the *In Utero*/Postnatal Gavage Studies of AZT in C3B6.129F1-*Trp53*^{tm1Brd} N12 Haploinsufficient Mice

	Male	Female
Doses in aqueous methylcellulose/ Tween® 80 by gavage		
30-Week Study[a]	F_0: 0 or 240 mg/kg per day F_1: 0 or 120/240 mg/kg per day	F_0: 0 or 240 mg/kg per day F_1: 0 or 120/240 mg/kg per day
45-Week Study[a]	F_0: 0, 80, 160, or 240 mg/kg per day F_1: 0, 40/80, 80/160, or 120/240 mg/kg per day	F_0: 0, 80, 160, or 240 mg/kg per day F_1: 0, 40/80, 80/160, or 120/240 mg/kg per day
45-Week Stop-Study	F_0: 0 or 240 mg/kg per day F_1: 0 or 40 mg/kg per day postnatal days 1-8	F_0: 0 or 240 mg/kg per day F_1: 0 or 40 mg/kg per day postnatal days 1-8
Body weights	Dosed groups less than 0 mg/kg groups	Dosed groups less than 0 mg/kg groups, except in 45-week stop-study
Survival rates		
30-Week Study	27/27, 21/26	24/27, 26/26
45-Week Study	24/27, 21/27, 27/27, 22/27	23/26, 23/27, 24/27, 21/27
45-Week Stop-Study	23/24, 22/25	25/26, 23/26
Nonneoplastic effects		
30-Week Study	None	None
45-Week Study	None	None
45-Week Stop-Study	None	None
Neoplastic effects		
30-Week Study	None	None
45-Week Study	Liver: hepatocellular adenoma (3/26, 2/27, 6/27, 9/27)	None
45-Week Stop-Study	Liver: hepatocelluar adenoma (3/24, 5/25); hepatocellular adenoma or carcinoma (3/24, 7/25)	None
Equivocal findings		
30-Week Study	Malignant lymphoma: (0/27, 3/26)	None
45-Week Study	None	Malignant lymphoma: (0/26, 0/27, 1/27, 3/27)
45-Week Stop-Study	None	None
Level of evidence of carcinogenic activity	Clear evidence	Equivocal evidence
Genetic toxicology		
Micronucleated normochromatic erythrocytes and reticulocytes		
30-Week Study	Positive in males and females at 30 weeks of age	
45-Week Study	Positive in males and females at PND 1, PND 10, and PND 28	

[a] Pups in the 30- and 45-week studies were administered AZT doses that were half that of the maternal dose on post natal days 1 through 10. Pup doses were the same as maternal doses from post natal day 11 until the end of the studies.

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 3′-azido-3′-deoxythymidine 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.

Stephen M. Roberts, Ph.D., Chairperson
College of Veterinary Medicine
University of Florida
Gainesville, FL

Jane Alcorn, D.V.M., Ph.D.
University of Saskatchewan
Saskatchewan, Canada

Lucy M. Anderson, Ph.D., Consultant
Catonsville, MD

Hillary M. Carpenter, III, Ph.D.
Minnesota Department of Health
St. Paul, MN

Russell C. Cattley, V.M.D., Ph.D.
College of Veterinary Medicine
Auburn University
Auburn, AL

Michael R. Elwell, D.V.M., Ph.D., Primary Reviewer
Covance Laboratories, Inc.
Chantilly, VA

Jon C. Mirsalis, Ph.D.
SRI International
Menlo Park, CA

Ofelia A. Olivero, Ph.D., Primary Reviewer
National Cancer Institute
Bethesda, MD

Lisa A. Peterson, Ph.D.
University of Minnesota
Minneapolis, MN

Michael V. Pino, D.V.M., Ph.D.
Sanofi
Bridgewater, NJ

Keith A. Soper, Ph.D., Primary Reviewer
Merck Research Laboratories
West Point, PA

SUMMARY OF PEER REVIEW PANEL COMMENTS

On February 9, 2012, the draft Report on the studies of 3′-azido-3′-deoxythymidine 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 present study is the first study to use the model with perinatal exposure. Dr. Leakey reviewed the experimental design for the main study and stop-study, dosing AZT alone once per day from gestational day 12 to 9 months of age. He noted that the model was found to be sensitive enough to detect carcinogenesis, with a treatment-related tumor profile similar to that seen in the B6C3F1 mouse, mainly liver tumors and lymphomas. There was no evidence of clustering of lesions within litters.

The proposed conclusions for GMM 14 were *clear evidence of carcinogenic activity* of AZT in male heterozygous F1 p53$^{+/-}$ mice and *equivocal evidence of carcinogenic activity* of AZT in female heterozygous F1 p53$^{+/-}$ mice.

Dr. Elwell, the first primary reviewer, felt the studies were well designed, and he had no scientific criticisms. He felt that it would be useful to have some discussion in the report of the differences, if any, between the perinatal exposure and the exposure for the full 45 weeks. He asked if the occurrence of lymphoma in the stop-study should also be mentioned in "other findings." He suggested that "other findings" that were dismissed or not brought forward to the summary or conclusions should be clarified in the discussion section. Based on the comment in the report on group size and statistical significance, he asked if the sample size should have been increased in the stop-study to improve the statistical ability of the study to discern small increases in tumor incidences. He said that there was indication of vagina examination only in the 30-week study tables, and asked whether that had also been examined in the 45-week study.

Dr. Soper, the second primary reviewer, agreed that the studies were well designed and well executed and agreed with the proposed conclusions.

Dr. Olivero, the third primary reviewer, expressed concern about the limited historical control database.

Dr. Leakey replied that when this study was written and evaluated, it was the only one of its kind, and thus the historical controls consisted of only 103 animals from the two companion studies and one other. He said that nonetheless, they were confident in the tumor diagnoses. He felt that the low tumor incidence was driven by haploinsufficiency rather than the actual dosing vehicle. He added that as the study series progresses, the historical control database would be built up. Regarding the stop-study, he noted that body weight within the latter stage of dosing does affect liver tumor incidence. He said that he would add some discussion of the "other findings" such as bone and brain tumors to offer more explanation as to why certain neoplasms were in the conclusions while others were not.

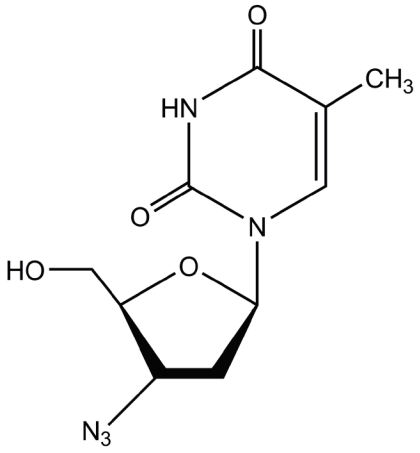
Dr. Olivero asked about the increase in hemoglobin. Dr. Leakey said that while it was statistically significant, it was not outside the physiological range. He said the investigators were expecting to see more anemia, as seen in industry studies where dosing was twice per day.

Dr. Mirsalis inquired about the appearance of the malignant lymphomas compared to those seen in B6C3F1 mice. Study Pathologist Dr. G.R. Olson, Toxicologic Pathology Associates, replied that the lymphomas looked much like those normally seen. However, in the second study, there were several undifferentiated tumors that needed to be further characterized. Dr. Elwell asked how the fatal malignant lymphomas compare to those in B6C3F1 mice at 45 weeks. Dr. Olson said they were the same as those seen in normal chronic studies.

Drs. Anderson and Olivero mentioned similar studies in CD1 mice, which had yielded very different results. Dr. Leakey said he would include their discussion in the report.

Dr. Elwell moved that the conclusions be accepted as written, and Dr. Mirsalis seconded the motion. The panel unanimously recommended acceptance of the conclusions as written.

INTRODUCTION



3′-AZIDO-3′-DEOXYTHYMIDINE

CAS No. 30516-87-1

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

Synonyms: AZT; 3′-azido-2′,3′-dideoxythymidine; azidodeoxythymidine; azidothymidine; 3′-azidothymidine; 3′-deoxy-3′-azidothymidine; 3′-deoxy-(8CI) (9CI); BW A509U; Compound S; ZDV; zidovudine
Trade Name: Retrovir®

Antiviral therapy is essential for treatment and prevention of human immunodeficiency virus (HIV) disease in adults and children and to prevent mother-to-child transmission of HIV (DHHS, 2009). The studies described in this report were designed to determine possible long-term sequelae from 3′-azido-3′-deoxythymidine (AZT) treatment, often used in combination with other antivirals, in preventing mother-to-child transmission of HIV.

CHEMICAL AND PHYSICAL PROPERTIES

AZT is a dideoxynucleoside of thymine and a structural analogue of 2′-deoxythymidine and is the most widely used and evaluated chemotherapeutic agent for the treatment of persons with acquired immune deficiency syndrome (AIDS) and persons seropositive for HIV. AZT is a white to off-white, odorless, crystalline solid and 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 10 mg AZT/mL solution) (NTP, 2006).

PRODUCTION, USE, AND HUMAN EXPOSURE

AZT was first synthesized by Horowitz *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 (FDA) 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 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, capsules of either 100 or 300 mg of AZT alone, combination capsules containing either 300 mg AZT and 150 mg of the NRTI 3TC (lamivudine, 2′-deoxy-3′-thiacytidine), and combination capsules containing 300 mg AZT and 150 mg of 3TC and 300 mg of the NRTI ABC [abacavir, (1S,4R)-4-(2-amino-6-(cyclopropylamino)-9H-purin-9-yl)-2-cyclopentene-1-methanol] (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 the triple drug combination Highly Active Antiretroviral Therapy (HAART) for infants and children infected with HIV (DHHS, 2008b). Typical pediatric doses range from 1.5 mg/kg in intravenous infusion or oral doses of 2 mg/kg every 12 hours for term neonates to 100 mg three times daily for children weighing more than 30 kg (DHHS, 2008b). AZT is also included as part of a therapeutic regimen to prevent mother-to-child transmission of HIV and 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 a primary drug of choice for combination HAART or prophylactic treatment in pregnant women who are HIV positive and is the primary drug of choice for intravenous infusion during labor (PHS, 2008).

In 2007, it was estimated that 30 to 36 million people worldwide were living with HIV infections, of whom approximately half were women (UNAIDS, 2008). During 2007, 2.2 to 3.2 million new infections occurred, while 1.8 to 2.3 million people died. The 2007 estimates suggest that there are 0.69 to 1.9 million people living with HIV in the United States, of whom 140,000 to 400,000 are women (UNAIDS, 2008). Earlier estimates (Steinbrook, 2004; 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.

PHARMACOLOGY

The antiviral activity of AZT depends on its conversion to a nucleotide triphosphate (3′-azido-2′,3′-dideoxythymidine 5′-triphosphate; AZTTP). AZT enters mammalian cells by non-facilitated diffusion (Zimmerman *et al.,* 1987), and it is then phosphorylated in successive reactions catalyzed in proliferating cells primarily by thymidine kinase 1, thymidylate kinase, and nucleoside diphosphokinase present in cell cytosol (Avramis *et al.,* 1989; Törnevik *et al.,* 1995; Bradshaw *et al.,* 2005). The resulting nucleoside triphosphate, 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).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

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).

TOXICITY

Experimental Animals

In animals, AZT causes hematologic toxicities (Thompson *et al.,* 1991) and impaired function of the electron transport chain in cardiac (Walker *et al.,* 2004) and skeletal (Lamperth *et al.,* 1991) muscle mitochondria. 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 in 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 as a result of its inhibition of mitochondrial thymidine kinase 2 (Lynx and McKee 2006; Lynx *et al.,* 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 non-mitotic 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 pharmacological 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 exposed to approximately 29 to 102 mg AZT/kg body weight per day in drinking water 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 exposed to 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 exposure; no alterations were seen in rats after 120 days of exposure (Corcuera Pindado *et al.,* 1994). Sprague-Dawley rats given intraperitoneal injections of approximately 17 to 51 mg AZT/kg body weight 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 exposed to AZT in drinking water at doses of approximately 180 to 200 mg AZT/kg body weight 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 was found. The National Toxicology Program (NTP) (1999) did not report cardiac toxicity in B6C3F1/N mice administered 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. However, in an NTP study where Swiss (CD-1®) mouse pups were exposed *in utero*, via lactation, and by direct gavage on postnatal days (PND) 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), which were similar to those observed in human neonates exposed to antiretroviral drugs (Divi *et al.,* 2007). In skeletal muscle, these abnormalities were characterized as abnormally shaped mitochondria with disrupted cristae (Gerschenson *et al.,* 2000). In heart muscle, small mitochondria in myocytes with myofibrillar loss and abnormal alignment of sarcomeres were observed.

Humans

Exposure to AZT has resulted in myelosuppression and anemia in some human patients as 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 murine 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; DHHS, 2008a). 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 include vascular necrosis, decreased bone density, and skin rashes (DHHS, 2008a). A recent study suggests that mitochondrial damage in children of mothers taking AZT may persist for up to 2 years after birth; however, HIV infection by itself may lead to cardiac toxicity (Artandi *et al.,* 2000; Raidel *et al.,* 2002).

REPRODUCTIVE TOXICITY AND TERATOGENICITY

Experimental Animals

AZT has been shown to cross the placenta of mice (Child *et al.,* 1991) and of 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 overt 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 live-born 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

day (GD) 6 until 18 had reduced body weight gain, anemia, and increased late fetal deaths. The live-born offspring showed no developmental abnormalities or teratogenic effects.

When pregnant CD-1® mice were administered daily intragastric doses of 25 mg AZT/kg body weight per day from GD 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 live-born 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 postblastocyst development at doses similar to human therapeutic doses (Toltzis *et al.,* 1991; DHHS, 2008b).

Humans

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 National Institutes of Health (NIH) panel cautions 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).

CARCINOGENICITY

Experimental Animals

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 AZT/kg body weight per day for up to 2 years (Ayers *et al.,* 1996a). Because of anemia, the high dose was reduced to 450 mg/kg per day at day 91; on day 278, the high dose was again reduced to 300 mg/kg per day. Squamous cell carcinoma of the vagina occurred in two females receiving 300 mg/kg; no vaginal neoplasms/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 at day 90, where they remained for the rest of the 22-month study of AZT.

The only neoplasms associated with administration of AZT in the mice were vaginal squamous cell carcinoma in five females receiving 40 mg/kg, vaginal squamous cell papilloma in one female receiving 30 mg/kg and in one female receiving 40 mg/kg, and one vaginal squamous polyp in a female receiving 40 mg/kg. Although the incidences of hyperplasia of the vaginal epithelium were not increased above that in the controls, the severity of this lesion increased with increasing dose. In order to clarify the role of AZT in producing vaginal neoplasms, AZT was administered intravaginally to CD-1® mice for 22 months (Ayers *et al.,* 1996b). Higher incidences of vaginal neoplasms occurred than were seen in the AZT oral gavage study in CD-1® mice. 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 neoplasms 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 neoplasms seen in mice would not necessarily be predictive of vaginal neoplasms in humans.

The NTP's 2-year chronic studies of AZT and AZT/interferon were conducted in B6C3F1/N 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 body weight, given in two equal doses, 5 days per week for 105 weeks; 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 80 male and 80 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 (2/197, 0/49, 5/45, 11/49) or in combination with α-interferon A/D (0/49, 0/44, 5/48, 6/48). 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), Swiss (CD-1®) mice were administered 0, 50, 100, 200, or 300 mg AZT/kg body weight per day by oral gavage from 10 to 14 days prior to conception until GD 19, and up to 4 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 (14/50, 20/50, 13/50, 7/37, 8/32; Poly-3 survival-adjusted rates of 40.2%, 54.5%, 40.5%, 66.3%, 72.9%). There was no evidence of carcinogenic activity in F_1 female mice exposed transplacentally to AZT at 50, 100, 200, or 300 mg/kg.

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. A dose-dependent increase in tumor multiplicity in the lung, liver, and female reproductive organs occurred. However, in a transplacental carcinogenicity study using lower 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). Some of the pups from these litters were then continued on AZT treatment by daily gavage at doses of 20 or 40 mg/kg per day for 24 months. AZT tumor findings were limited to the vaginal epithelium.

A more recent study exposed B6C3F1 mice and Fischer 344 rats to relatively high doses (80, 240, 480 mg/kg) of AZT during gestation and evaluated the animals for tumor incidence at 24 months of age (Walker *et al.,* 2007). In male mice, there were statistically significant increases in the incidences of hemangiosarcoma in all three dosed groups and of hepatocellular carcinoma in the highest dose group. In female mice there was a statistically significant increase in the incidence of neoplasms of the uterus in the 480 mg/kg group. In female rats, the incidences of mononuclear cell leukemia were increased in all three dosed groups.

Humans

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 (Antiretroviral Pregnancy Registry, 2003). An NIH panel has recommended long-term follow-up in children receiving *in utero* exposure to AZT and other antiretroviral drugs (Corcuera Pindado *et al.,* 1994).

GENETIC TOXICITY

AZT is a DNA-reactive chemical that is positive in the *Salmonella* 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 erythrocytes (markers of chromosomal damage) in rats and mice exposed through various combinations of routes and exposure durations (Phillips *et al.,* 1991; Dertinger *et al.,* 1996; Von Tungeln *et al.,* 2002; Bishop *et al.,* 2004b; Witt *et al.,* 2004). 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 body weight

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 recent study by Dobrovolsky *et al.* (2007) using C3B6F$_1$-*Trp53*^{tm1Brd} p53$^{+/-}$ haploinsufficient mice reported that transplacental followed by neonatal exposure to AZT increased the reticulocyte micronucleus frequency 4.8-, 7.1-, and 11.3-fold when measured on PND 1 with maternal doses of 48, 80, and 160 mg AZT/kg body weight 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 AZT/kg body weight 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 *Erythrocebus 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).

A study conducted in neonatal CD-1® mice reported that treatment with AZT (150 mg/kg per day) for 4 weeks resulted in reduced mtDNA copy number and increased mtDNA lesions in heart mitochondria evaluated on PND 28 (Chan *et al.,* 2007). Females appeared to be more susceptible to damage than males.

In humans, HAART designed to stop mother-to-child HIV transmission has been reported to increase frequencies of micronuleated reticulocytes in blood samples from mothers and their babies taken from the cord blood and during the first postnatal week. Percent micronucleated reticulocyte frequencies were up to 10-fold greater in blood from infants exposed to HAART incorporating AZT than from those exposed to HAART that did not include AZT (Witt *et al.,* 2007).

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 when 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 NTRIs (predominantly AZT) as compared to frequencies from children of HIV-infected mothers who were not exposed to NTRIs. 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.

BACKGROUND ON GENETICALLY MODIFIED MICE USED IN THE AZT STUDIES

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 these studies. 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 these studies, 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 Laboratory Animals and Services (Germantown, NY)]. The heterozygous F1 p53$^{+/-}$ mouse was selected for the 30- and 45-week studies 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/N mouse used in NTP studies (NTP, 2013). Together, these factors provided a rational basis for selection of this GMM test model. In addition, the NTP study reported on senna also used the C3B6.129F1/Tac-*Trp53*^{tm1Brd} N12 haploinsufficient GMM model (NTP, 2012), and the background rate for spontaneous tumors in the control group C3B6.129F1-*Trp53*^{tm1Brd} haploinsufficient mice in both studies (AZT and senna) was 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 (Steinbrook, 2004; UNAIDS, 2008).

In the United States, AZT is a primary drug of choice for treating pregnant women who are HIV positive to prevent transmission of the virus to their children. AZT is either used alone or in combination with other antiretroviral drugs. Because AZT has only 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 in the previous sections, AZT has been shown to be both genotoxic and a rodent carcinogen.

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, in preventing mother-to-child transmission of HIV. The C3B6.129F1-*Trp*53^{tm1Brd} N12 haploinsufficient mouse was selected for these studies because previous NTP studies had shown that p53 mutations were associated with AZT-induced carcinogenesis processes (NTP, 2006). 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 carcinogenesis studies. The studies were therefore designed to determine whether genetically modified mice that are haploinsufficient for the p53 gene would provide a suitable model for (1) further evaluating the potential carcinogenicity of AZT and (2) determining whether current and future HAART drug combinations incorporating AZT may potentiate the harmful side effects of AZT. The current study was conducted in conjunction with ongoing NTP-sponsored 2-year studies of AZT, alone or in combination with other HAART drugs, in B6C3F1 mice exposed either during gestation, via maternal gavage, or during gestational exposure followed by neonatal exposure via direct oral gavage to the pups (NTP, 2013).

Doses of AZT were selected based on range-finding studies conducted in B6C3F1 mice performed in conjunction with the previously mentioned 2-year studies. The heterozygous F1 p53$^{+/-}$ mice were exposed to AZT transplacentally via maternal dosing at the same doses that were used for the 2-year studies (NTP, 2013). These transplacental AZT doses were 80, 160, or 240 mg/kg per day from GD 12 though 18 given to the dams as a single daily gavage. These doses fall within the range of doses used in other animal studies that varied from 20 mg/kg per day (Ayers *et al.*, 1997) to 800 mg/kg (Olivero *et al.*, 1997). Pregnant women receiving HAART typically receive doses of 8 to 9 mg/kg per day (NTP, 2013). According to traditional dose scaling calculations (Freireich *et al.*, 1966), this is approximately equivalent to 100 mg/kg per day for adult mice and by the same scaling formulas the selected dam doses of 80, 160, and 240 mg AZT/kg body weight per day were estimated as equivalent to daily AZT doses of 6.5, 13.0, and 19.5 mg/kg, respectively, for a pregnant woman. In the current studies, dosing was continued by daily oral gavage of the pups throughout postnatal development until scheduled evaluation at either 30 or 45 weeks of age. Pup dosing was initiated on PND 1, but the doses and dose volumes were reduced by half from PND 1 to PND 10 to compensate for immaturity of detoxification systems. Continuous dosing until evaluation was used to maximize exposure. A 45-week evaluation time was selected so that the age of the mice would match that of other GMM studies that utilized p53 haploinsufficient mice (e.g., NTP, 2007e) where dosing was initiated at 6 to 7 weeks of age and continued for 39 weeks. Additional mice from the 0/0/0 mg/kg and 240/120/240 mg/kg groups were evaluated at 30 weeks of age (30-week study) to help establish the rate of tumor progression in heterozygous F1 p53$^{+/-}$ mice. Additional mice that were dosed only up to PND 8 (45-week stop-study) were also incorporated into the current studies. This dosing regime (0/0 and 240/40 mg/kg per day) was identical to that used in the concurrent ongoing 2-year studies in B6C3F1 mice and allowed a direct comparison between the studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

AZT

3′-Azido-3′-deoxythymidine (AZT) was obtained from Cipla Ltd., Mumbai Central (Mumbai, India) in one lot (FX4159) used in the 30- and 45-week studies and the 45-week stop-study. 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). Reports on analyses performed in support of the AZT studies are on file at the NCTR.

Lot FX4159 of the chemical, a white crystalline powder, was identified as AZT by the study laboratory using proton nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe-electron ionization mass spectrometry, and high-performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry. All spectra were consistent with literature spectra and the structure of AZT. The melting point range of lot FX4159 was determined to be 122.5° to 123.4° C by Galbraith Laboratories, Inc.; these values were consistent with those reported in the literature for AZT crystallized from water.

Karl Fischer titration and elemental analyses of lot FX4159 were performed by Galbraith Laboratories, Inc., and the study laboratory determined the purity of the bulk chemical by HPLC. Karl Fischer titration indicated less than 0.46% water. Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were in agreement with the theoretical values for AZT. HPLC detected no impurity peaks by comparisons to spectra from previously characterized AZT standards, and the purity of lot FX4159 was determined to be 100% under the conditions of the assay.

Dosing Vehicle

The vehicle used for dose formulations in the 30- and 45-week studies and the 45-week stop-study was a 0.2% methylcellulose/0.1% Tween® 80 aqueous solution. Methylcellulose was obtained from Sigma-Aldrich® Corporation (St. Louis, MO) in one lot (014K0081). Identity studies of lot 014K0081 were performed by the study laboratory using proton and carbon-13 NMR spectroscopy; the results of these analyses were consistent with those obtained previously for a methylcellulose standard obtained from Fischer Scientific (Pittsburgh, PA). Tween® 80 was obtained from Sigma-Aldrich® Corporation in one lot (073K00641).

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing AZT with the dosing vehicle to give the required concentrations. The dose formulations were stored at room temperature in sealed amber glass bottles for up to 29 days.

Stability studies of 0.05 and 0.20 mg/mL formulations were performed by the study laboratory using HPLC. Stability was confirmed for at least 29 days for formulations stored in sealed amber glass bottles at room temperature.

Periodic analyses of the dose formulations of AZT were conducted by the study laboratory using HPLC. Accuracy of dose delivery from the dosing apparatus was also periodically determined using HPLC. Of the dose formulations analyzed and used during the studies, 209 of 211 were within 10% of the target concentrations (Table F2). For the dose accuracy of delivered doses, 17 of the 20 samples analyzed were within 10% of the target doses (Table F3).

STUDY DESIGNS

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 AZT 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). The doses for this study were selected to overlap human exposures.

Male and female heterozygous F1 $p53^{+/-}$ mice were exposed to AZT *in utero* on GDs 12 through 18, then administered AZT by gavage from postnatal day (PND) 1 through 30 weeks of age (30-week study), 45 weeks of age (45-week study), or PND 8 (45-week stop-study). Mice in 0 mg/kg groups 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 8, when the dosing volume was 5 mL/kg. Mice were dosed once daily until PND 28, then once daily 5 days per week. Dosing was performed using a Hamilton Microlab 500 series pump. Full details of this dosing technique have been described by Lewis *et al.* (2010). A summary of the dose groups is presented in Table 1.

For the continuous dosing studies, pregnant dams were administered 0, 80, 160, or 240 mg AZT/kg body weight per day on GDs 12 through 18. Litters were culled to six pups, which were administered 0, 40, 80, or 120 mg/kg as a single daily gavage on PNDs 1 through 10, then 0, 80, 160, or 240 mg/kg as a single daily gavage until PND 28, when they were assigned to either the 30-week or 45-week studies (Figure 1), or culled for genotoxicity studies (Appendix B). Litters that had less than six live pups on PND 1 were also dosed and used for these studies.

For the 30-week study, groups of 26 or 27 male and 26 or 27 female pups from the 0/0/0 or 240/120/240 mg/kg dose groups were assigned to the study on PND 28 and were administered either 0 or 240 mg/kg in a single daily gavage, 5 days/week until the end of the study (Figure 1).

For the 45-week study, groups of 27 male and 26 or 27 female pups from the 0/0/0, 80/40/80, 160/80/160, or 240/120/240 mg/kg dose groups were assigned to the study on PND 28 and were administered either 0, 80, 160, or 240 mg/kg in a single daily gavage, 5 days/week until the end of the study (Figure 1).

For the 45-week stop-study, pregnant dams were administered 0 or 240 mg AZT/kg body weight per day on GDs 12 through 18. Litters were culled to six pups, which were administered 0 or 40 mg/kg as a single daily gavage on PNDs 1 through 8. Groups of 24 or 25 male and 25 or 26 female pups were assigned 0/0 or 240/40 mg/kg dose groups on PND 28 and then maintained on study until 45 weeks of age without further dosing (Figure 2). The neonatal dose of 40 mg/kg was used to match that used in a 2-year study in B6C3F1 mice that will be presented elsewhere. Where possible, no more than two littermates per sex were assigned to each dose group. The individual litter assignments are presented in Appendix G.

TABLE 1
Summary of Dose Groups for Mice Treated with AZT

Treatment Group	Daily Dose				
	0/0 or 0/0/0 mg/kg	240/40 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
30-Week Study[a]					
Dams or litters	34[b]	—	—	—	36[b]
Males	27	—	—	—	26
Females	27	—	—	—	26
45-Week Study[a]					
Dams or litters	34[b]	—	18	16	36[b]
Males	27	—	27	27	27
Females	26	—	27	27	27
45-Week Stop-Study[c]					
Dams or litters	12	14	—	—	—
Males	24	25	—	—	—
Females	26	26	—	—	—

[a] Pups in the 30- and 45-week studies were administered AZT doses that were half that of the maternal dose on PNDs 1 through 10. Pup doses were then doubled and animals received that dose from PND 11 until the end of the studies.
[b] Pups from the same litters were assigned to either the 30-week study or the 45-week study.
[c] For the 45-week stop-study, pups were exposed to 240 mg/kg *in utero* on GDs 12 through 18, then to 40 mg/kg on PNDs 1 through 8.

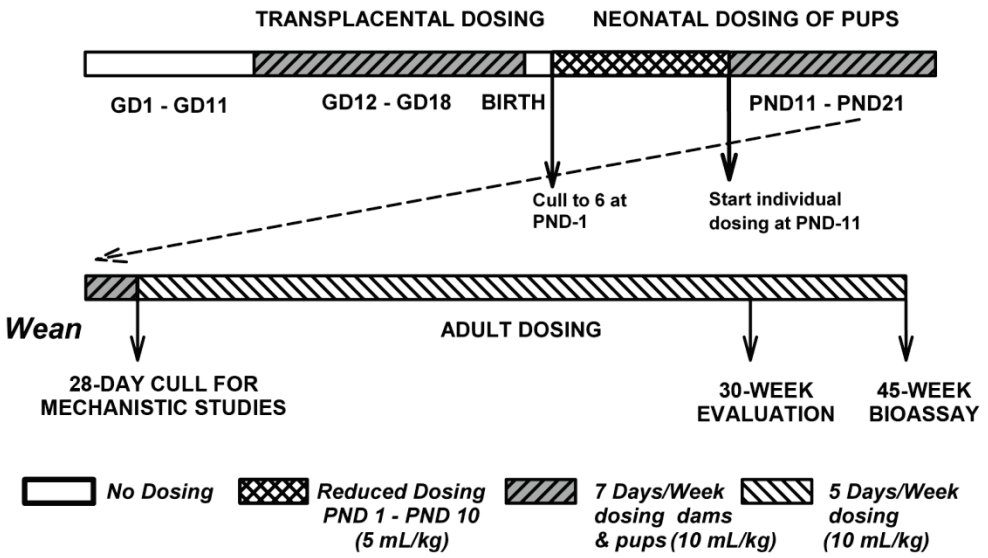


FIGURE 1
Dosing Schedules for 30-Week and 45-Week Studies

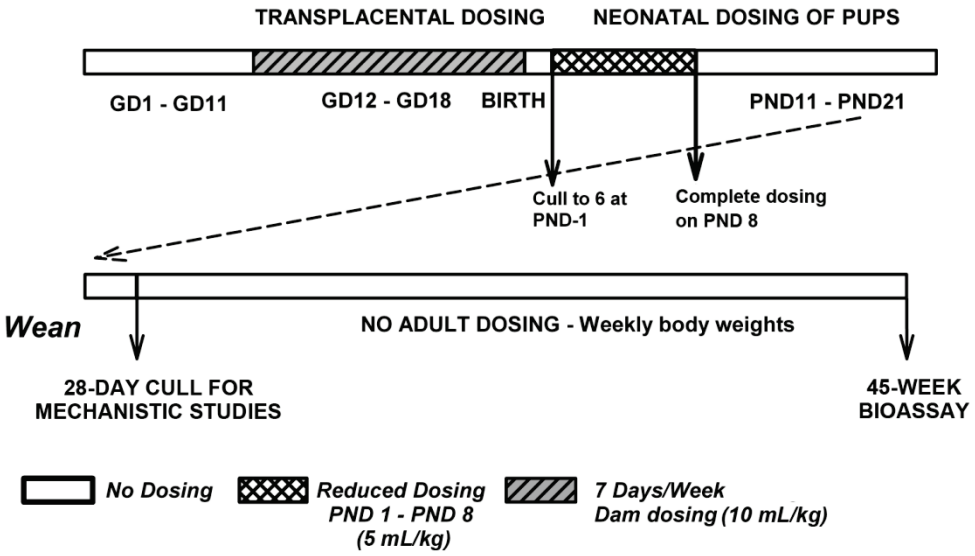


FIGURE 2
Dosing Schedule for 45-Week Stop-Study

Source and Specification of Animals

Female C3H/HeN wild-type mice and male homozygous, p53-null C57BL/6(N12)*Trp53*$^{(-/-)}$ p53-null mice were obtained under an academic breeding license from Taconic Farms, Inc. (Germantown, NY). The animals were quarantined for 14 days prior to being assigned to the study. Each male was mated with up to six females in succession and 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 returned to the breeding pool on GD 10. Dosing of pregnant mice was initiated on GD 12. 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 determined, and the litter was culled to six pups of equal sex ratio when possible. On PND 28, excess pups were culled for mechanistic studies that will be reported elsewhere.

Animal Maintenance

Mouse dams were housed individually with litters until PND 21; pups were weaned, then housed individually beginning PND 29. Feed and water were available *ad libitum*. In order to monitor the health of animals, blood was drawn from two sentinel mice at 3 and 20 weeks and from four sentinel mice at 37, 47, and 56 weeks after receipt of the 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. Pups were weaned on PND 21, and were group housed as a litter until PND 28 when mice assigned to the study were housed individually.

Clinical Examinations and Pathology

Animals were observed twice daily. Body weights were recorded daily for pregnant dams and for litters until PND 21, then individual pups were weighed weekly until the end of the studies; clinical findings were recorded twice weekly.

At PND 160, blood was collected via the saphenous vein from eight males and eight females in the 0/0/0 mg/kg groups and 16 males and 16 females in the 240/120/240 mg/kg groups from the 30- and 45-week studies to monitor for macrocytic anemia. Blood was also collected from surviving male and female mice in the 30-week study at study termination via cardiac puncture under carbon dioxide anesthesia for hematology and clinical chemistry. Blood samples for clinical chemistry were allowed to clot then centrifuged. The serum was removed and frozen at –60° C until analysis. Blood samples for hematology were collected in EDTA and analysis was performed on the day of collection. Automated hematology was performed using an ABX Pentra 60 C+ hematology analyzer (ABX, Irvine, CA). Clinical chemistry analyses were conducted on an Alfa Wassermann ALERA analyzer (Alfa Wassermann, West Caldwell, NJ) with reagents manufactured and/or supplied by Alfa Wassermann or Catachem (Bridgeport, CT). The parameters measured are listed in Table 2.

Necropsies and microscopic examinations were performed on all mice. The brain (45-week study and 45-week stop-study) heart, left and right kidney, liver, and lung (30-week study) 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 Acquisition 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 and all potential target organs, which included the liver of male mice and the lymph nodes, spleen, and thymus of male and female mice. 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 Studies of AZT

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 1st breeders was 11/17/2004; first conception dates:
30-Week Study: 11/18/2004
45-Week Study: 11/18/2004
45-Week Stop-Study: 03/10/2005

Duration of Dosing
30-Week Study: GDs 12 through 18, then PND 1 through 30 weeks of age
45-Week Study: GDs 12 through 18, then PND 1 through 45 weeks of age
45-Week Stop-Study: GDs 12 through 18, then PNDs 1 through 8

Date of Last Dose
30-Week Study: 01/24/2006
45-Week Study: 02/28/2006
45-Week Stop-Study: 06/21/2005

Date of Necropsy
30-Week Study: 01/25/2006
45-Week Study: 03/01/2006
45-Week Stop-Study: 04/26/2006

Age at Necropsy
30 (30-week study) or 45 (45-week study and 45-week stop-study) weeks for terminal kill; early deaths and moribund animals were also necropsied

Size of Study Groups
30-Week Study: 26 or 27 males and 26 or 27 females
45-Week Study: 27 males and 26 or 27 females
45-Week Stop-Study: 24 or 25 males and 26 females

Animals per Cage
One male and one female during breeding; dams housed individually with litters until PND 21; pups housed with littermates PNDs 21-28 then individually beginning PND 28

TABLE 2
Experimental Design and Materials and Methods in the *In Utero*/Postnatal Gavage Studies of AZT

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*

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.), changed weekly

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

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

Doses
30-Week Study: GDs 12-18, 0 or 240 mg/kg per day with a dose volume of 10 mL/kg; PNDs 1-10, 0 or 120 mg/kg per day with a dose volume of 5 mL/kg; PND 11 to 30 weeks of age, 0 or 240 mg/kg per day with a dose volume of 10 mL/kg

45-Week Study: GDs 12-18, 0, 80, 160, or 240 mg/kg with a dose volume of 10 mL/kg; PNDs 1-10, 0, 40, 80, or 120 mg/kg per day with a dose volume of 5 mL/kg; PND 11 to 45 weeks of age, 0, 80, 160, or 240 mg/kg per day with a dose volume of 10 mL/kg

45-Week Stop-Study: GDs 12-18, 0 or 240 mg/kg per day with a dose volume of 10 mL/kg; PNDs 1-8, 0 or 40 mg/kg per day with a dose volume of 5 mL/kg, then undosed to 45 weeks of age

Dosing was once daily through PND 28 then once a day, 5 days/week from PND 29 to 30 or 45 weeks of age

Method of Distribution
Animals were weight ranked, then assigned to vehicle control or dosed cages randomly

Type and Frequency of Observation
Observed twice daily; body weights recorded daily for pregnant dams and for litters until PND 21, individual pups weekly thereafter; clinical findings were recorded twice weekly

Method of Kill
CO_2 asphyxiation

Necropsy
All mice were necropsied. Organs weighed were brain (45-week study and 45-week stop-study), heart, left and right kidney, liver, and lung (30-week study)

TABLE 2
Experimental Design and Materials and Methods in the *In Utero*/Postnatal Gavage Studies of AZT

Clinical Pathology
At PND 160, blood was collected via the saphenous vein from eight males and eight females in the 0/0/0 mg/kg groups and 16 males and 16 females in the 240/120/240 mg/kg groups from the 30- and 45-week studies to monitor for macrocytic anemia. Blood was also collected from surviving male and female mice in the 30-week study at study termination via cardiac puncture under carbon dioxide anesthesia for hematology and clinical chemistry.
Hematology: *PND 160* – hematocrit, hemoglobin, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and erythrocyte count and dilution width; *30 Weeks* – hematocrit, hemoglobin, erythrocyte and platelet counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and leukocyte count and differentials
Clinical chemistry: creatinine and blood urea nitrogen

Histopathology
Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder, heart, Harderian gland, 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, skeletal muscle, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.

STATISTICAL METHODS

Survival

Weaned pups reaching terminal kill were censored from analysis. Kaplan-Meier estimates (Kaplan and Meier, 1958) of mean survival times were calculated for each sex-by-treatment group and the Kaplan-Meier curves were plotted. For each sex and dosing regimen (30-week, 45-week, and 45-week stop-study) combination, four proportional hazards models (Cox, 1972) were used to test the effect of the dose (linear trend and comparison to control). The four models were unadjusted for litter using standard Cox model, unadjusted for litter using a sandwich variance estimate (Binder, 1992), adjusted for litter using a sandwich variance estimate, and adjusted for sires using a sandwich variance estimate. The second model was necessary to truly gauge the impact of the litter correlation. All survival analysis P values are two sided. Unless otherwise noted, statistical significance was set at the 5% level.

Body Weight Analysis

The body weight data for each animal were rasterized to evenly-spaced time points (every 4 weeks) via LOESS 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 analyzed using a mixed effects model by sex and age (in weeks). This was done to facilitate proper modeling of the intralitter correlation and the inherent variance heteroscedasticity with age. To capture the growth dynamics, additional modeling captured the initial growth rate and the late growth rate. The model treated body weight as a function of treatment group. Dunnett's method (Dunnett, 1955) was used to compare body weight in the dosed animals to body weight in the control animals at each scored age. This model was run unadjusted for litters, adjusted for litters, and adjusted for sires. Plots are presented using naive means and standard errors.

Analysis of Continuous Variables

For organ weights in the 30-week study and the 45-week stop-study and for hematology and clinical chemistry data, dosed groups were compared to the control groups using Student's *t*-test. For organ weights in the 45-week study, groups were analyzed by a two-tailed Dunnett's test run under the SAS General Linear Models Program.

Calculation of Neoplasm and Nonneoplastic Lesion Incidences and Severities

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, A4, A6, A7, A9, A10, A12, A13, A15, A16, and A18 as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For 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. For dam- and sire-adjusted correlation models, the Poly-3 weighted generalized linear model was used to generate estimated correlation-adjusted incidences and these are given along with the relevant test P value.

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) is typically used to assess treatment effects on neoplastic and nonneoplastic lesion prevalence. This test is a

survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. The variance correction of Bieler and Williams (1993) is usually used to account for the extra-binomial variability induced by using a stochastic denominator in the Cochran-Armitage test. Pairwise comparisons in this test are accomplished by reanalyzing the treatment groups in pairs. This framework limits the Poly-k test to one-way designs with no correlation. This model was run for these studies for each dosing regimen (30-week, 45-week, and 45-week stop-study) and does not adjust for intralitter correlation.

To adjust for intralitter correlation, the Poly-k test was revised. Bieler and Williams (1993) used the fact that the Cochran-Armitage test can be envisioned as a binomial-weighted regression in the derivation of their variance correction. If we begin with this paradigm, we can generalize it to view the Cochran-Armitage test as a generalized linear model with binomial variation and an identity link function (McCullagh and Nelder, 1989). This model can be used with the Poly-k weights to allow more complex designs including litter correlations using generalized estimating equations (Liang and Zeger, 1986) and factorial effects as well as alternative link functions.

Several issues arise in this situation. First, estimated variances will be group-specific rather than null hypothesis-specific. Bieler and Williams (1993) mentioned that group-specific variances had caused their correction to be less stable in simulations and opted for the null hypothesis variance. Using group-specific variances certainly has the effect of creating uniform groups and causing estimation problems in typical NTP datasets. Specifying the null hypothesis deviance function might address this problem but this was not done for the current studies. Instead, for these analyses, a more traditional method of bumping uniform groups was used. The identity link did not appear to be very sensitive to the amount of the bump; especially in comparison to the logit link which was sensitive to bump size. Therefore the identity link was used.

The second difficulty comes in estimating correlations. Poly-k refits the pairwise models in order to estimate the pairwise effects. This is not applicable in factorial analyses and is problematic in litter correlation analyses since it allows the litter correlation to be different among the different analyses. Thus all the data were used to estimate the correlations that should be considered as common across treatment groups.

The common correlation leads to the third issue; Poly-k is ultimately a linear regression. However, we can consider the generalized linear model to be an ANOVA model with design effects and use contrasts to generate the linear trend and pairwise comparisons or, indeed, any factorial contrast we may wish to examine. This ANOVA-style method is not quite in keeping with traditional Poly-k.

For each dosing-regimen and lesion pool, three analyses of these data were analyzed: traditional Poly-3, dam-adjusted Poly-3, and sire-adjusted Poly-3. For each lesion pool and drug combination, the sire- or dam-adjusted analysis fits a lesion-present flag to dose level using a generalized linear model with binomial distribution, identity link function, and Poly-3 observation weights. Uniform treatment groups were bumped away from uniformity by adding an uncorrelated dummy lesion flag observation with value=0.005 and Poly-3 weight=0.005. Correlation within sire or dam was achieved by invoking a generalized estimating equation-based exchangeable correlation among sire- or dam-mates. This completed the model. Suitable contrasts were used to test the relevant hypotheses. One-sided results were generated in keeping with NTP standards. The sire-adjusted analyses were generated in the same manner differing only in the specification of the correlation group variable.

It should be emphasized again that the implementation details of the correlation Poly-k methods are different from the Bieler and Williams' variance-adjusted Poly-k test (Bieler and Williams, 1993). Particularly, the variance is not quantal-adjusted, is group-specific rather than null hypothesis-specific, 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. Although the correlation method appears to generate similar results, it does represent a significant departure from the standard Poly-k method that the NTP has used.

In addition, to incorporate lesion severity scores, the distribution-free (but unadjusted for age and unadjusted for litter correlation) 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.

QUALITY ASSURANCE METHODS

The 30- and 45-week studies and the 45-week stop-study were conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, records from these studies including protocol 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; daily animal room logs and a copy of the laboratory study will be submitted to the NCTR Archives.

GENETIC TOXICOLOGY

Mouse Peripheral Blood Micronucleus Test Protocol

Blood was collected from excess heterozygous F1 $p53^{+/-}$ mice that were culled on PND 1, PND 10, or PND 28 in the 45-week study or from mice in the 30-week study at terminal kill (Appendix B). Micronucleated cells were identified and quantified using a MicroFlow^{PLUS} mouse kit (Litron Laboratories, Rochester, NY) (Dertinger *et al.,* 2006). The frequencies of micronucleated reticulocytes and micronucleated normochromatic erythrocytes were determined in blood samples collected on PND 1 following transplacental dosing from GDs 12 through 18 (Figure 1) or within 6 hours after dosing for older mice. Mouse peripheral blood was diluted with anticoagulant, fixed in –80° C methanol, and stained with three fluorochromes for flow analysis. 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. Flow cytometry was performed on a FACScan^{TM} (Beckton-Dickinson, San Jose, CA) equipped with a 488 nm argon ion laser and fluorescence detectors. Data acquisition for each sample stopped automatically after 20,000 reticulocytes were detected by the flow cytometer. The flow cytometry collection parameters for the samples were set as described in the Litron instruction manual. Differences in reticulocyte micronucleus frequency between dose groups were analyzed using a two-tailed Dunnett's test. At 30 weeks, when data from only 0/0/0 mg/kg and 240/120/240 mg/kg groups were available, the statistical analysis consisted of a two-tailed Student's *t*-test. When data were available from more than one pup/sex from a given litter, the data were averaged and incorporated as one data point.

RESULTS

LITTER SUCCESS AND PUP SURVIVAL

As shown in Table 3, AZT at the doses used was relatively nontoxic to both the pregnant dams and neonatal heterozygous F1 $p53^{+/-}$ mouse pups. There were no apparent treatment-related effects on both litter success and pup survival to postnatal day (PND) 28, which was greater than 90% in all dose groups.

30-WEEK STUDY

Survival

Estimates of 30-week survival probabilities for male and female mice are shown in Table 4 and Figure 3. Survival of males in the 240/120/240 mg/kg group was significantly less than that of the 0/0/0 mg/kg group.

TABLE 3
Litter Parameters and Pup Survival for Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

	0/0/0 (0/0) mg/kg[a]	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 (240/40) mg/kg[a]
Total dams pregnant	50	22	19	53
Dams which did not litter	4	4	3	3
Total litters	46	18	16	50
Total pups born	314	141	105	351
Average born per litter	6.8	7.8	6.8	7.0
Number of males born[b]	152 (48.4)	69 (48.9)	56 (53.3)	174 (49.6)
Sex ratio[c]	1:0.97	1:1.01	1:0.88	1:0.94
Pups born dead (%)	14 (4.5)	2 (1.4)	0 (0)	13 (3.7)
% Survival PND 1 – PND 10[d]	92.8	98.1	97.8	95.3
% Survival PND 11 – PND 28[d]	96.7	91.1	98.9	93.8

[a] Litters dosed to provide stop-study pups are included in the litter and pup birth data but are excluded from the pup survival estimates. Postnatal survival for stop-study pups was 98.6% and 96.5% between PND 1 and PND 10 for the 0/0 mg/kg group and 240/40 mg/kg groups, respectively, and 98.6% and 100% between PND 11 and PND 28 for the 0/0 mg/kg group and 240/40 mg/kg groups, respectively.

[b] Excludes pups born dead, percent of total live pups given in parentheses

[c] Male:female

[d] Excludes pups culled on PND 1 and stop-study litters

TABLE 4
Survival of Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Male		
Animals initially in study	27	26
Number of litters/group	22	21
Moribund	0	4
Natural deaths	0	1
Mice surviving to study termination	27	21
Percent probability of survival to end of study[a]	100.0	80.8
Mean survival (days)	229.3	214.5
Survival analysis[b]		P=0.034
Female		
Animals initially in study	27	26
Number of litters/group	22	23
Moribund	2	0
Natural death	1	0
Mice surviving to study termination	24	26
Percent probability of survival to end of study	88.9	100.0
Mean survival (days)	217.4	228.7
Survival analysis		P=0.258N

[a] Kaplan-Meier determinations
[b] Results of the life table pairwise comparisons (Cox, 1972) with the 0/0/0 mg/kg group. A lower mortality in a dosed group is indicated by **N**.

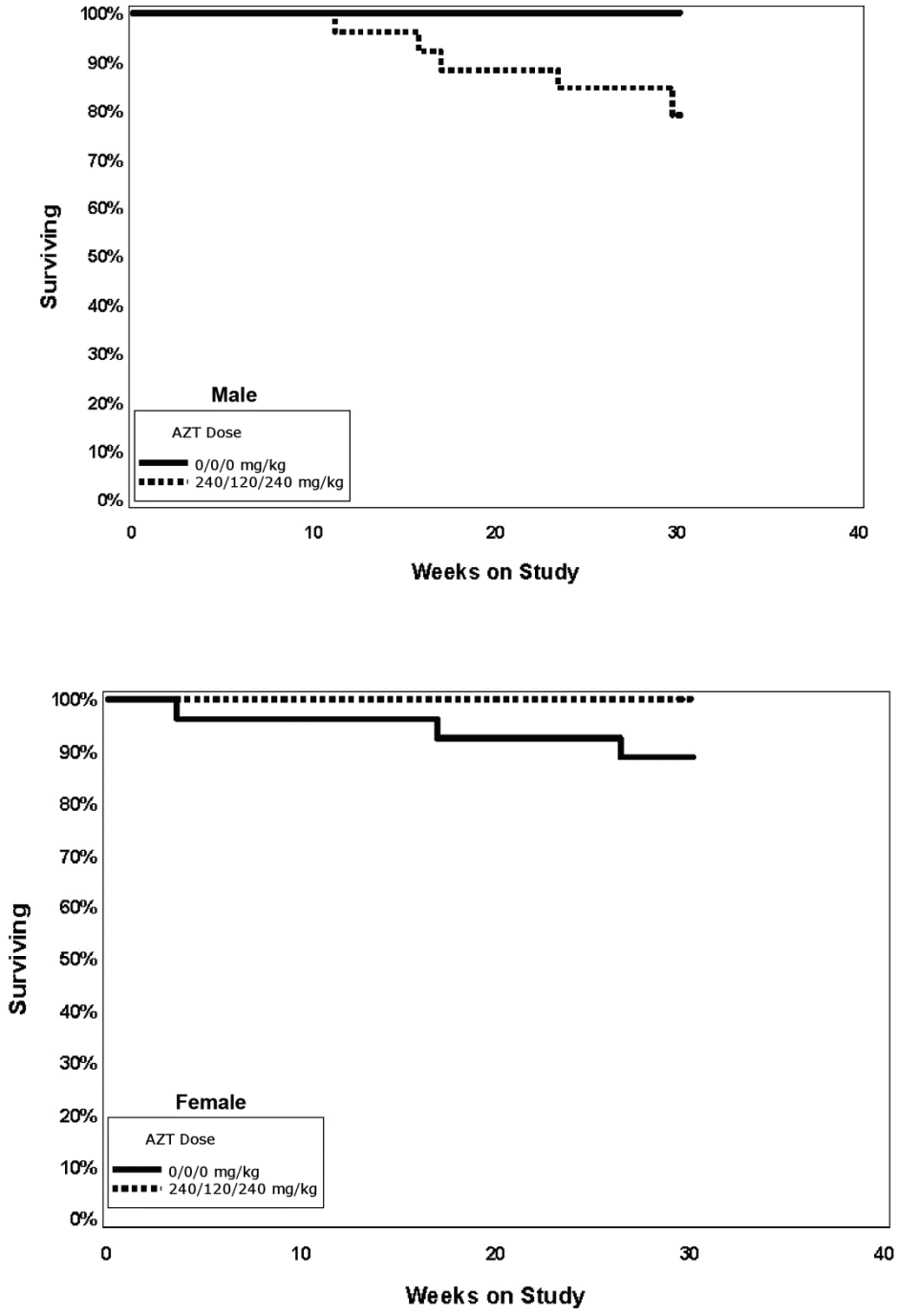


FIGURE 3
Kaplan-Meier Survival Curves for Mice Administered AZT by Gavage in the 30-Week *In Utero*/Postnatal Study of AZT

Body Weights, Organ Weights, and Clinical Pathology

Growth curves for male and female mice are shown in Figure 4. For male body weights, dose, time, and the interaction of dose and time were significant effects (Table D1). Males administered AZT had significantly lower body weights compared to the 0/0/0 mg/kg group during the duration of the study (Tables D2 and D3). For females, there were significant dose and time effects; however the interaction of dose and time was not significant. Throughout the study, females administered AZT had significantly lower body weights compared to those of the 0/0/0 mg/kg group.

Absolute kidney weights were significantly less in 240/120/240 mg/kg males and females compared to the 0/0/0 mg/kg groups, but the relative kidney weights were similar (Table E1). Mean cell volume and mean cell hemoglobin in dosed females and mean cell volume in dosed males were significantly increased at the end of the study (Table C1); when severe, these changes are characteristic of macrocytic anemia. However, these increases were within normal physiological ranges for mice, suggesting minimal macrocytosis rather than pathologic macrocytic anemia.

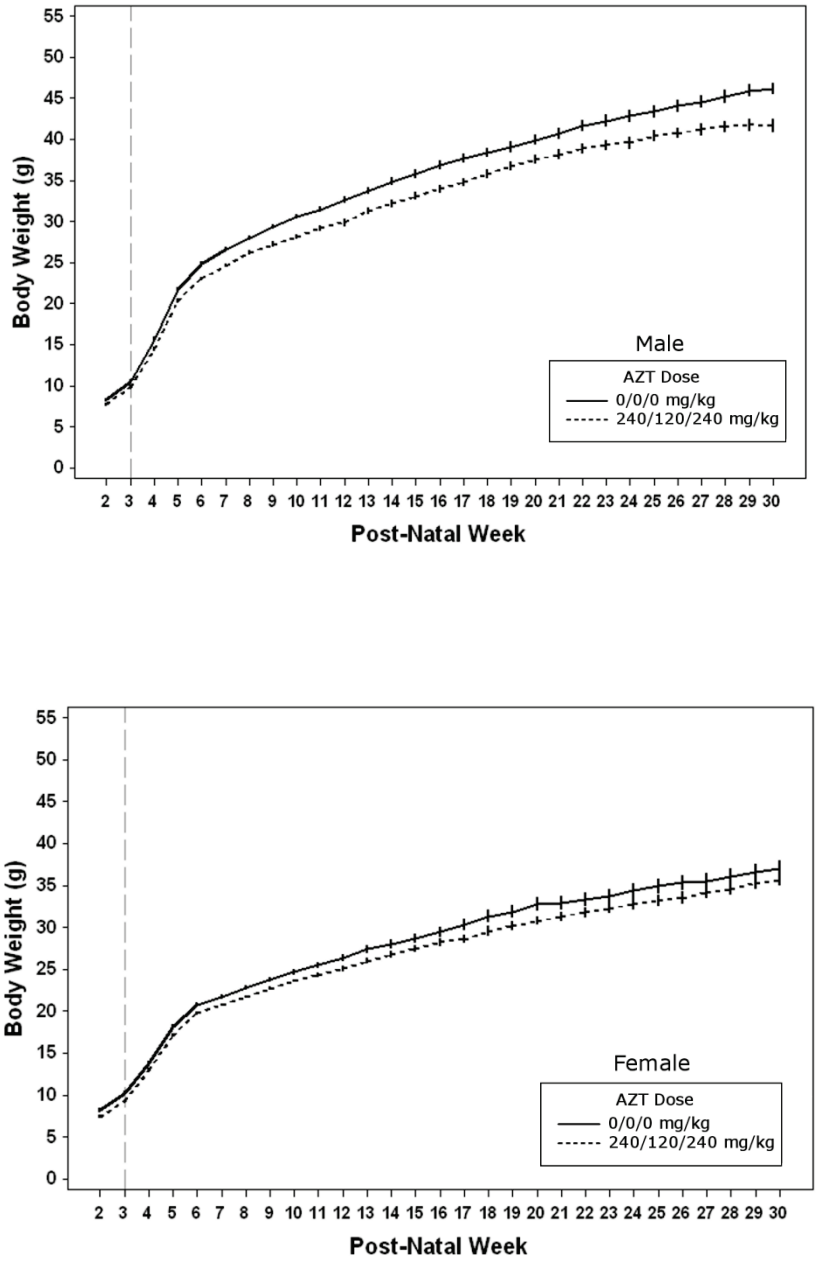


FIGURE 4
Growth Curves for Mice Administered AZT by Gavage in the 30-Week *In Utero*/Postnatal Study of AZT

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms of the bone and brain. Significant changes in the incidences of nonneoplastic lesions included lymphocytic cellular infiltration of the kidney and lymphocytic cellular infiltration of the salivary gland, which were both reduced in male and female mice administered 240/120/240 mg/kg relative to the incidences in the 0/0/0 mg/kg groups. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analysis of selected primary neoplasms are presented in Appendix A (Tables A1 through A6).

Malignant Lymphoma: In male mice administered 240/120/240 mg/kg, the incidence of malignant lymphoma was increased compared to that in the 0/0/0 mg/kg group and this contributed to the increased mortality in treated male mice. Although the increased incidence of malignant lymphoma was not statistically significant when evaluated with a standard Poly-3 analysis, it was significant (P=0.005) when adjustments for litter correlations were made (Tables 5, A1, A2, and G6).

Other Findings: The AZT-treated male mice exhibited low incidences of bone and brain neoplasms; these lesions did not occur in the 0/0/0 mg/kg males (Tables 5, A1, and A2).

Litter Correlation Analysis: When statistical analysis incorporating correlation of influence among littermates was used to compute incidences of neoplasms (Appendix G), statistical significance generally increased (i.e., P values decreased) suggesting that there was no positive correlation between tumor incidence and litter. While in most cases these changes were insufficient to decrease P values for the incidences of neoplasms below the 5% definition of significance, the significance of the incidence of malignant lymphoma was altered by this analysis (Tables 5 and G6).

TABLE 5
Incidences of Malignant Neoplasms in Male Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Femur[a]	27	26
Osteosarcoma[b]	0	1
Cerebrum	27	26
Astrocytoma Malignant	0	1
Olfactory Lobe, Neuroblastoma	0	1
All Organs		
Malignant Lymphoma		
Overall rate[c]	0/27 (0.0%)	3/26 (11.5%)
Adjusted rate[d]	0.0%	11.9%
Terminal rate[e]	0/27 (0.0%)	0/21 (0.0%)
First incidence (days)	—[g]	84
Poly-3 test[f]		P=0.102
Litter-adjusted Poly-3 rate	0.0%	14.4%
Litter-adjusted Poly-3 test		P=0.005

[a] Number of animals with tissue examined microscopically
[b] Number of animals with neoplasm
[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 dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and the 240/120/240 mg/kg group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[g] Not applicable; no neoplasms in animal group

45-WEEK STUDY

Survival

Estimates of 45-week survival probabilities for male and female mice are shown in Table 6 and Figure 5. Compared to the 0/0/0 mg/kg groups, there was no significant difference in survival across AZT dose groups in either males or females.

Body Weights, Organ Weights, and Hematology

Growth curves for male and female mice are shown in Figure 6. Exposure to AZT caused small decreases in body weight relative to vehicle control mice. These decreases were not statistically significant for any dose group in either males or females when compared for each week of the study (Table D6). However, the overall comparison was significant for dose (P=0.002 and P=0.014 for males and females respectively, Table D4). In addition, there were significant negative linear trends with dose, such that for each increment in AZT dose there was, on average, a 6.3 and 4.2 g decrease in body weight for male and female mice, respectively (Table D5).

Absolute brain weights were significantly less in 240/120/240 mg/kg males and in all three dosed groups of females compared to those in the 0/0/0 mg/kg groups, but relative brain weights were not significantly different (Table E2). Mean cell volume and mean cell hemoglobin evaluated at 160 days were significantly increased in 240/120/240 mg/kg males and females (Table C2), which is characteristic of macrocytic anemia; however, as with the mice evaluated at 30 weeks, these increases were within the normal physiological ranges for mice, suggesting minimal macrocytosis rather than pathologic macrocytic anemia.

TABLE 6
Survival of Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Male				
Animals initially in the study	27	27	27	27
Number of litters/group	24	14	15	25
Moribund	2	4	0	2
Natural deaths	1	2	0	3
Animals surviving to study termination	24	21	27	22
Percent probability of survival to end of study[a]	88.9	77.8	100.0	81.5
Mean survival (days)	316.7	298.4	335.5	311.3
Survival analysis[b]		P=0.241	P=0.184N	P=0.458
Female				
Animals initially in the study	26	27	27	27
Number of litters/group	23	13	15	25
Moribund	2	2	1	3
Natural deaths	1	2	2	3
Animals surviving to study termination	23	23	24	21
Percent probability of survival to end of study	88.5	85.2	88.9	77.8
Mean survival (days)	326.7	316.7	323.9	316.2
Survival analysis		P=0.701	P=0.928N	P=0.334

[a] Kaplan-Meier determinations
[b] Results of the life table pairwise comparisons (Cox, 1972) with the 0/0/0 mg/kg group. A lower mortality in a dosed group is indicated by **N**.

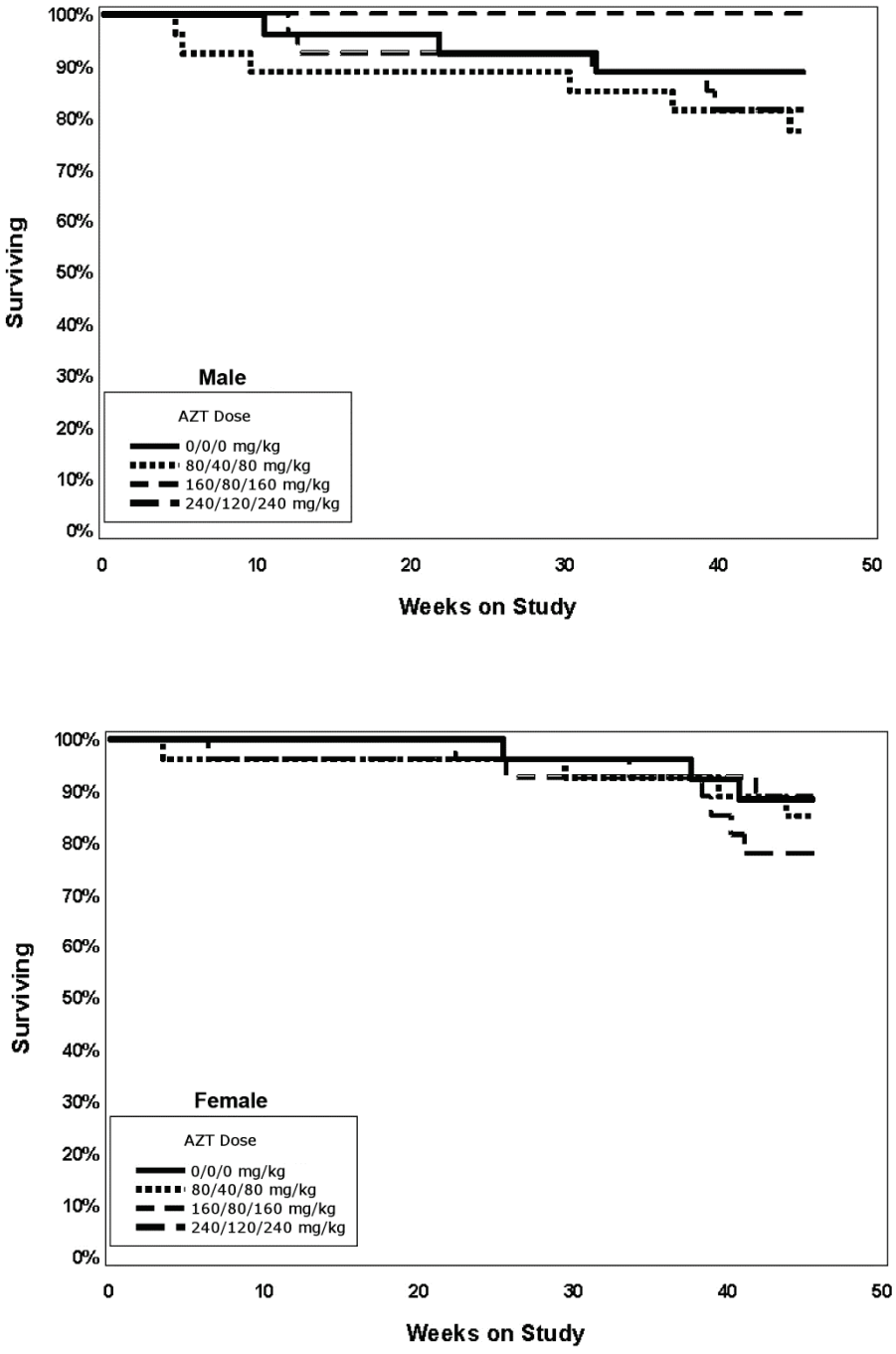


FIGURE 5
Kaplan-Meier Survival Curves for Mice Administered AZT by Gavage in the 45-Week *In Utero*/Postnatal Study of AZT

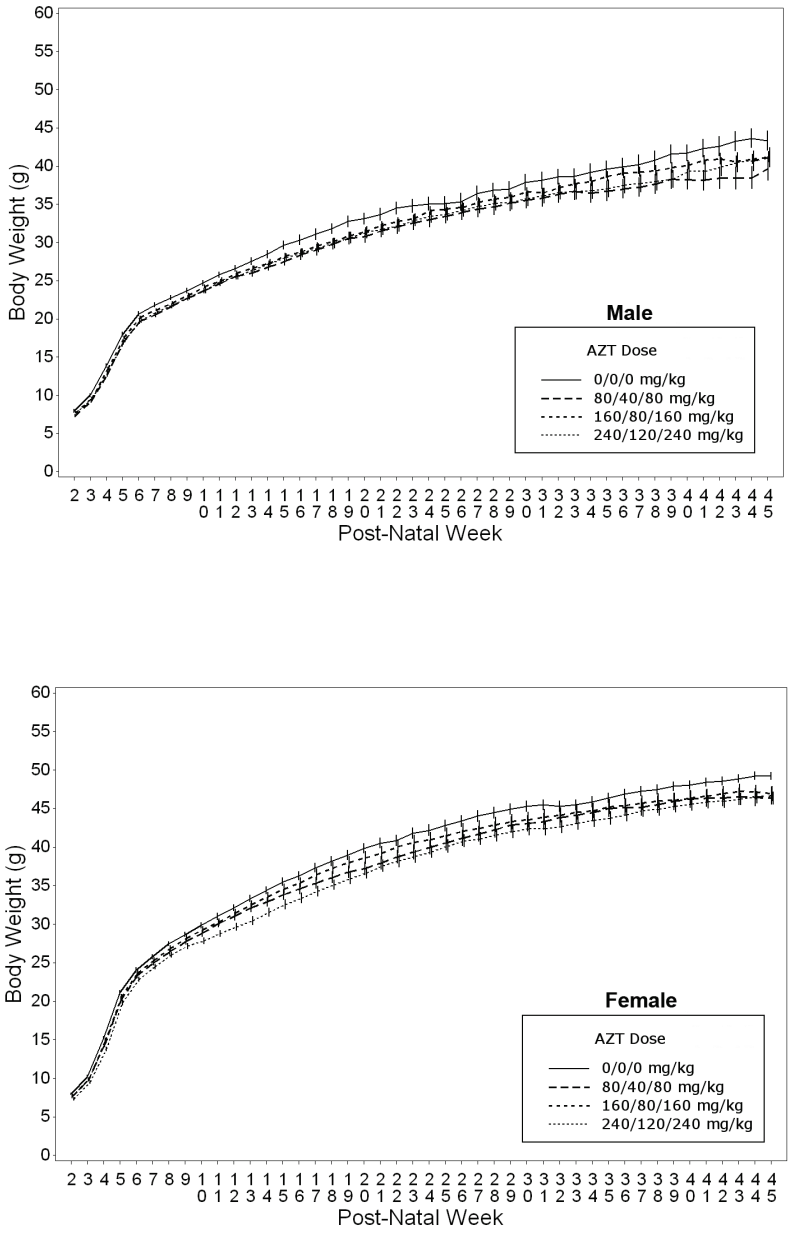


FIGURE 6
Growth Curves for Mice Administered AZT by Gavage in the 45-Week *In Utero*/Postnatal Study of AZT

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the liver in male mice, of malignant lymphoma and other malignant neoplasms in females, and nonneoplastic lesions of the preputial gland in male mice and of the pancreas and bone marrow in females. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analysis of selected primary neoplasms are presented in Appendix A (Tables A7 through A12). Historical control incidences for neoplasms in heterozygous F1 $p53^{+/-}$ mice are shown in Appendix I.

Liver: The incidences of hepatocellular adenoma in males occurred with a positive trend, and the incidence in the 240/120/240 mg/kg group was significantly greater than that in the 0/0/0 mg/kg group (Tables 7, A7, and A8). A single hepatocellular carcinoma occurred in the 80/40/80 mg/kg group.

TABLE 7
Incidences of Hepatocellular Neoplasms in Male Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Hepatocellular Adenoma[a]				
Overall rate[b]	3/26 (11.5%)	2/27 (7.4%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate[c]	12.3%	8.8%	22.2%	36.5%
Terminal rate[d]	3/24 (12.5%)	2/21 (9.5%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test[e]	P=0.013	P=0.531N	P=0.288	P=0.048
Hepatocellular Adenoma or Carcinoma				
Overall rate	3/26 (11.5%)	3/27 (11.1%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate	12.3%	13.2%	22.2%	36.5%
Terminal rate	3/24 (12.5%)	3/21 (14.3%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test	P=0.019	P=0.634	P=0.288	P=0.048

(T) Terminal kill

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

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

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

[d] Observed incidence at terminal kill

[e] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group 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 dosed group is indicated by **N**.

Malignant Lymphoma: A significant positive trend occurred in the incidences of malignant lymphoma in female mice; this neoplasm occurred in the 160/80/160 and 240/120/240 mg/kg groups, but not in the 0/0/0 mg/kg group. When analyzed with the standard Poly-3 test, no significant differences in incidences were found between the treated groups and the 0/0/0 mg/kg group (Tables 8 and A11).

Other Malignant Neoplasms: Both male and female heterozygous F1 $p53^{+/-}$ mice exhibited low incidences of several malignant neoplasms, which appear to be characteristic of this strain. In females, some of these neoplasms, including osteosarcoma and mammary gland adenocarcinoma exhibited higher incidences in treated groups than in the 0/0/0 mg/kg group (Tables A10 and A11). These contributed to increases (not significant) in the overall incidences of malignant neoplasms in 160/80/160 and 240/120/240 mg/kg females (Table A11). There were no incidences of vaginal epithelial neoplasms in any dose group (Table A10). Complete lists of all neoplastic lesions observed in each male or female mouse used in the study are shown in Tables G1 and G2.

Nonneoplastic lesions: Incidences of several nonneoplastic lesions were increased in dosed groups relative to the 0/0/0 mg/kg groups. In males, preputial gland degeneration was observed only in the 240/120/240 mg/kg group (0/0/0 mg/kg, 0/27; 80/40/80 mg/kg, 0/26; 160/80/160 mg/kg, 0/27; 240/120/240 mg/kg, 3/26; Table A9), and in females, incidences of lymphocytic cellular infiltration of the pancreas (0/26, 0/27, 1/27, 3/26, Table A12) and myeloid cell hyperplasia of bone marrow (0/26, 0/26, 4/26, 1/26, Table A12) showed dose-related increases.

Litter Correlation Analysis: When statistical analysis incorporating correlation of influence among littermates was used to compute incidences of neoplasms (Appendix G), statistical significance generally increased (i.e., P values decreased) suggesting that there was no positive correlation between tumor incidence and litter. In most cases, this did not decrease P values below the 5% definition of significance. However, in the case of malignant lymphoma in female mice, significance of the 3/27 incidence in the 240/120/240 mg/kg group relative to the 0/0/0 mg/kg group incidence (0/26) increased from P=0.115 for the non-litter-adjusted Poly-3 test to P=0.033 for the litter-adjusted Poly-3 test (Tables 8 and G10). Incidences of preputial gland degeneration in males (Table G13), and lymphocytic cellular infiltration of the pancreas and myeloid cell hyperplasia of the bone marrow in females (Table G16) exhibited statistically significant increases when litter-adjusted evaluations were performed, but not when the naive Poly-3 evaluations were performed (Tables A9 and A12).

TABLE 8
Incidences of Malignant Lymphoma in Female Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Malignant Lymphoma[a]				
Overall rate[b]	0/26 (0.0%)	0/27 (0.0%)	1/27 (3.7%)	3/27 (11.1%)
Adjusted rate[c]	0.0%	0.0%	4.0%	12.2%
Terminal rate[d]	0/23 (0.0%)	0/23 (0.0%)	1/24 (4.2%)	2/21 (9.5%)
First incidence (days)	—[f]	—	322 (T)	241
Poly-3 test[e]	P=0.020	—[g]	P=0.505	P=0.115
Litter-adjusted Poly-3 rate	0.0%	0.0%	4.0%	12.2%
Litter-adjusted Poly-3 test	P=0.023	—	P=0.148	P=0.033

(T) Terminal kill

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

[b] Number of animals with malignant lymphoma per number of animals examined microscopically

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

[d] Observed incidence at terminal kill

[e] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

45-WEEK STOP-STUDY

Survival

Estimates of 45-week survival probabilities for male and female mice are shown in Table 9 and Figure 7. There was no effect of AZT administration on survival in males or females.

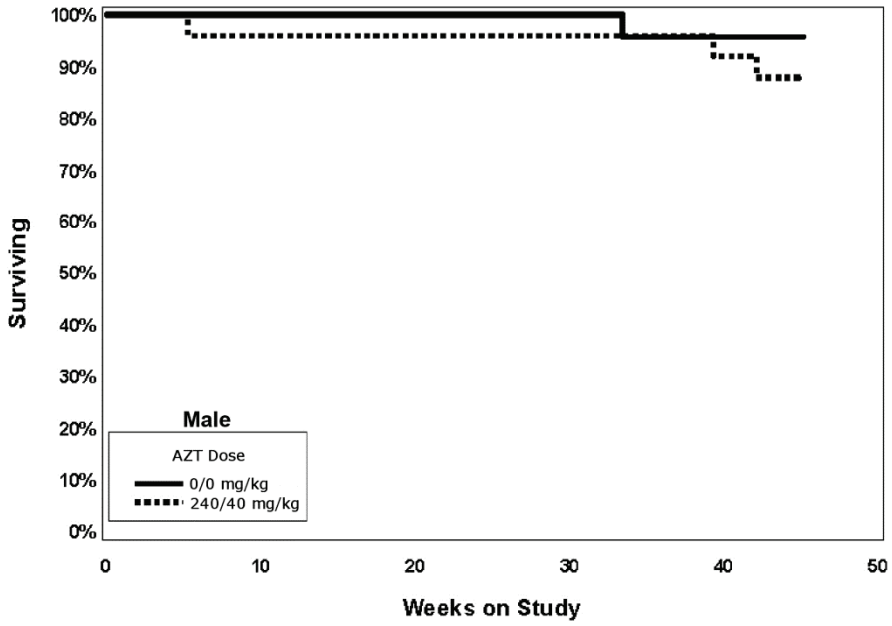
Body Weights and Organ Weights

Growth curves for male and female mice are shown in Figure 8. There were significant dose and time effects on body weight in males and a significant time effect in females (Table D7). Dosed male mice had significantly lower body weights compared to the 0/0 mg/kg group (Tables D8 and D9). Absolute brain weights of dosed groups of males and females were significantly less than those of the 0/0 mg/kg group, though relative brain weights were not significantly altered (Table E3).

TABLE 9
Survival of Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Male		
Animals initially in study	24	25
Number of litters/group	13	14
Moribund	1	2
Natural death	0	1
Mice surviving to study termination	23	22
Percent probability of survival to end of study[a]	92.0	88.0
Mean survival (days)	330.9	320.2
Survival analysis[b]		P=0.276
Female		
Animals initially in study	26	26
Number of litters/group	13	14
Moribund	0	3
Natural death	1	0
Mice surviving to study termination	25	23
Percent probability of survival to end of study	96.2	88.5
Mean survival (days)	331.5	326.6
Survival analysis		P=0.261

[a] Kaplan-Meier determinations
[b] Results of the life table pairwise comparisons (Cox, 1972) with the 0/0 mg/kg group



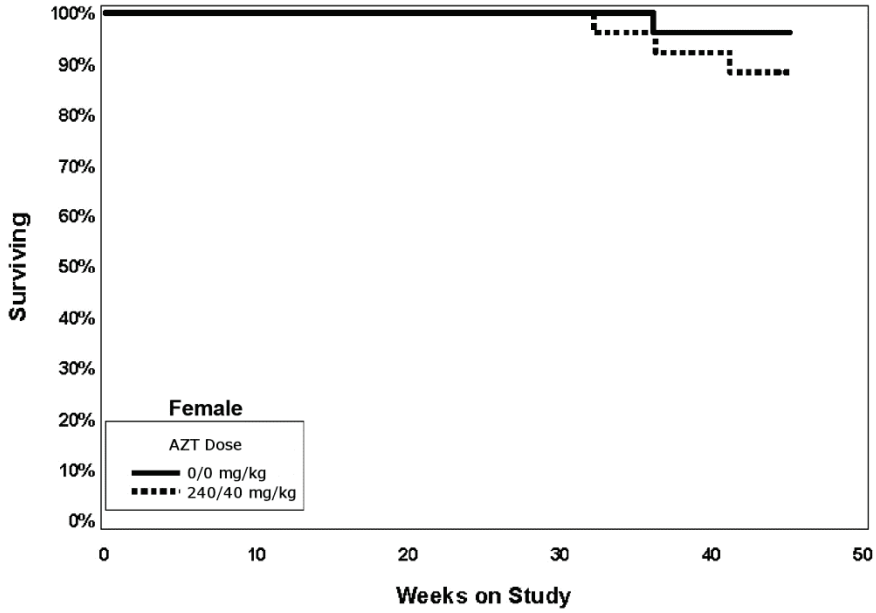
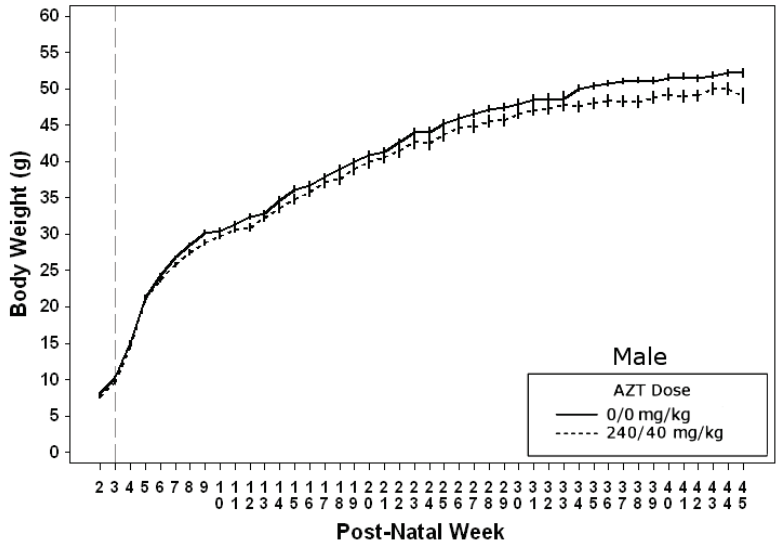


FIGURE 7
Kaplan-Meier Survival Curves for Mice Administered AZT by Gavage in the 45-Week *In Utero*/Postnatal Stop-Study of AZT



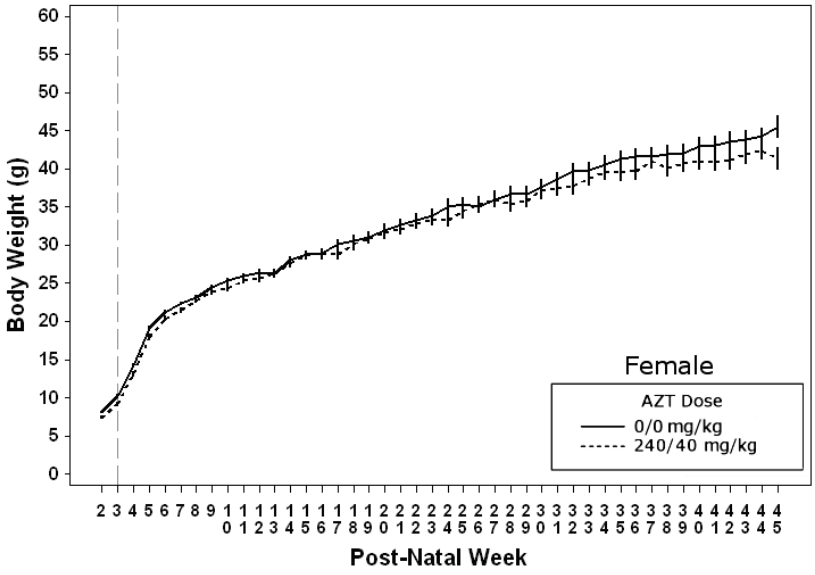


FIGURE 8
Growth Curves for Mice Administered AZT by Gavage in the 45-Week *In Utero*/Postnatal Stop-Study of AZT

Pathology and Statistical Analyses

Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Appendix A (Tables A13 through A18). There were no significant changes in the incidences of neoplasms in male or female mice in the 45-week stop-study.

Liver: The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were slightly increased in 240/40 mg/kg males [hepatocellular adenoma: 0/0 mg/kg, 3/24; 240/40 mg/kg, 5/25; hepatocellular adenoma or carcinoma (combined): 3/24, 7/25; Table A14]. These increases occurred despite a significant decrease in body weight, suggesting that if a larger sample size had been used, a significant increase in the incidences of hepatocellular neoplasms might have been observed.

Litter Correlation Analysis: When statistical analysis incorporating correlation of influence among littermates was used to compute incidences of neoplasms (Appendix G), statistical significance generally increased (i.e., P values decreased) suggesting that there was no positive correlation between tumor incidence and litter. However, these changes were insufficient to decrease P values for the incidences of neoplasms below the 5% definition of significance.

GENETIC TOXICOLOGY

In pups that were evaluated on PND 1, having been exposed to AZT transplacentally from GD 12 through GD 18, there was a significant decrease in the percentage of reticulocytes in the red blood cell population in the 160 and 240 mg/kg groups of male pups and in all three exposed groups of female pups (Table B1). In both sexes, this was associated with a significant negative dose trend. AZT exposure did not significantly alter the percentage of reticulocytes in the red blood cell population at the later evaluation time points (Tables B2, B3, and B4). Both the percentage of micronucleated normochromatic erythrocytes (NCEs) and the percentage of micronucleated reticulocytes (RETs) were significantly increased relative to the corresponding 0, 0/0, or 0/0/0 mg/kg group values in 1-day-old pups exposed to 160 or 240 mg/kg (except percent micronucleated NCEs in 160 mg/kg females); in 10-day-old pups administered 80/40, 160/80, or 240/120 mg/kg (except percent micronucleated RETs in 80/40 mg/kg females); in 28-day-old pups administered 80/40/80, 160/80/160, or 240/120/240 mg/kg (except percent micronucleated NCEs in 80/40/80 mg/kg females); and in 30-week-old mice administered 240/120/240 mg/kg.

DISCUSSION AND CONCLUSIONS

While the advent of Highly Active Antiretroviral Therapy (HAART) has dramatically decreased rates of morbidity and mortality in people infected with the human immunodeficiency virus (HIV), the long-term toxicological consequences of such therapy are unknown (PHS, 2008). 3′-Azido-3′-deoxythymidine (AZT) is a key component of HAART, and while it has been previously studied in cancer bioassays, it has not been studied in combination with other antiretroviral drugs. A major objective of the current studies was to develop and evaluate an animal model that could be used for investigating the potential carcinogenicity of AIDS drug combinations, and the studies utilized the heterozygous F1 $p53^{+/-}$ mouse model for the first time in an NTP study.

This genetically modified mouse used 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*$^{tm1Brd(-/-)}$ (N12) 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 handling of their pups during dosing. 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 genetically modified model studies are designed to provide rapid screens for potent carcinogens because the genetic modifications generally decrease the time to tumor manifestation. They are not designed to screen for rare tumors, which requires a larger sample size and extensive historical control data. Increased incidences of both malignant lymphoma and hepatocellular adenoma were observed following exposure to AZT in the current heterozygous F1 $p53^{+/-}$ mouse studies despite the lack of increased incidences of vaginal epithelial tumors which have been reported to occur in rodents exposed to AZT in 2-year evaluations (Ayers *et al.*, 1996a; NTP, 1999). The B6.129-*Trp53*$^{tm1Brd(+/-)}$ (N12) p53 haploinsufficient mouse model, which was used in previous NTP GMM studies, exhibited a very low incidence of liver neoplasms and was unresponsive to exposure to dichloroacetate (NTP, 2007e), 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).

In the 45-week study, the incidences of hepatocellular adenoma occurred with a positive trend in male heterozygous F1 $p53^{+/-}$ mice and the incidence in the 240/120/240 mg/kg group was significantly increased. In addition, the male mice in the 45-week stop-study, exposed to AZT by maternal gavage from gestational day (GD) 12 through 18 and by direct gavage from postnatal day (PND) 1 through 8, exhibited increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined), but not at a level that reached statistical significance within the study design. However, these increased incidences of hepatocellular neoplasms should also be considered to be related to AZT administration. While incidences of hepatocellular neoplasms were not increased in female heterozygous F1 $p53^{+/-}$ mice, there was a positive trend in the incidences of malignant lymphoma. Thus, this mouse model appears to show sensitivity comparable to that of the B6C3F1 mouse that has recently been reported to develop increased incidences of hepatocellular carcinoma when exposed to high concentrations of AZT during gestation (Walker *et al.*, 2007). This heterozygous F1 $p53^{+/-}$ mouse is therefore superior to the B6.129-*Trp53*$^{tm1Brd(+/-)}$ (N12) p53 haploinsufficient mouse for use in GMM studies designed to evaluate potentially hepatocarcinogenic chemicals.

The increased incidences of malignant lymphoma in male heterozygous F1 $p53^{+/-}$ mice continuously exposed to AZT for 30 weeks (Table 5) and in female heterozygous F1 $p53^{+/-}$ mice continuously exposed to AZT for 45 weeks (Table 8) could also be treatment related. Historical incidences for malignant lymphoma in 40- and 45-week studies are 3/101 and 3/102 for male and female heterozygous F1 $p53^{+/-}$ mice, respectively (Appendix I). Conversely, in a previous study, prenatal exposure to AZT reduced the incidence of malignant lymphoma in female CD-1 mice (Diwan *et al.,* 1999). This suggests that postnatal and adult exposure to AZT is required to increase the incidence of malignant

lymphoma in mice. Low incidences of malignant neoplasms of the bone (osteosarcoma) and nervous tissue (neuroblastoma, astrocytoma) were also observed in male or female heterozygous F1 $p53^{+/-}$ mice. Incidences were not significantly increased by AZT exposure. These neoplasms are rare in B6C3F1 mice and may arise as a consequence of p53 haploinsufficiency or be related to treatment. The NTP historical control incidences for osteosarcoma are 5/101 and 3/102 for male and female heterozygous F1 $p53^{+/-}$ mice, respectively (Appendix I).

AZT exposure has been linked to development of macrocytic anemia and lactic acidosis in both experimental animals and human AIDS patients (Ayers *et al.,* 1996b). While significant elevations of mean cell hemoglobin and mean cell volume were observed in the heterozygous F1 $p53^{+/-}$ mice continuously exposed to 240/120/240 mg/kg for 30 weeks (terminal evaluation) and/or 160 days, the elevated values remained within the normal ranges, suggesting that the highest doses of AZT used in these studies were below the threshold required to produce macrocytic anemia in this strain of mouse.

While early rodent cancer bioassays of AZT using low doses (20 and 40 mg AZT/kg per day) did not report significant carcinogenic activity (Ayers 1988; Ayers *et al.,* 1997), NTP studies have reported carcinogenic activity when higher doses have been used. For example, B6C3F1/N mice exposed to 60 or 120 mg AZT/kg body weight by gavage for 2 years exhibited increased incidences of vaginal squamous cell carcinoma (females) and Harderian gland adenomas (males) (NTP, 1999). While no increases in the incidences of hepatocellular neoplasms were reported in that study, data interpretation was complicated by high background liver neoplasm incidences associated with active *Helicobacter hepaticus* infection. In a followup 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 neoplasms [alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined)] at 2-years of age. A study by 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.

The mechanisms for the carcinogenic effects of AZT in rodents may 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, 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 the increased incidence of neoplasms 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.,* 1996a). 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. However, 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 AZT/kg was reported to be 44 minutes (Williams *et al.,* 2003).

AZT has a relatively high rate of incorporation into nuclear and mitochondrial DNA of liver and lung tissue after transplacental exposure (Olivero *et al.,* 1997), and this may explain why these tissues are targets for transplacental and neonatal carcinogenicity of AZT. AZT exposure has been reported to evoke a wide range of genotoxic effects in both *in vitro* and *in vivo* test systems (Olivero, 2007, 2008). In genotoxicity studies run in conjunction with this bioassay, reticulocyte micronucleus frequency was increased 5.6-, 12.1-, and 19.1-fold in 1-day-old heterozygous F1 $p53^{+/-}$ mice given 80, 160, or 240 mg AZT/kg per day, respectively, during gestation, and increased 9.1-, 24.7-, and 13.3-fold in 10-day-old heterozygous F1 $p53^{+/-}$ mice neonatally exposed to 40, 80, or 120 mg AZT/kg per day, respectively.

While there have been no reports of AZT causing cancer in humans, a recent epidemiology study reported that patients with HIV infection or acquired immunodeficiency syndrome (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. Cancer takes time to develop,

and further follow-up of patients is required to determine any association between AZT (and other retroviral 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 alone or in combination with other therapeutic agents. It has the advantage of 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 neoplasia.

CONCLUSIONS

Under the conditions of these gavage studies, there was *clear evidence of carcinogenic activity** of AZT in male heterozygous F1 $p53^{+/-}$ mice based on the occurrence of hepatocellular neoplasms (predominantly adenomas) after 45 weeks of administration. The occurrence of malignant lymphoma may have been related to AZT administration for 30 weeks. There was *equivocal evidence of carcinogenic activity* of AZT in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of malignant lymphoma after 45 weeks of administration.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Peer Review Panel comments and the public discussion on this Report appears on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN HETEROZYGOUS F1 p53$^{+/-}$ MICE IN THE *IN UTERO*/POSTNATAL GAVAGE STUDIES OF AZT

TABLE A1
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
Disposition Summary		
Animals initially in study	27	26
Early deaths		
Moribund		3
Survivors		
Died last week of study		2
Terminal kill	27	21
Animals examined microscopically	27	26
Alimentary System		
Gallbladder	(26)	(26)
Intestine large, cecum	(27)	(26)
Intestine small, jejunum	(27)	(26)
Liver	(27)	(26)
Hepatocellular adenoma	1 (4%)	1 (4%)
Pancreas	(27)	(26)
Salivary glands	(27)	(26)
Stomach, glandular	(27)	(26)
Cardiovascular System		
Blood vessel	(26)	(25)
Heart	(27)	(26)
Endocrine System		
Adrenal cortex	(27)	(26)
Thyroid gland	(26)	(26)
General Body System		
None		
Genital System		
Epididymis	(27)	(26)
Preputial gland	(27)	(26)
Prostate	(27)	(26)
Seminal vesicle	(27)	(26)
Testes	(27)	(26)
Hematopoietic System		
Bone marrow	(27)	(26)
Lymph node	(0)	(1)
Lymph node, mandibular	(26)	(26)
Lymph node, mesenteric	(27)	(25)
Spleen	(27)	(26)
Thymus	(26)	(26)

TABLE A1
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Integumentary System		
None		
Musculoskeletal System		
Bone, femur	(27)	(26)
Osteosarcoma		1 (4%)
Nervous System		
Brain, brain stem	(27)	(26)
Astrocytoma malignant, metastatic, brain, cerebrum		1 (4%)
Brain, cerebellum	(27)	(26)
Astrocytoma malignant, metastatic, brain, cerebrum		1 (4%)
Brain, cerebrum	(27)	(26)
Astrocytoma malignant		1 (4%)
Olfactory lobe, neuroblastoma		1 (4%)
Respiratory System		
Lung	(27)	(26)
Nose	(27)	(26)
Neuroblastoma, metastatic, brain, cerebrum		1 (4%)
Special Senses System		
Eye	(27)	(26)
Harderian gland	(27)	(26)
Urinary System		
Kidney	(27)	(26)
Urinary bladder	(27)	(26)
Systemic Lesions		
Multiple organs[b]	(27)	(26)
Lymphoma malignant		3 (12%)
Neoplasm Summary		
Total animals with primary neoplasms[c]	1	7
Total primary neoplasms	1	7
Total animals with benign neoplasms	1	1
Total benign neoplasms	1	1
Total animals with malignant neoplasms		6
Total malignant neoplasms		6
Total animals with metastatic neoplasms		2
Total metastatic neoplasms		3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[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 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Liver: Hepatocellular Adenoma		
Overall rate[a]	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate[b]	3.7%	4.4%
Terminal rate[c]	1/27 (3.7%)	1/21 (4.8%)
First incidence (days)	217 (T)	213 (T)
Poly-3 test[d]		P=0.722
All Organs: Malignant Lymphoma		
Overall rate	0/27 (0.0%)	3/26 (11.5%)
Adjusted rate	0.0%	11.9%
Terminal rate	0/27 (0.0%)	0/21 (0.0%)
First incidence (days)	—[e]	84
Poly-3 test		P=0.102
All Organs: Osteosarcoma		
Overall rate	0/27 (0.0%)	1/26 (3.8%)
Adjusted rate	0.0%	4.4%
Terminal rate	0/27 (0.0%)	1/21 (4.8%)
First incidence (days)	—	213 (T)
Poly-3 test		P=0.467
All Organs: Benign Neoplasms		
Overall rate	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate	3.7%	4.4%
Terminal rate	1/27 (3.7%)	1/21 (4.8%)
First incidence (days)	217 (T)	213 (T)
Poly-3 test		P=0.722
All Organs: Malignant Neoplasms		
Overall rate	0/27 (0.0%)	6/26 (23.1%)
Adjusted rate	0.0%	23.8%
Terminal rate	0/27 (0.0%)	2/21 (9.5%)
First incidence (days)	—	84
Poly-3 test		P=0.008
All Organs: Benign or Malignant Neoplasms		
Overall rate	1/27 (3.7%)	7/26 (26.9%)
Adjusted rate	3.7%	27.8%
Terminal rate	1/27 (3.7%)	3/21 (14.3%)
First incidence (days)	217 (T)	84
Poly-3 test		P=0.017

(T) Terminal kill

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

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

[c] Observed incidence at terminal kill

[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

[e] Not applicable; no neoplasms in animal group

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
Disposition Summary		
Animals initially in study	27	26
Early deaths		
Moribund		3
Survivors		
Died last week of study		2
Terminal kill	27	21
Animals examined microscopically	27	26
Alimentary System		
Gallbladder	(26)	(26)
Intestine large, cecum	(27)	(26)
Hyperplasia, lymphoid	3 (11%)	1 (4%)
Intestine small, jejunum	(27)	(26)
Liver	(27)	(26)
Fatty change		1 (4%)
Vacuolization cytoplasmic	8 (30%)	4 (15%)
Pancreas	(27)	(26)
Salivary glands	(27)	(26)
Infiltration cellular, lymphocyte	5 (19%)	
Stomach, glandular	(27)	(26)
Cardiovascular System		
Blood vessel	(26)	(25)
Heart	(27)	(26)
Cardiomyopathy	2 (7%)	
Endocrine System		
Adrenal cortex	(27)	(26)
Subcapsular, hyperplasia	2 (7%)	2 (8%)
Thyroid gland	(26)	(26)
Cyst		1 (4%)
Infiltration cellular, lymphocyte		1 (4%)
General Body System		
None		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Genital System		
Epididymis	(27)	(26)
Preputial gland	(27)	(26)
Atrophy		1 (4%)
Prostate	(27)	(26)
Inflammation, suppurative		1 (4%)
Seminal vesicle	(27)	(26)
Lumen, dilatation		1 (4%)
Testes	(27)	(26)
Seminiferous tubule, degeneration		1 (4%)
Hematopoietic System		
Bone marrow	(27)	(26)
Myeloid cell, hyperplasia		1 (4%)
Lymph node	(0)	(1)
Lymph node, mandibular	(26)	(26)
Hyperplasia, lymphoid	1 (4%)	1 (4%)
Lymph node, mesenteric	(27)	(25)
Hyperplasia, lymphoid	19 (70%)	16 (64%)
Hyperplasia, plasma cell	1 (4%)	
Spleen	(27)	(26)
Hematopoietic cell proliferation	1 (4%)	2 (8%)
Hyperplasia, lymphoid	2 (7%)	
Thymus	(26)	(26)
Atrophy		1 (4%)
Hyperplasia, lymphoid		2 (8%)
Integumentary System		
None		
Musculoskeletal System		
Bone, femur	(27)	(26)
Nervous System		
Brain, brain stem	(27)	(26)
Brain, cerebellum	(27)	(26)
Brain, cerebrum	(27)	(26)
Respiratory System		
Lung	(27)	(26)
Abscess		1 (4%)
Nose	(27)	(26)
Inflammation, suppurative	1 (4%)	1 (4%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Special Senses System		
Eye	(27)	(26)
Harderian gland	(27)	(26)
Epithelium, hyperplasia		1 (4%)
Urinary System		
Kidney	(27)	(26)
Bacterium		1 (4%)
Infiltration cellular, lymphocyte	7 (26%)	2 (8%)
Inflammation, chronic active		1 (4%)
Nephropathy		1 (4%)
Pelvis, dilatation		2 (8%)
Transitional epithelium, hyperplasia	1 (4%)	1 (4%)
Urinary bladder	(27)	(26)
Lumen, dilatation	1 (4%)	2 (8%)

TABLE A4
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
Disposition Summary		
Animals initially in study	27	26
Early deaths		
Moribund	1	
Natural death	1	
Survivors		
Died last week of study	1	
Terminal kill	24	26
Animals examined microscopically	27	26
Alimentary System		
Esophagus	(23)	(25)
Gallbladder	(24)	(25)
Sarcoma		1 (4%)
Intestine large, cecum	(26)	(26)
Polyp	1 (4%)	
Intestine large, rectum	(26)	(26)
Intestine small, ileum	(26)	(26)
Liver	(26)	(26)
Sarcoma		1 (4%)
Pancreas	(26)	(26)
Salivary glands	(26)	(26)
Stomach, glandular	(26)	(26)
Cardiovascular System		
Heart	(26)	(26)
Endocrine System		
Adrenal cortex	(26)	(26)
Adrenal medulla	(26)	(26)
Parathyroid gland	(22)	(21)
Pituitary gland	(26)	(26)
Thyroid gland	(26)	(25)
General Body System		
Tissue NOS	(0)	(1)
Genital System		
Ovary	(26)	(26)
Uterus	(26)	(26)
Endometrium, polyp stromal	1 (4%)	
Vagina	(27)	(26)

TABLE A4
Summary of the Incidence of Neoplasms in Female Heterozygous F1 $p53^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Hematopoietic System		
Bone marrow	(26)	(26)
Lymph node	(3)	(1)
Lymph node, mandibular	(26)	(25)
Lymph node, mesenteric	(24)	(25)
Sarcoma, metastatic, liver		1 (4%)
Spleen	(26)	(26)
Sarcoma, metastatic, liver		1 (4%)
Thymus	(26)	(26)
Integumentary System		
Mammary gland	(24)	(24)
Adenocarcinoma	1 (4%)	
Skin	(26)	(26)
Basal cell carcinoma		1 (4%)
Musculoskeletal System		
None		
Nervous System		
Brain, cerebrum	(26)	(26)
Respiratory System		
Lung	(26)	(26)
Adenocarcinoma, metastatic, mammary gland	1 (4%)	
Basal cell carcinoma, metastatic, skin		1 (4%)
Special Senses System		
Harderian gland	(26)	(26)
Urinary System		
Kidney	(26)	(26)
Urinary bladder	(26)	(26)
Systemic Lesions		
Multiple organs[b]	(27)	(26)
Lymphoma malignant	1 (4%)	1 (4%)

TABLE A4
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Neoplasm Summary		
Total animals with primary neoplasms[c]	4	3
Total primary neoplasms	4	4
Total animals with benign neoplasms	2	
Total benign neoplasms	2	
Total animals with malignant neoplasms	2	3
Total malignant neoplasms	2	4
Total animals with metastatic neoplasms	1	2
Total metastatic neoplasms	1	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[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 Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
All Organs: Malignant Lymphoma		
Overall rate[a]	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate[b]	4.0%	3.8%
Terminal rate[c]	0/24 (0.0%)	1/26 (3.8%)
First incidence (days)	192	213 (T)
Poly-3 test[d]		P=0.753N
All Organs: Benign Neoplasms		
Overall rate	2/27 (7.4%)	0/26 (0.0%)
Adjusted rate	8.0%	0.0%
Terminal rate	2/24 (8.3%)	0/26 (0.0%)
First incidence (days)	211 (T)	—[e]
Poly-3 test		P=0.225N
All Organs: Malignant Neoplasms		
Overall rate	2/27 (7.4%)	3/26 (11.5%)
Adjusted rate	7.9%	11.5%
Terminal rate	1/24 (4.2%)	3/26 (11.5%)
First incidence (days)	192	213 (T)
Poly-3 test		P=0.515
All Organs: Benign or Malignant Neoplasms		
Overall rate	4/27 (14.8%)	3/26 (11.5%)
Adjusted rate	15.9%	11.5%
Terminal rate	3/24 (12.5%)	3/26 (11.5%)
First incidence (days)	192	213 (T)
Poly-3 test		P=0.482N

(T) Terminal kill

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

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

[c] Observed incidence at terminal kill

[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg 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**.

[e] Not applicable; no neoplasms in animal group

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
Disposition Summary		
Animals initially in study	27	26
Early deaths		
Moribund kill	1	
Natural death	1	
Survivors		
Died last week of study	1	
Terminal kill	24	26
Animals examined microscopically	27	26
Alimentary System		
Esophagus	(23)	(25)
Adventitia, foreign body	1 (4%)	
Adventitia, necrosis	1 (4%)	
Gallbladder	(24)	(25)
Intestine large, cecum	(26)	(26)
Hyperplasia, lymphoid		2 (8%)
Intestine large, rectum	(26)	(26)
Intestine small, ileum	(26)	(26)
Liver	(26)	(26)
Infiltration cellular, lymphocyte		1 (4%)
Inflammation, chronic active	3 (12%)	2 (8%)
Tension lipidosis	1 (4%)	3 (12%)
Vacuolization cytoplasmic	1 (4%)	1 (4%)
Pancreas	(26)	(26)
Infiltration cellular, lymphocyte		1 (4%)
Inflammation, chronic active		1 (4%)
Salivary glands	(26)	(26)
Infiltration cellular, lymphocyte	14 (54%)	7 (27%)
Stomach, glandular	(26)	(26)
Cardiovascular System		
Heart	(26)	(26)
Cardiomyopathy	1 (4%)	
Pericardium, inflammation, chronic	1 (4%)	
Pericardium, inflammation, chronic active		1 (4%)
Pericardium, mineralization		1 (4%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Endocrine System		
Adrenal cortex	(26)	(26)
Accessory adrenal cortical nodule	1 (4%)	1 (4%)
Vacuolization cytoplasmic	2 (8%)	
Subcapsular, hyperplasia	23 (88%)	24 (92%)
Adrenal medulla	(26)	(26)
Parathyroid gland	(22)	(21)
Infiltration cellular, lymphocyte	1 (5%)	
Pituitary gland	(26)	(26)
Thyroid gland	(26)	(25)
Cyst	1 (4%)	
Infiltration cellular, lymphocyte	1 (4%)	1 (4%)
General Body System		
Tissue NOS	(0)	(1)
Abscess		1 (100%)
Genital System		
Ovary	(26)	(26)
Cyst		1 (4%)
Uterus	(26)	(26)
Endometrium, hyperplasia, cystic	1 (4%)	1 (4%)
Lumen, dilatation	2 (8%)	2 (8%)
Vagina	(27)	(26)
Hematopoietic System		
Bone marrow	(26)	(26)
Myeloid cell, hyperplasia	3 (12%)	2 (8%)
Lymph node	(3)	(1)
Mediastinal, hyperplasia, lymphoid	2 (67%)	
Mediastinal, infiltration cellular, plasma cell	1 (33%)	
Lymph node, mandibular	(26)	(25)
Hyperplasia, lymphoid	3 (12%)	2 (8%)
Lymph node, mesenteric	(24)	(25)
Hyperplasia, lymphoid	7 (29%)	11 (44%)
Spleen	(26)	(26)
Depletion lymphoid	1 (4%)	
Hematopoietic cell proliferation	3 (12%)	2 (8%)
Hyperplasia, lymphoid		2 (8%)
Thymus	(26)	(26)
Hyperplasia, lymphoid	3 (12%)	2 (8%)
Hyperplasia, plasma cell	1 (4%)	
Necrosis	1 (4%)	
Adventitia, inflammation, chronic	1 (4%)	
Epithelial cell, hyperplasia	1 (4%)	

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Integumentary System		
Mammary gland	(24)	(24)
Skin	(26)	(26)
Musculoskeletal System		
None		
Nervous System		
Brain, cerebrum	(26)	(26)
Cyst epithelial inclusion	1 (4%)	
Respiratory System		
Lung	(26)	(26)
Abscess	2 (8%)	
Infiltration cellular, lymphocyte	1 (4%)	
Inflammation, chronic active		1 (4%)
Mediastinum, inflammation, chronic active		1 (4%)
Mediastinum, mineralization		1 (4%)
Mediastinum, necrosis	1 (4%)	
Special Senses System		
Harderian gland	(26)	(26)
Infiltration cellular, lymphocyte	1 (4%)	
Urinary System		
Kidney	(26)	(26)
Infiltration cellular, lymphocyte	8 (31%)	3 (12%)
Inflammation, chronic active	1 (4%)	
Urinary bladder	(26)	(26)
Infiltration cellular, lymphocyte	3 (12%)	1 (4%)

TABLE A7
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Disposition Summary				
Animals initially in study	27	27	27	27
Early deaths				
Moribund	1	1		1
Natural death	1	2		1
Survivors				
Died last week of study	1	3		3
Terminal kill	24	21	27	22
Animals examined microscopically	27	27	27	27
Alimentary System				
Esophagus	(27)	(26)	(27)	(27)
Gallbladder	(26)	(25)	(27)	(25)
Intestine large, cecum	(26)	(26)	(27)	(25)
Intestine large, colon	(26)	(26)	(27)	(25)
Intestine large, rectum	(26)	(26)	(27)	(26)
Intestine small, duodenum	(26)	(25)	(27)	(25)
Liver	(26)	(27)	(27)	(27)
Hepatocellular adenoma	3 (12%)	2 (7%)	6 (22%)	9 (33%)
Hepatocellular carcinoma		1 (4%)		
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Mesentery	(0)	(2)	(1)	(0)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (50%)		
Pancreas	(27)	(27)	(27)	(27)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Salivary glands	(27)	(26)	(27)	(27)
Stomach, forestomach	(26)	(26)	(27)	(27)
Squamous cell carcinoma		1 (4%)		
Stomach, glandular	(26)	(26)	(27)	(25)
Adenocarcinoma		1 (4%)		
Cardiovascular System				
Blood vessel	(27)	(27)	(27)	(27)
Heart	(27)	(27)	(27)	(27)
Endocrine System				
Adrenal cortex	(27)	(27)	(27)	(27)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Adrenal medulla	(25)	(26)	(24)	(26)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Islets, pancreatic	(27)	(27)	(27)	(27)
Parathyroid gland	(16)	(20)	(21)	(22)
Pituitary gland	(25)	(27)	(27)	(27)
Thyroid gland	(26)	(27)	(27)	(26)

TABLE A7
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
General Body System				
Tissue NOS	(2)	(1)	(0)	(1)
Sarcoma	1 (50%)			
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (100%)		
Genital System				
Coagulating gland	(0)	(1)	(0)	(0)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (100%)		
Epididymis	(27)	(27)	(27)	(26)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Preputial gland	(27)	(26)	(27)	(26)
Prostate	(27)	(27)	(27)	(25)
Seminal vesicle	(26)	(27)	(27)	(26)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Testes	(27)	(26)	(27)	(26)
Hematopoietic System				
Bone marrow	(27)	(26)	(27)	(26)
Lymph node	(0)	(1)	(0)	(1)
Lymph node, mandibular	(27)	(25)	(27)	(26)
Lymph node, mesenteric	(26)	(23)	(26)	(25)
Spleen	(26)	(25)	(27)	(25)
Thymus	(27)	(24)	(27)	(24)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Integumentary System				
Skin	(27)	(27)	(27)	(27)
Subcutaneous tissue, fibrosarcoma				1 (4%)
Musculoskeletal System				
Bone	(1)	(0)	(0)	(0)
Humerus, osteosarcoma	1 (100%)			
Bone, femur	(27)	(27)	(27)	(27)
Osteosarcoma	1 (4%)			
Skeletal muscle	(27)	(27)	(27)	(27)
Diaphragm, squamous cell carcinoma, metastatic, stomach, forestomach		1 (4%)		
Nervous System				
Brain, cerebellum	(27)	(27)	(27)	(27)
Brain, cerebrum	(27)	(27)	(27)	(27)
Olfactory lobe, neuroblastoma		1 (4%)		1 (4%)

TABLE A7
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Respiratory System				
Lung	(27)	(27)	(27)	(27)
Alveolar/bronchiolar carcinoma		1 (4%)		
Osteosarcoma, metastatic, bone, femur	1 (4%)			
Nose	(27)	(27)	(27)	(25)
Neuroblastoma, metastatic, brain, cerebrum		1 (4%)		1 (4%)
Special Senses System				
Harderian gland	(27)	(27)	(27)	(27)
Adenoma	1 (4%)			
Urinary System				
Kidney	(26)	(27)	(27)	(26)
Urinary bladder	(27)	(27)	(27)	(26)
Systemic Lesions				
Multiple organs[b]	(27)	(27)	(27)	(27)
Lymphoma malignant	1 (4%)	2 (7%)	1 (4%)	1 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	8	7	7	12
Total primary neoplasms	8	9	7	12
Total animals with benign neoplasms	4	2	6	9
Total benign neoplasms	4	2	6	9
Total animals with malignant neoplasms	4	6	1	3
Total malignant neoplasms	4	7	1	3
Total animals with metastatic neoplasms	1	2		1
Total metastatic neoplasms	1	12		1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[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 Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Liver: Hepatocellular Adenoma				
Overall rate[a]	3/26 (11.5%)	2/27 (7.4%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate[b]	12.3%	8.8%	22.2%	36.5%
Terminal rate[c]	3/24 (12.5%)	2/21 (9.5%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test[d]	P=0.013	P=0.531N	P=0.288	P=0.048
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/26 (11.5%)	3/27 (11.1%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate	12.3%	13.2%	22.2%	36.5%
Terminal rate	3/24 (12.5%)	3/21 (14.3%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test	P=0.019	P=0.634	P=0.288	P=0.048
All Organs: Malignant Lymphoma				
Overall rate	1/27 (3.7%)	2/27 (7.4%)	1/27 (3.7%)	1/27 (3.7%)
Adjusted rate	3.9%	8.5%	3.7%	4.1%
Terminal rate	0/24 (0.0%)	1/21 (4.8%)	1/27 (3.7%)	0/22 (0.0%)
First incidence (days)	159	217	321 (T)	94
Poly-3 test	P=0.502N	P=0.473	P=0.746N	P=0.754
All Organs: Osteosarcoma				
Overall rate	2/27 (7.4%)	0/27 (0.0%)	0/27 (0.0%)	0/27 (0.0%)
Adjusted rate	8.2%	0.0%	0.0%	0.0%
Terminal rate	2/24 (8.3%)	0/21 (0.0%)	0/27 (0.0%)	0/22 (0.0%)
First incidence (days)	319 (T)	—[e]	—	—
Poly-3 test	P=0.050N	P=0.251N	P=0.214N	P=0.242N
All Organs: Benign Neoplasms				
Overall rate	4/27 (14.8%)	2/27 (7.4%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate	16.3%	8.8%	22.2%	36.5%
Terminal rate	4/24 (16.7%)	2/21 (9.5%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test	P=0.031	P=0.367N	P=0.430	P=0.099
All Organs: Malignant Neoplasms				
Overall rate	4/27 (14.8%)	6/27 (22.2%)	1/27 (3.7%)	3/27 (11.1%)
Adjusted rate	15.8%	25.0%	3.7%	12.2%
Terminal rate	3/24 (12.5%)	3/21 (14.3%)	1/27 (3.7%)	2/22 (9.1%)
First incidence (days)	159	217	321 (T)	94
Poly-3 test	P=0.176N	P=0.328	P=0.155N	P=0.516N

TABLE A8
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	8/27 (29.6%)	7/27 (25.9%)	7/27 (25.9%)	12/27 (44.4%)
Adjusted rate	31.5%	29.1%	25.9%	46.8%
Terminal rate	7/24 (29.2%)	4/21 (19.0%)	7/27 (25.9%)	9/22 (40.9%)
First incidence (days)	159	217	320 (T)	94
Poly-3 test	P=0.185	P=0.550N	P=0.444N	P=0.205

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg 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**.
[e] Not applicable; no neoplasms in animal group

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Disposition Summary				
Animals initially in study	27	27	27	27
Early deaths				
Moribund	1	1		1
Natural death	1	2		1
Survivors				
Died last week of study	1	3		3
Terminal kill	24	21	27	22
Animals examined microscopically	27	27	27	27
Alimentary System				
Esophagus	(27)	(26)	(27)	(27)
Foreign body		1 (4%)		
Adventitia, inflammation, suppurative		1 (4%)		
Adventitia, necrosis		1 (4%)		
Gallbladder	(26)	(25)	(27)	(25)
Mineralization				1 (4%)
Intestine large, cecum	(26)	(26)	(27)	(25)
Hyperplasia, lymphoid		2 (8%)	2 (7%)	3 (12%)
Intestine large, colon	(26)	(26)	(27)	(25)
Epithelium, hyperplasia				1 (4%)
Intestine large, rectum	(26)	(26)	(27)	(26)
Hyperplasia, lymphoid				1 (4%)
Intestine small, duodenum	(26)	(25)	(27)	(25)
Inflammation, chronic active			1 (4%)	
Liver	(26)	(27)	(27)	(27)
Basophilic focus	1 (4%)	1 (4%)		1 (4%)
Cytomegaly	14 (54%)	14 (52%)	13 (48%)	9 (33%)
Hematopoietic cell proliferation	1 (4%)			
Mixed cell focus				1 (4%)
Tension lipidosis	3 (12%)			1 (4%)
Vacuolization cytoplasmic	20 (77%)	17 (63%)	23 (85%)	13 (48%)
Mesentery	(0)	(2)	(1)	(0)
Fat, fibrosis			1 (100%)	
Pancreas	(27)	(27)	(27)	(27)
Cytoplasmic alteration			1 (4%)	
Inflammation, chronic active			1 (4%)	1 (4%)
Pigmentation			1 (4%)	
Acinar cell, degeneration	1 (4%)			
Salivary glands	(27)	(26)	(27)	(27)
Infiltration cellular, lymphocyte	7 (26%)	4 (15%)	2 (7%)	5 (19%)
Stomach, forestomach	(26)	(26)	(27)	(27)
Epithelium, hyperplasia	1 (4%)			
Epithelium, hyperplasia, squamous		1 (4%)		
Stomach, glandular	(26)	(26)	(27)	(25)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Cardiovascular System				
Blood vessel	(27)	(27)	(27)	(27)
Heart	(27)	(27)	(27)	(27)
Inflammation, suppurative		1 (4%)		
Polyarteritis			1 (4%)	
Pericardium, inflammation, chronic active	1 (4%)			
Endocrine System				
Adrenal cortex	(27)	(27)	(27)	(27)
Accessory adrenal cortical nodule	1 (4%)			
Cyst	1 (4%)			
Hypertrophy	1 (4%)		1 (4%)	
Subcapsular, hyperplasia	6 (22%)	6 (22%)	7 (26%)	6 (22%)
Adrenal medulla	(25)	(26)	(24)	(26)
Islets, pancreatic	(27)	(27)	(27)	(27)
Hyperplasia			1 (4%)	
Parathyroid gland	(16)	(20)	(21)	(22)
Cyst				1 (5%)
Pituitary gland	(25)	(27)	(27)	(27)
Thyroid gland	(26)	(27)	(27)	(26)
Cyst	1 (4%)	1 (4%)		
Degeneration		1 (4%)		
Ectopic thymus		1 (4%)		
Follicular cell, hyperplasia			1 (4%)	
General Body System				
Tissue NOS	(2)	(1)	(0)	(1)
Mediastinum, abscess	1 (50%)			
Thoracic, thrombosis				1 (100%)
Genital System				
Coagulating gland	(0)	(1)	(0)	(0)
Epididymis	(27)	(27)	(27)	(26)
Hypospermia		1 (4%)		1 (4%)
Preputial gland	(27)	(26)	(27)	(26)
Degeneration				3 (12%)
Infiltration cellular, lymphocyte	1 (4%)			
Inflammation, suppurative		1 (4%)		
Inflammation, chronic active				1 (4%)
Duct, dilatation		1 (4%)		
Prostate	(27)	(27)	(27)	(25)
Seminal vesicle	(26)	(27)	(27)	(26)
Inflammation, chronic				1 (4%)
Testes	(27)	(26)	(27)	(26)
Interstitial cell, hyperplasia		1 (4%)		
Seminiferous tubule, degeneration		1 (4%)	1 (4%)	1 (4%)

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Hematopoietic System				
Bone marrow	(27)	(26)	(27)	(26)
Myeloid cell, hyperplasia	2 (7%)	2 (8%)	1 (4%)	
Lymph node	(0)	(1)	(0)	(1)
Lymph node, mandibular	(27)	(25)	(27)	(26)
Hyperplasia, lymphoid	1 (4%)	1 (4%)	1 (4%)	2 (8%)
Hyperplasia, plasma cell			1 (4%)	
Lymph node, mesenteric	(26)	(23)	(26)	(25)
Hematopoietic cell proliferation				1 (4%)
Hyperplasia, lymphoid	13 (50%)	12 (52%)	17 (65%)	14 (56%)
Hyperplasia, plasma cell			1 (4%)	
Infiltration cellular, histiocyte			1 (4%)	
Infiltration cellular, polymorphonuclear				1 (4%)
Spleen	(26)	(25)	(27)	(25)
Hematopoietic cell proliferation	4 (15%)	2 (8%)	5 (19%)	1 (4%)
Hemorrhage				1 (4%)
Hyperplasia, lymphoid	2 (8%)	5 (20%)	6 (22%)	2 (8%)
Thymus	(27)	(24)	(27)	(24)
Atrophy	1 (4%)		1 (4%)	
Hyperplasia, lymphoid	1 (4%)	1 (4%)		
Integumentary System				
Skin	(27)	(27)	(27)	(27)
Abscess				1 (4%)
Cyst epithelial inclusion			1 (4%)	
Hemorrhage				1 (4%)
Musculoskeletal System				
Bone	(1)	(0)	(0)	(0)
Bone, femur	(27)	(27)	(27)	(27)
Skeletal muscle	(27)	(27)	(27)	(27)
Diaphragm, foreign body		1 (4%)		
Diaphragm, inflammation, granulomatous		1 (4%)		
Nervous System				
Brain, cerebellum	(27)	(27)	(27)	(27)
Hemorrhage				1 (4%)
Brain, cerebrum	(27)	(27)	(27)	(27)
Mineralization	4 (15%)	1 (4%)	2 (7%)	

TABLE A9
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Respiratory System				
Lung	(27)	(27)	(27)	(27)
Congestion	1 (4%)			1 (4%)
Infiltration cellular, lymphocyte		1 (4%)		
Infiltration cellular, polymorphonuclear				1 (4%)
Inflammation, chronic active	1 (4%)			
Mediastinum, inflammation, suppurative		1 (4%)		
Mediastinum, necrosis		1 (4%)		
Nose	(27)	(27)	(27)	(25)
Exudate	1 (4%)			
Inflammation, suppurative	1 (4%)			
Special Senses System				
Harderian gland	(27)	(27)	(27)	(27)
Urinary System				
Kidney	(26)	(27)	(27)	(26)
Casts protein		1 (4%)		1 (4%)
Cyst	1 (4%)	1 (4%)		
Infiltration cellular, lymphocyte	6 (23%)	11 (41%)	6 (22%)	4 (15%)
Pelvis, dilatation				1 (4%)
Pelvis, inflammation, chronic				1 (4%)
Urinary bladder	(27)	(27)	(27)	(26)
Lumen, dilatation		1 (4%)	2 (7%)	2 (8%)

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[a]

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Disposition Summary				
Animals initially in study	26	27	27	27
Early deaths				
Moribund	1			1
Natural death		1	1	
Survivors				
Died last week of study	2	3	2	5
Terminal kill	23	23	24	21
Animals examined microscopically	26	27	27	27
Alimentary System				
Esophagus	(26)	(27)	(27)	(27)
Intestine large, cecum	(25)	(27)	(25)	(24)
Intestine large, colon	(26)	(26)	(27)	(25)
Intestine large, rectum	(25)	(27)	(27)	(25)
Intestine small, ileum	(25)	(27)	(25)	(25)
Intestine small, jejunum	(25)	(27)	(25)	(25)
Liver	(26)	(27)	(27)	(27)
Sarcoma	1 (4%)			
Mesentery	(1)	(0)	(1)	(1)
Pancreas	(26)	(27)	(27)	(26)
Salivary glands	(26)	(27)	(26)	(27)
Stomach, forestomach	(26)	(27)	(27)	(27)
Squamous cell papilloma		1 (4%)		
Cardiovascular System				
Heart	(26)	(27)	(27)	(26)
Endocrine System				
Adrenal cortex	(26)	(27)	(27)	(26)
Adenoma			1 (4%)	
Parathyroid gland	(21)	(22)	(22)	(22)
Pituitary gland	(26)	(27)	(27)	(27)
Thyroid gland	(25)	(27)	(27)	(26)
General Body System				
Tissue NOS	(0)	(1)	(0)	(1)
Thoracic, osteosarcoma		1 (100%)		
Genital System				
Clitoral gland	(25)	(26)	(26)	(27)
Ovary	(26)	(27)	(27)	(26)
Luteoma	1 (4%)			
Uterus	(26)	(27)	(27)	(27)
Endometrium, polyp stromal		1 (4%)		
Vagina	(25)	(27)	(27)	(24)

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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Hematopoietic System				
Bone marrow	(26)	(26)	(26)	(26)
Sarcoma	1 (4%)			
Lymph node	(1)	(1)	(0)	(3)
Lymph node, mandibular	(26)	(27)	(25)	(26)
Sarcoma	1 (4%)			
Lymph node, mesenteric	(26)	(26)	(26)	(27)
Sarcoma	1 (4%)			
Spleen	(26)	(26)	(27)	(27)
Sarcoma	1 (4%)			
Thymus	(24)	(26)	(23)	(27)
Integumentary System				
Mammary gland	(25)	(26)	(27)	(27)
Adenocarcinoma			2 (7%)	1 (4%)
Skin	(26)	(27)	(27)	(27)
Fibrosarcoma				1 (4%)
Squamous cell carcinoma			1 (4%)	
Musculoskeletal System				
Bone	(0)	(1)	(0)	(1)
Cranium, osteoma				1 (100%)
Rib, osteosarcoma		1 (100%)		
Bone, femur	(26)	(27)	(27)	(27)
Osteosarcoma	1 (4%)		2 (7%)	1 (4%)
Skeletal muscle	(26)	(27)	(27)	(27)
Nervous System				
Brain, brain stem	(26)	(27)	(27)	(27)
Brain, cerebrum	(26)	(27)	(27)	(27)
Sarcoma	1 (4%)			
Peripheral nerve	(2)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)
Respiratory System				
Lung	(26)	(27)	(26)	(27)
Alveolar/bronchiolar adenoma		1 (4%)		
Osteosarcoma, metastatic, bone, femur				1 (4%)
Mediastinum, sarcoma	1 (4%)			
Nose	(26)	(27)	(27)	(27)
Osteosarcoma			1 (4%)	
Sarcoma	1 (4%)			
Special Senses System				
Eye	(25)	(25)	(26)	(24)
Harderian gland	(26)	(27)	(27)	(25)
Adenoma			1 (4%)	

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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Urinary System				
Kidney	(26)	(27)	(27)	(27)
Renal tubule, adenoma		1 (4%)		
Ureter	(0)	(0)	(1)	(0)
Urinary bladder	(26)	(27)	(27)	(26)
Systemic Lesions				
Multiple organs[b]	(26)	(27)	(27)	(27)
Histiocytic sarcoma		1 (4%)		1 (4%)
Lymphoma malignant			1 (4%)	3 (11%)
Mesothelioma malignant	1 (4%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	4	7	8	8
Total primary neoplasms	11	7	9	8
Total animals with benign neoplasms	1	4	2	1
Total benign neoplasms	1	4	2	1
Total animals with malignant neoplasms	3	3	6	7
Total malignant neoplasms	10	3	7	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
[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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
All Organs: Malignant Lymphoma				
Overall rate[a]	0/26 (0.0%)	0/27 (0.0%)	1/27 (3.7%)	3/27 (11.1%)
Adjusted rate[b]	0.0%	0.0%	4.0%	12.2%
Terminal rate[c]	0/23 (0.0%)	0/23 (0.0%)	1/24 (4.2%)	2/21 (9.5%)
First incidence (days)	—[e]	—	322 (T)	241
Poly-3 test[d]	P=0.020	—[f]	P=0.505	P=0.115
All Organs: Histiocytic Sarcoma				
Overall rate	0/26 (0.0%)	1/27 (3.7%)	0/27 (0.0%)	1/27 (3.7%)
Adjusted rate	0.0%	3.9%	0.0%	4.1%
Terminal rate	0/23 (0.0%)	0/23 (0.0%)	0/24 (0.0%)	0/21 (0.0%)
First incidence (days)	—	212	—	278
Poly-3 test	P=0.378	P=0.509	—	P=0.500
All Organs: Osteosarcoma				
Overall rate	1/26 (3.8%)	2/27 (7.4%)	3/27 (11.1%)	1/27 (3.7%)
Adjusted rate	4.0%	7.9%	12.0%	4.1%
Terminal rate	0/23 (0.0%)	1/23 (4.3%)	3/24 (12.5%)	0/21 (0.0%)
First incidence (days)	269	282	320 (T)	287
Poly-3 test	P=0.486	0.504	0.305	0.756
All Organs: Mesothelioma				
Overall rate	1/26 (3.8%)	0/27 (0.0%)	0/27 (0.0%)	0/27 (0.0%)
Adjusted rate	4.0%	0.0%	0.0%	0.0%
Terminal rate	0/23 (0.0%)	0/23 (0.0%)	0/24 (0.0%)	0/21 (0.0%)
First incidence (days)	291	—	—	—
Poly-3 test	P=0.186N	P=0.499N	P=0.497N	P=0.506N
All Organs: Benign Neoplasms				
Overall rate	1/26 (3.8%)	4/27 (14.8%)	2/27 (7.4%)	1/27 (3.7%)
Adjusted rate	4.1%	16.1%	8.0%	4.2%
Terminal rate	1/23 (4.3%)	4/23 (17.4%)	2/24 (8.3%)	1/21 (4.8%)
First incidence (days)	320 (T)	319 (T)	322 (T)	321 (T)
Poly-3 test	P=0.452N	P=0.177	P=0.509	P=0.757
All Organs: Malignant Neoplasms				
Overall rate	3/26 (11.5%)	3/27 (11.1%)	6/27 (22.2%)	7/27 (25.9%)
Adjusted rate	11.5%	11.6%	23.7%	27.3%
Terminal rate	0/23 (0.0%)	1/23 (4.3%)	5/24 (20.8%)	3/21 (14.3%)
First incidence (days)	184	212	298	241
Poly-3 test	P=0.053	P=0.663	P=0.220	P=0.139

TABLE A11
Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	4/26 (15.4%)	7/27 (25.9%)	8/27 (29.6%)	8/27 (29.6%)
Adjusted rate	15.4%	27.0%	31.6%	31.2%
Terminal rate	1/23 (4.3%)	5/23 (21.7%)	7/24 (29.2%)	4/21 (19.0%)
First incidence (days)	184	212	298	241
Poly-3 test	P=0.105	P=0.248	P=0.149	P=0.154

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group 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**.
[e] Not applicable; no neoplasms in animal group
[f] 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[a]

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Disposition Summary				
Animals initially in study	26	27	27	27
Early deaths				
Moribund	1			1
Natural death		1	1	
Survivors				
Died last week of study	2	3	2	5
Terminal kill	23	23	24	21
Animals examined microscopically	26	27	27	27
Alimentary System				
Esophagus	(26)	(27)	(27)	(27)
Adventitia, necrosis		1 (4%)		
Intestine large, cecum	(25)	(27)	(25)	(24)
Hyperplasia, lymphoid	2 (8%)	2 (7%)	3 (12%)	2 (8%)
Epithelium, hyperplasia			1 (4%)	
Intestine large, colon	(26)	(26)	(27)	(25)
Intestine large, rectum	(25)	(27)	(27)	(25)
Hyperplasia, lymphoid		1 (4%)	1 (4%)	
Intestine small, ileum	(25)	(27)	(25)	(25)
Intestine small, jejunum	(25)	(27)	(25)	(25)
Liver	(26)	(27)	(27)	(27)
Basophilic focus				1 (4%)
Hematopoietic cell proliferation			1 (4%)	
Infiltration cellular, lymphocyte				1 (4%)
Inflammation, chronic active		2 (7%)	1 (4%)	
Necrosis			1 (4%)	
Tension lipidosis	3 (12%)	5 (19%)	7 (26%)	4 (15%)
Vacuolization cytoplasmic	6 (23%)	4 (15%)	3 (11%)	10 (37%)
Mesentery	(1)	(0)	(1)	(1)
Hemorrhage	1 (100%)			1 (100%)
Fat, necrosis			1 (100%)	1 (100%)
Pancreas	(26)	(27)	(27)	(26)
Cytoplasmic alteration				1 (4%)
Infiltration cellular, lymphocyte			1 (4%)	3 (12%)
Acinar cell, degeneration			1 (4%)	1 (4%)
Salivary glands	(26)	(27)	(26)	(27)
Infiltration cellular, lymphocyte	15 (58%)	14 (52%)	10 (38%)	10 (37%)
Stomach, forestomach	(26)	(27)	(27)	(27)
Cardiovascular System				
Heart	(26)	(27)	(27)	(26)
Bacterium		1 (4%)		
Congestion			2 (7%)	
Inflammation, chronic active	1 (4%)		1 (4%)	1 (4%)
Pericardium, necrosis		1 (4%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Endocrine System				
Adrenal cortex	(26)	(27)	(27)	(26)
Subcapsular, hyperplasia	25 (96%)	25 (93%)	27 (100%)	24 (92%)
Parathyroid gland	(21)	(22)	(22)	(22)
Cyst		1 (5%)		
Pituitary gland	(26)	(27)	(27)	(27)
Thyroid gland	(25)	(27)	(27)	(26)
Cyst		1 (4%)		
Degeneration	1 (4%)	2 (7%)		
Ectopic thymus		2 (7%)		1 (4%)
General Body System				
Tissue NOS	(0)	(1)	(0)	(1)
Mediastinum, abscess				1 (100%)
Mediastinum, foreign body				1 (100%)
Genital System				
Clitoral gland	(25)	(26)	(26)	(27)
Degeneration				1 (4%)
Ovary	(26)	(27)	(27)	(26)
Cyst		1 (4%)	1 (4%)	1 (4%)
Pigmentation				1 (4%)
Bilateral, cyst			1 (4%)	
Uterus	(26)	(27)	(27)	(27)
Edema				1 (4%)
Infiltration cellular, polymorphonuclear	1 (4%)	3 (11%)	3 (11%)	3 (11%)
Endometrium, hyperplasia, cystic	3 (12%)	4 (15%)	5 (19%)	5 (19%)
Lumen, dilatation	10 (38%)	12 (44%)	14 (52%)	8 (30%)
Vagina	(25)	(27)	(27)	(24)
Hematopoietic System				
Bone marrow	(26)	(26)	(26)	(26)
Myeloid cell, hyperplasia			4 (15%)	1 (4%)
Lymph node	(1)	(1)	(0)	(3)
Lumbar, hyperplasia, lymphoid	1 (100%)			1 (33%)
Lymph node, mandibular	(26)	(27)	(25)	(26)
Hyperplasia, lymphoid			2 (8%)	
Lymph node, mesenteric	(26)	(26)	(26)	(27)
Hyperplasia, lymphoid	8 (31%)	6 (23%)	6 (23%)	6 (22%)
Spleen	(26)	(26)	(27)	(27)
Hematopoietic cell proliferation	5 (19%)	4 (15%)	5 (19%)	8 (30%)
Hyperplasia, lymphoid	4 (15%)	5 (19%)	7 (26%)	6 (22%)
Thymus	(24)	(26)	(23)	(27)
Bacterium		1 (4%)		
Foreign body		1 (4%)		
Hyperplasia, lymphoid	3 (13%)	2 (8%)	3 (13%)	2 (7%)
Inflammation, chronic active				1 (4%)
Necrosis		1 (4%)		
Integumentary System				
Mammary gland	(25)	(26)	(27)	(27)
Alveolus, hyperplasia		1 (4%)		
Skin	(26)	(27)	(27)	(27)

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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Musculoskeletal System				
Bone	(0)	(1)	(0)	(1)
Bone, femur	(26)	(27)	(27)	(27)
Skeletal muscle	(26)	(27)	(27)	(27)
Atrophy	2 (8%)			
Cyst	1 (4%)			
Nervous System				
Brain, brain stem	(26)	(27)	(27)	(27)
Gliosis		1 (4%)		
Brain, cerebrum	(26)	(27)	(27)	(27)
Compression				1 (4%)
Infiltration cellular, lymphocyte		1 (4%)		
Mineralization		1 (4%)	4 (15%)	2 (7%)
Peripheral nerve	(2)	(0)	(0)	(0)
Axon, degeneration	2 (100%)			
Spinal cord	(1)	(0)	(0)	(0)
Axon, degeneration	1 (100%)			
Respiratory System				
Lung	(26)	(27)	(26)	(27)
Abscess				1 (4%)
Bacterium		1 (4%)		
Foreign body				1 (4%)
Inflammation, chronic active				1 (4%)
Necrosis				1 (4%)
Mediastinum, foreign body		1 (4%)		
Mediastinum, necrosis		1 (4%)		
Nose	(26)	(27)	(27)	(27)
Special Senses System				
Eye	(25)	(25)	(26)	(24)
Cataract	1 (4%)			
Harderian gland	(26)	(27)	(27)	(25)
Urinary System				
Kidney	(26)	(27)	(27)	(27)
Casts protein	1 (4%)	1 (4%)	3 (11%)	2 (7%)
Cyst	1 (4%)			1 (4%)
Glomerulosclerosis				1 (4%)
Hydronephrosis			1 (4%)	
Infiltration cellular, lymphocyte	9 (35%)	6 (22%)	5 (19%)	4 (15%)
Inflammation, suppurative			1 (4%)	
Metaplasia, osseous		1 (4%)		
Mineralization	1 (4%)			
Nephropathy			1 (4%)	1 (4%)
Ureter	(0)	(0)	(1)	(0)
Inflammation, suppurative			1 (100%)	
Lumen, dilatation			1 (100%)	
Urinary bladder	(26)	(27)	(27)	(26)
Infiltration cellular, lymphocyte	1 (4%)	4 (15%)	2 (7%)	1 (4%)

TABLE A13
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

	0/0 mg/kg	240/40 mg/kg
Disposition Summary		
Animals initially in study	24	25
Early deaths		
Natural death		1
Survivors		
Died last week of study	1	2
Terminal kill	23	22
Animals examined microscopically	24	25
Alimentary System		
Intestine large, cecum	(24)	(24)
Intestine large, rectum	(24)	(24)
Liver	(24)	(25)
Carcinoma, metastatic, pancreas	1 (4%)	
Hepatocellular adenoma	3 (13%)	5 (20%)
Hepatocellular carcinoma		2 (8%)
Pancreas	(23)	(25)
Acinar cell, carcinoma	1 (4%)	
Salivary glands	(24)	(25)
Cardiovascular System		
Blood vessel	(24)	(24)
Heart	(24)	(25)
Endocrine System		
Adrenal cortex	(24)	(25)
Islets, pancreatic	(23)	(25)
Thyroid gland	(24)	(24)
General Body System		
Tissue NOS	(0)	(1)
Sarcoma, metastatic, skin		1 (100%)
Genital System		
Epididymis	(24)	(24)
Preputial gland	(24)	(24)
Prostate	(24)	(25)
Seminal vesicle	(24)	(25)
Testes	(24)	(24)
Hematopoietic System		
Bone marrow	(24)	(25)
Lymph node, mesenteric	(24)	(24)
Spleen	(24)	(24)
Thymus	(23)	(22)

TABLE A13
Summary of the Incidence of Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Integumentary System		
Skin	(24)	(25)
Subcutaneous tissue, sarcoma		1 (4%)
Musculoskeletal System		
None		
Nervous System		
Brain, cerebrum	(24)	(25)
Respiratory System		
Lung	(24)	(25)
Alveolar/bronchiolar adenoma	2 (8%)	
Nose	(24)	(25)
Special Senses System		
Harderian gland	(24)	(25)
Adenoma	1 (4%)	
Urinary System		
Kidney	(24)	(25)
Urinary bladder	(24)	(24)
Systemic Lesions		
Multiple organs[b]	(24)	(25)
Lymphoma malignant		2 (8%)
Neoplasm Summary		
Total animals with primary neoplasms[c]	7	10
Total primary neoplasms	7	10
Total animals with benign neoplasms	6	5
Total benign neoplasms	6	5
Total animals with malignant neoplasms	1	5
Total malignant neoplasms	1	5
Total animals with metastatic neoplasms	1	1
Total metastatic neoplasms	1	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A14
Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Liver: Hepatocellular Adenoma		
Overall rate[a]	3/24 (12.5%)	5/25 (20.0%)
Adjusted rate[b]	12.8%	21.3%
Terminal rate[c]	3/23 (13.0%)	5/22 (22.7%)
First incidence (days)	317 (T)	317 (T)
Poly-3 test[d]		P=0.352
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	3/24 (12.5%)	7/25 (28.0%)
Adjusted rate	12.8%	29.8%
Terminal rate	3/23 (13.0%)	7/22 (31.8%)
First incidence (days)	317 (T)	317 (T)
Poly-3 test		P=0.143
All Organs: Malignant Lymphoma		
Overall rate	0/24 (0.0%)	2/25 (8.0%)
Adjusted rate	0.0%	8.5%
Terminal rate	0/23 (0.0%)	1/22 (4.5%)
First incidence (days)	—[e]	300
Poly-3 test		P=0.237
All Organs: Benign Neoplasms		
Overall rate	6/24 (25.0%)	5/25 (20.0%)
Adjusted rate	25.6%	21.3%
Terminal rate	6/23 (26.1%)	5/22 (22.7%)
First incidence (days)	317 (T)	317 (T)
Poly-3 test		P=0.498N
All Organs: Malignant Neoplasms		
Overall rate	1/24 (4.2%)	5/25 (20.0%)
Adjusted rate	4.2%	20.8%
Terminal rate	0/23 (0.0%)	3/22 (13.6%)
First incidence (days)	240	281
Poly-3 test		P=0.093
All Organs: Benign or Malignant Neoplasms		
Overall rate	7/24 (29.2%)	10/25 (40.0%)
Adjusted rate	29.2%	41.7%
Terminal rate	6/23 (26.1%)	8/22 (36.4%)
First incidence (days)	240	281
Poly-3 test		P=0.276

(T) Terminal kill

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

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

[c] Observed incidence at terminal kill

[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group 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**.

[e] Not applicable; no neoplasms in animal group

TABLE A15
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

	0/0 mg/kg	240/40 mg/kg
Disposition Summary		
Animals initially in study	24	25
Early deaths		
Natural death		1
Survivors		
Died last week of study	1	2
Terminal kill	23	22
Animals examined microscopically	24	25
Alimentary System		
Intestine large, cecum	(24)	(24)
Hyperplasia, lymphoid	3 (13%)	1 (4%)
Intestine large, rectum	(24)	(24)
Degeneration, cystic	1 (4%)	
Liver	(24)	(25)
Cytomegaly	18 (75%)	14 (56%)
Eosinophilic focus		1 (4%)
Inflammation, chronic active	1 (4%)	1 (4%)
Necrosis	2 (8%)	1 (4%)
Tension lipidosis	3 (13%)	2 (8%)
Vacuolization cytoplasmic	23 (96%)	18 (72%)
Pancreas	(23)	(25)
Cytoplasmic alteration	1 (4%)	
Salivary glands	(24)	(25)
Infiltration cellular, lymphocyte	8 (33%)	8 (32%)
Cardiovascular System		
Blood vessel	(24)	(24)
Heart	(24)	(25)
Endocrine System		
Adrenal cortex	(24)	(25)
Hypertrophy		1 (4%)
Subcapsular, hyperplasia	7 (29%)	7 (28%)
Islets, pancreatic	(23)	(25)
Hyperplasia	1 (4%)	2 (8%)
Thyroid gland	(24)	(24)
Cyst		1 (4%)
Ectopic thymus	1 (4%)	
General Body System		
Tissue NOS	(0)	(1)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A15
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Genital System		
Epididymis	(24)	(24)
Hypospermia		1 (4%)
Serosa, inflammation, chronic active	1 (4%)	
Serosa, necrosis	1 (4%)	
Preputial gland	(24)	(24)
Abscess		1 (4%)
Degeneration	1 (4%)	
Duct, dilatation		1 (4%)
Prostate	(24)	(25)
Infiltration cellular, lymphocyte	1 (4%)	1 (4%)
Epithelium, hyperplasia	1 (4%)	
Seminal vesicle	(24)	(25)
Serosa, inflammation, chronic active	1 (4%)	
Testes	(24)	(24)
Spermatocele		1 (4%)
Interstitial cell, hyperplasia	2 (8%)	4 (17%)
Seminiferous tubule, degeneration	2 (8%)	2 (8%)
Hematopoietic System		
Bone marrow	(24)	(25)
Myeloid cell, hyperplasia		2 (8%)
Lymph node, mesenteric	(24)	(24)
Hyperplasia, lymphoid	15 (63%)	12 (50%)
Spleen	(24)	(24)
Depletion lymphoid	1 (4%)	
Hematopoietic cell proliferation	2 (8%)	3 (13%)
Hyperplasia, lymphoid	5 (21%)	7 (29%)
Thymus	(23)	(22)
Degeneration	1 (4%)	
Hyperplasia, lymphoid		1 (5%)
Necrosis		1 (5%)
Integumentary System		
Skin	(24)	(25)
Musculoskeletal System		
None		
Nervous System		
Brain, cerebrum	(24)	(25)
Mineralization	6 (25%)	3 (12%)

TABLE A15
Summary of the Incidence of Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Respiratory System		
Lung	(24)	(25)
Foreign body		1 (4%)
Infiltration cellular, polymorphonuclear		1 (4%)
Bronchiole, epithelium, hyperplasia		1 (4%)
Nose	(24)	(25)
Foreign body		1 (4%)
Inflammation, suppurative		1 (4%)
Special Senses System		
Harderian gland	(24)	(25)
Urinary System		
Kidney	(24)	(25)
Infiltration cellular, lymphocyte	6 (25%)	6 (24%)
Mineralization		1 (4%)
Capsule, inflammation, chronic active	1 (4%)	
Pelvis, dilatation		1 (4%)
Renal tubule, regeneration	2 (8%)	3 (12%)
Urinary bladder	(24)	(24)
Infiltration cellular, lymphocyte		1 (4%)

TABLE A16
Summary of the Incidence of Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

	0/0 mg/kg	240/40 mg/kg
Disposition Summary		
Animals initially in study	26	26
Survivors		
Died last week of study	1	3
Terminal kill	25	23
Animals examined microscopically	26	26
Alimentary System		
Intestine large, cecum	(25)	(26)
Liver	(25)	(26)
Salivary glands	(25)	(26)
Cardiovascular System		
Heart	(25)	(26)
Endocrine System		
Adrenal cortex	(26)	(26)
Thyroid gland	(26)	(26)
General Body System		
None		
Genital System		
Ovary	(26)	(26)
Teratoma malignant	1 (4%)	
Yolk sac carcinoma	1 (4%)	
Uterus	(26)	(26)
Polyp stromal	1 (4%)	1 (4%)
Sarcoma stromal		1 (4%)
Vagina	(26)	(25)
Hematopoietic System		
Bone marrow	(25)	(26)
Lymph node	(0)	(2)
Lymph node, mandibular	(25)	(25)
Lymph node, mesenteric	(25)	(26)
Spleen	(26)	(26)
Thymus	(25)	(25)
Integumentary System		
Mammary gland	(26)	(26)
Adenoma	1 (4%)	

TABLE A16
Summary of the Incidence of Neoplasms in Female C3B6 Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Musculoskeletal System		
None		
Nervous System		
Brain, brain stem	(26)	(26)
Astrocytoma malignant		1 (4%)
Brain, cerebellum	(26)	(26)
Astrocytoma malignant		1 (4%)
Brain, cerebrum	(26)	(26)
Respiratory System		
Lung	(26)	(26)
Yolk sac carcinoma, metastatic, ovary	1 (4%)	
Special Senses System		
Harderian gland	(25)	(26)
Urinary System		
Kidney	(25)	(26)
Urinary bladder	(25)	(26)
Systemic Lesions		
Multiple organs[b]	(26)	(26)
Leukemia, granulocytic		1 (4%)
Neoplasm Summary		
Total animals with primary neoplasms[c]	4	4
Total primary neoplasms	4	5
Total animals with benign neoplasms	2	1
Total benign neoplasms	2	1
Total animals with malignant neoplasms	2	3
Total malignant neoplasms	2	4
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
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A17
Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
All Organs: Granulocytic Leukemia		
Overall rate[a]	0/26 (0.0%)	1/26 (3.8%)
Adjusted rate[b]	0.0%	4.1%
Terminal rate[c]	0/25 (0.0%)	1/23 (4.3%)
First incidence (days)	—[e]	320 (T)
Poly-3 test[d]		P=0.493
All Organs: Benign Neoplasms		
Overall rate	2/26 (7.7%)	1/26 (3.8%)
Adjusted rate	7.8%	4.1%
Terminal rate	2/25 (8.0%)	1/23 (4.3%)
First incidence (days)	320 (T)	318 (T)
Poly-3 test		P=0.512N
All Organs: Malignant Neoplasms		
Overall rate	2/26 (7.7%)	3/26 (11.5%)
Adjusted rate	7.7%	11.9%
Terminal rate	1/25 (4.0%)	2/23 (8.7%)
First incidence (days)	259	260
Poly-3 test		P=0.484
All Organs: Benign or Malignant Neoplasms		
Overall rate	4/26 (15.4%)	4/26 (15.4%)
Adjusted rate	15.4%	15.9%
Terminal rate	3/25 (12.0%)	3/23 (13.0%)
First incidence (days)	259	260
Poly-3 test		P=0.628

(T) Terminal kill

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

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

[c] Observed incidence at terminal kill

[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group 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**.

[e] Not applicable; no neoplasms in animal group

TABLE A18
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

	0/0 mg/kg	240/40 mg/kg
Disposition Summary		
Animals initially in study	26	26
Survivors		
Died last week of study	1	3
Terminal kill	25	23
Animals examined microscopically	26	26
Alimentary System		
Intestine large, cecum	(25)	(26)
Hyperplasia, lymphoid	3 (12%)	
Liver	(25)	(26)
Basophilic focus		1 (4%)
Infiltration cellular, lymphocyte	2 (8%)	1 (4%)
Inflammation, chronic active	2 (8%)	
Tension lipidosis	3 (12%)	4 (15%)
Vacuolization cytoplasmic	12 (48%)	6 (23%)
Salivary glands	(25)	(26)
Infiltration cellular, lymphocyte	15 (60%)	12 (46%)
Cardiovascular System		
Heart	(25)	(26)
Endocrine System		
Adrenal cortex	(26)	(26)
Subcapsular, hyperplasia	23 (88%)	24 (92%)
Thyroid gland	(26)	(26)
Degeneration		1 (4%)
Ectopic thymus	2 (8%)	1 (4%)
General Body System		
None		
Genital System		
Ovary	(26)	(26)
Cyst	4 (15%)	1 (4%)
Uterus	(26)	(26)
Infiltration cellular, polymorphonuclear	1 (4%)	
Endometrium, hyperplasia, cystic	4 (15%)	3 (12%)
Lumen, dilatation	16 (62%)	13 (50%)
Vagina	(26)	(25)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A18
Summary of the Incidence of Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Hematopoietic System		
Bone marrow	(25)	(26)
Lymph node	(0)	(2)
Renal, hyperplasia, lymphoid		1 (50%)
Lymph node, mandibular	(25)	(25)
Hyperplasia, lymphoid		1 (4%)
Lymph node, mesenteric	(25)	(26)
Hyperplasia, lymphoid	4 (16%)	3 (12%)
Spleen	(26)	(26)
Hematopoietic cell proliferation	2 (8%)	2 (8%)
Hyperplasia, lymphoid	6 (23%)	6 (23%)
Thymus	(25)	(25)
Cyst, multiple		1 (4%)
Hyperplasia, lymphoid	1 (4%)	3 (12%)
Integumentary System		
Mammary gland	(26)	(26)
Musculoskeletal System		
None		
Nervous System		
Brain, brain stem	(26)	(26)
Brain, cerebellum	(26)	(26)
Brain, cerebrum	(26)	(26)
Mineralization	2 (8%)	4 (15%)
Respiratory System		
Lung	(26)	(26)
Infiltration cellular, lymphocyte		1 (4%)
Inflammation, chronic active		1 (4%)
Special Senses System		
Harderian gland	(25)	(26)
Urinary System		
Kidney	(25)	(26)
Infiltration cellular, lymphocyte	9 (36%)	5 (19%)
Urinary bladder	(25)	(26)
Infiltration cellular, lymphocyte	2 (8%)	3 (12%)
Lumen, dilatation	1 (4%)	

APPENDIX B
GENETIC TOXICOLOGY

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

	% Reticulocytes	% Micronucleated NCE	% Micronucleated RET
Male			
0 mg/kg[b]	7	7	7
Mean ± standard error	19.9 ± 2.6	0.16 ± 0.03	0.29 ± 0.04
P value[c]	P=0.007N	P<0.0001	P<0.0001
80 mg/kg	7	7	7
Mean ± standard error	14.4 ± 1.3	0.81 ± 0.13	1.44 ± 0.36
P value	[P=0.189]	[P=0.094]	[P=0.228]
160 mg/kg	7	7	7
Mean ± standard error	12.0 ± 1.2	1.26 ± 0.22	3.61 ± 0.60
P value	P=0.039N	P=0.0064	P=0.0016
240 mg/kg	9	9	9
Mean ± standard error	11.8 ± 2.5	2.20 ± 0.35	4.84 ± 0.87
P value	P=0.024N	P<0.0001	P<0.0001
Female			
0 mg/kg	6	6	6
Mean ± standard error	19.7 ± 1.6	0.13 ± 0.04	0.27 ± 0.07
P value	P<0.001N	P=0.0006	P=0.0003
80 mg/kg	9	9	9
Mean ± standard error	11.9 ± 1.1	0.96 ± 0.13	1.69 ± 0.22
P value	P=0.002N	[P=0.298]	[P=0.314]
160 mg/kg	12	12	12
Mean ± standard error	11.0 ± 1.0	1.28 ± 0.16	3.27 ± 0.49
P value	P<0.001N	[P=0.152]	P=0.044
240 mg/kg	9	9	9
Mean ± standard error	10.1 ± 1.6	3.27 ± 1.00	5.82 ± 1.71
P value	P<0.001N	P=0.0007	P=0.0008

[a] Dams were dosed from gestational day (GD) 12 through GD 18 with the listed doses; NCE = normochromatic erythrocytes; RET = reticulocytes

[b] Number examined

[c] Beneath the 0 mg/kg group percentage is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that exposed group. Two-tailed Dunnett's tests were used for the % reticulocyte values and one-tailed Dunnett's tests were used for the % micronucleated NCE and % micronucleated RET values. Nonsignificant P values are indicated with brackets.

TABLE B2
Frequency of Micronuclei in Peripheral Blood Erythrocytes and Reticulocytes in 10-Day-Old Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	% Reticulocytes	% Micronucleated NCE	% Micronucleated RET
Male			
0/0 mg/kg[b]	5	5	5
Mean ± standard error	14.1 ± 0.5	0.32 ± 0.05	0.31 ± 0.05
P value[c]	[P=0.099]	P<0.0001	P<0.0001
80/40 mg/kg	6	6	6
Mean ± standard error	17.0 ± 1.4	7.23 ± 0.66	2.89 ± 0.27
P value	[P=0.55]	P<0.0001	P=0.0014
160/80 mg/kg	11	11	11
Mean ± standard error	18.8 ± 1.2	10.33 ± 0.63	7.04 ± 0.42
P value	[P=0.14]	P<0.0001	P<0.0001
240/120 mg/kg	8	8	8
Mean ± standard error	18.0 ± 2.3	4.94 ± 0.62	3.34 ± 0.48
P value	[P=0.28]	P<0.0001	P =0.0001
Female			
0/0 mg/kg	6	3	3
Mean ± standard error	19.2 ± 4.0	0.27 ± 0.07	0.29 ± 0.75
P value	[P=0.29]	P<0.0001	P=0.0030
80/40 mg/kg	6	9	6
Mean ± standard error	18.0 ± 0.9	7.14 ± 0.38	2.68 ± 0.29
P value	[P=0.98]	P<0.0001	[P=0.21]
160/80 mg/kg	13	13	13
Mean ± standard error	22.2 ± 1.8	9.85 ± 0.56	7.96 ± 0.87
P value	[P=0.79]	P<0.0001	P=0.0002
240/120 mg/kg	6	6	6
Mean ± standard error	23.2 ± 4.1	6.56 ± 1.11	5.04 ± 1.36
P value	[P=0.68]	P=0.0002	P=0.0230

[a] Pups which had been exposed transplacentally as for Table B1 were dosed with AZT at half the adult dose from postnatal day (PND) 1 until evaluation on PND 10; NCE = normochromatic erythrocytes; RET = reticulocytes

[b] Number examined

[c] Beneath the 0/0 mg/kg group percentage is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group. Two-tailed Dunnett's tests were used for the % reticulocyte values and one-tailed Dunnett's tests were used for the % micronucleated NCE and % micronucleated RET values. Nonsignificant P values are indicated with brackets.

TABLE B3
Frequency of Micronuclei in Peripheral Blood Erythrocytes and Reticulocytes in 28-Day-Old Heterozygous F1 p53$^{+/-}$ Mouse Pups in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	% Reticulocytes	% Micronucleated NCE	% Micronucleated RET
Male			
0/0/0 mg/kg[b]	8	8	8
Mean ± standard error	8.03 ± 0.41	0.16 ± 0.02	0.22 ± 004
P value[c]	[P=0.19]	P<0.0001	P<0.0001
80/40/80 mg/kg	9	9	9
Mean ± standard error	6.90 ± 0.33	1.76 ± 0.07	0.98 ± 0.05
P value	[P=0.08]	P<0.0001	P<0.0001
160/80/160 mg/kg	14	14	14
Mean ± standard error	7.48 ± 0.28	2.70 ± 0.14	1.57 ± 0.11
P value	[P=0.34]	P<0.0001	P<0.0001
240/120/240 mg/kg	9	9	9
Mean ± standard error	7.17 ± 0.34	3.22 ± 0.33	1.49 ± 0.13
P value	[P=0.22]	P<0.0001	P<0.0001
Female			
0/0/0 mg/kg	9	9	9
Mean ± standard error	6.46 ± 0.90	0.13 ± 0.01	0.18 ± 0.03
P value	[P=0.20]	P<0.0001	P<0.0001
80/40/80 mg/kg	9	9	9
Mean ± standard error	6.55 ± 0.66	1.63 ± 0.09	0.95 ± 0.18
P value	[P=1.00]	[P=0.16]	P=0.0030
160/80/160 mg/kg	13	13	13
Mean ± standard error	6.86 ± 0.51	2.50 ± 0.08	1.40 ± 0.11
P value	[P=0.99]	P=0.0184	P<0.0001
240/120/240 mg/kg	9	9	9
Mean ± standard error	8.98 ± 2.51	4.62 ± 1.49	1.39 ± 0.25
P value	[P=0.42]	P=0.0001	P<0.0001

[a] Pups which had been dosed as for Table B2 were dosed with the full dose of AZT from postnatal day (PND) 11 until evaluation on PND 28; NCE = normochromatic erythrocytes; RET = reticulocytes

[b] Number examined

[c] Beneath the 0/0/0 mg/kg group percentage is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group. Two-tailed Dunnett's tests were used for the % reticulocyte values and one-tailed Dunnett's tests were used for the % micronucleated NCE and % micronucleated RET values. Nonsignificant P values are indicated with brackets.

TABLE B4
Frequency of Micronuclei in Peripheral Blood Erythrocytes and Reticulocytes in 30-Week-Old Heterozygous F1 $p53^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	% Reticulocytes	% Micronucleated NCE	% Micronucleated RET
Male			
0/0/0 mg/kg[b]	13	13	13
Mean ± standard error	1.67 ± 0.14	0.41 ± 0.12	0.58 ± 0.12
240/120/240 mg/kg	13	13	13
Mean ± standard error	1.75 ± 0.19	1.10 ± 0.17	2.28 ± 0.61
P value[c]	[P=0.74]	P=0.0029	P=0.0166
Female			
0/0/0 mg/kg	13	13	13
Mean ± standard error	1.86 ± 0.23	0.44 ± 0.21	0.51 ± 0.13
240/120/240 mg/kg	10	10	10
Mean ± standard error	1.93 ± 0.65	1.31 ± 0.16	2.48 ± 0.19
P value[c]	[P=0.91]	P=0.0037	P<0.0001

[a] Blood samples were taken from mice from the 30-week study at terminal kill; NCE = normochromatic erythrocytes; RET = reticulocytes
[b] Number examined
[c] Beneath the dosed group incidences are the P values corresponding to pairwise comparison between the 0/0/0 mg/kg group and the dosed group using a two-tailed Student=s *t*-test. Nonsignificant P values are indicated with brackets.

APPENDIX C
CLINICAL PATHOLOGY RESULTS

TABLE C1
Hematology and Clinical Chemistry for Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
Male		
Hematology		
n	27	20
Erythrocytes (10^6/mm^3)	9.8 ± 0.2	9.2 ± 0.2*
Hemoglobin (g/dL)	16.1 ± 0.1	16.1 ± 0.2
Hematocrit (%)	47.0 ± 0.9	46.3 ± 1.0
Mean cell volume (fL)	48.0 ± 0.3	50.7 ± 0.3**
Mean cell hemoglobin (pg)	16.6 ± 0.4	17.7 ± 0.4
Mean cell hemoglobin concentration (g/dL)	34.0 ± 1.2	35.0 ± 0.9
Leukocytes (10^3/mm^3)	2.8 ± 0.3	3.1 ± 0.3
Neutrophils (%)	5.1 ± 0.4	5.8 ± 0.6
Lymphocytes (%)	89.0 ± 0.8	86.1 ± 1.7
Monocytes (%)	6.3 ± 1.8	6.0 ± 1.1
Eosinophils (%)	0.6 ± 0.2	0.9 ± 0.2
Basophils (%)	0.7 ± 0.1	1.3 ± 0.2*
Platelets (10^3/mm^3)	884.4 ± 25.3	810.9 ± 48.6
Clinical Chemistry		
n	25	21
Creatinine (mg/dL)	0.8 ± 0.1	0.6 ± 0.1
Blood urea nitrogen (mg/dL)	29.4 ± 5.5	23.5 ± 5.5
Female		
Hematology		
n	23	24
Erythrocytes (10^6/mm^3)	9.6 ± 0.1	9.4 ± 0.1
Hemoglobin (g/dL)	15.8 ± 0.2	16.0 ± 0.1
Hematocrit (%)	46.1 ± 0.9	47.4 ± 0.5
Mean cell volume (fL)	47.8 ± 0.3	50.6 ± 0.2**
Mean cell hemoglobin (pg)	16.5 ± 0.2	17.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.5 ± 0.4	33.8 ± 0.2
Leukocytes (10^3/mm^3)	4.7 ± 0.4	3.9 ± 0.3
Neutrophils (%)	5.1 ± 0.3	5.2 ± 0.6
Lymphocytes (%)	87.0 ± 0.9	86.7 ± 1.2
Monocytes (%)	6.4 ± 0.5	6.1 ± 0.7
Eosinophils (%)	0.4 ± 0.1	0.5 ± 0.1
Basophils (%)	1.1 ± 0.2	1.5 ± 0.3
Platelets (10^3/mm^3)	732.1 ± 40.1	799.3 ± 32.4

TABLE C1
Hematology and Clinical Chemistry for Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Female (continued)		
Clinical Chemistry		
n	24	24
Creatinine (mg/dL)	0.5 ± 0.0	0.5 ± 0.0
Blood urea nitrogen (mg/dL)	14.7 ± 0.5	15.0 ± 0.5

* Significantly different (P≤0.05) from the 0/0/0 mg/kg group by a *t*-test
** P≤0.01
[a] Data are presented as mean ± standard error.

TABLE C2
Hematology Data for Heterozygous F1 p53$^{+/-}$ Mice at 160 Days in the 30- and 45-Week *In Utero*/Postnatal Gavage Studies of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
n	8	16
Male		
Erythrocytes (10^6/mm^3)	11.2 ± 0.1	10.3 ± 0.1**
Hemoglobin (g/dL)	18.4 ± 0.2	17.9 ± 0.2*
Hematocrit (%)	55.1 ± 0.7	53.5 ± 0.4
Mean cell volume (fL)	49.1 ± 0.2	52.1 ± 0.3**
Mean cell hemoglobin (pg)	16.4 ± 0.1	17.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.4 ± 0.1
Erythrocyte distribution width (%)	13.6 ± 0.1	13.9 ± 0.1*
Female		
Erythrocytes (10^6/mm^3)	11.4 ± 0.1	10.3 ± 0.2**
Hemoglobin (g/dL)	19.1 ± 0.2	17.9 ± 0.4**
Hematocrit (%)	56.6 ± 0.6	53.6 ± 1.0*
Mean cell volume (fL)	49.5 ± 0.2	52.1 ± 0.2**
Mean cell hemoglobin (pg)	16.7 ± 0.1	17.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	33.4 ± 0.1*
Erythrocyte distribution width (%)	13.4 ± 0.1	13.6 ± 0.1

* Significantly different (P≤0.05) from the 0/0/0 mg/kg group by a *t*-test
** P≤0.01
[a] Data are presented as mean ± standard error. Hematology was performed on subsets of the 160-day-old mice receiving 0/0/0 mg/kg or 240/120/240 mg/kg in order to monitor for macrocytic anemia. Mice from both the 30-week and 45-week studies were evaluated. This evaluation was performed because previous studies (Ayers *et al.*, 1996a) had reported that mice exposed to AZT doses greater than 40 mg/kg per day developed macrocytic anemia severe enough to necessitate reduction of the dose concentration.

APPENDIX D
BODY WEIGHT ANALYSES

TABLE D1
Tests of Main Effects and Interactions for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

Source of Variation	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	P Value[b]
Male				
Postnatal week	28	1,377	781.3	0.000
Dose	1	51	31.6	0.000
Postnatal week × dose	28	1,377	1.8	0.006
Female				
Postnatal week	28	1,386	449.1	0.000
Dose	1	51	5.6	0.022
Postnatal week × dose	28	1,386	0.5	0.979

[a] Statistical analysis by repeated measures ANOVA
[b] Probability of significant main effect (postnatal week and dose) or significant interaction (postnatal week × dose); significant at P≤0.05

TABLE D2
Overall Trend Tests for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

Contrast of Interest	Estimate	Standard Error	Degrees of Freedom	t Value	P Value[b]
Male					
AZT dose; linear trend	–1.2	0.52	51	–2.4	0.022
Female					
AZT dose; linear trend	–2.7	0.48	51	–5.6	0.000

[a] Statistical analysis by repeated measures ANOVA
[b] Probability of significant linear trend using contrasts; significant at P≤0.05

TABLE D3
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)[a]	P Value[b]	n	Body Weight (g)	P Value
2	0/0/0	27	8.3 ± 0.2		27	8.2 ± 0.2	
	240/120/240	26	7.7 ± 0.2	0.020	26	7.4 ± 0.2	0.005
3	0/0/0	27	10.5 ± 0.2		27	10.2 ± 0.3	
	240/120/240	26	9.8 ± 0.2	0.026	26	9.2 ± 0.2	0.002
4	0/0/0	27	15.6 ± 0.3		27	13.7 ± 0.3	
	240/120/240	26	14.4 ± 0.3	0.004	26	13.0 ± 0.2	0.040
5	0/0/0	27	21.7 ± 0.3		26	18.1 ± 0.3	
	240/120/240	26	20.3 ± 0.3	0.000	26	17.1 ± 0.2	0.007
6	0/0/0	27	24.8 ± 0.3		26	20.7 ± 0.3	
	240/120/240	26	23.0 ± 0.2	0.000	26	19.7 ± 0.2	0.005
7	0/0/0	27	26.6 ± 0.3		26	21.7 ± 0.3	
	240/120/240	26	24.6 ± 0.2	0.000	26	20.7 ± 0.2	0.012
8	0/0/0	27	28.0 ± 0.3		26	22.8 ± 0.3	
	240/120/240	26	26.2 ± 0.2	0.000	26	21.7 ± 0.2	0.003
9	0/0/0	27	29.4 ± 0.3		26	23.7 ± 0.3	
	240/120/240	26	27.2 ± 0.3	0.000	26	22.7 ± 0.2	0.012
10	0/0/0	27	30.6 ± 0.3		26	24.7 ± 0.3	
	240/120/240	26	28.1 ± 0.3	0.000	26	23.6 ± 0.2	0.009
11	0/0/0	27	31.4 ± 0.3		26	25.4 ± 0.4	
	240/120/240	26	29.2 ± 0.3	0.000	26	24.3 ± 0.2	0.013
12	0/0/0	27	32.6 ± 0.4		26	26.3 ± 0.4	
	240/120/240	26	29.8 ± 0.4	0.000	26	25.0 ± 0.3	0.020
13	0/0/0	27	33.7 ± 0.4		26	27.4 ± 0.5	
	240/120/240	25	31.2 ± 0.4	0.000	26	26.0 ± 0.3	0.017
14	0/0/0	27	34.9 ± 0.5		26	28.0 ± 0.5	
	240/120/240	25	32.2 ± 0.4	0.000	26	26.8 ± 0.4	0.071
15	0/0/0	27	35.8 ± 0.5		26	28.6 ± 0.6	
	240/120/240	25	33.1 ± 0.5	0.000	26	27.5 ± 0.4	0.112
16	0/0/0	27	36.8 ± 0.5		26	29.4 ± 0.6	
	240/120/240	25	33.9 ± 0.5	0.000	26	28.2 ± 0.5	0.125
17	0/0/0	27	37.7 ± 0.6		26	30.3 ± 0.7	
	240/120/240	24	34.8 ± 0.5	0.000	26	28.6 ± 0.5	0.040
18	0/0/0	27	38.4 ± 0.6		25	31.3 ± 0.7	
	240/120/240	23	35.8 ± 0.5	0.000	26	29.5 ± 0.6	0.097

TABLE D3
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
19	0/0/0	27	39.0 ± 0.6		25	31.8 ± 0.8	
	240/120/240	23	36.7 ± 0.6	0.002	26	30.2 ± 0.6	0.132
20	0/0/0	27	39.9 ± 0.6		25	32.7 ± 0.8	
	240/120/240	23	37.5 ± 0.6	0.002	26	30.8 ± 0.5	0.069
21	0/0/0	27	40.7 ± 0.7		25	32.9 ± 0.8	
	240/120/240	23	38.1 ± 0.6	0.001	26	31.2 ± 0.5	0.116
22	0/0/0	27	41.6 ± 0.7		25	33.3 ± 0.8	
	240/120/240	23	38.9 ± 0.6	0.001	26	31.7 ± 0.6	0.159
23	0/0/0	27	42.2 ± 0.7		25	33.6 ± 0.9	
	240/120/240	23	39.3 ± 0.6	0.001	26	32.3 ± 0.6	0.252
24	0/0/0	27	42.8 ± 0.7		25	34.4 ± 0.9	
	240/120/240	23	39.5 ± 0.7	0.001	26	32.8 ± 0.6	0.173
25	0/0/0	27	43.4 ± 0.8		25	34.9 ± 0.9	
	240/120/240	22	40.4 ± 0.7	0.001	26	33.1 ± 0.7	0.145
26	0/0/0	27	44.1 ± 0.7		25	35.3 ± 0.9	
	240/120/240	22	40.7 ± 0.7	0.000	26	33.5 ± 0.7	0.135
27	0/0/0	27	44.5 ± 0.8		25	35.5 ± 0.9	
	240/120/240	22	41.2 ± 0.7	0.000	26	34.1 ± 0.7	0.259
28	0/0/0	27	45.2 ± 0.8		24	36.0 ± 1.0	
	240/120/240	22	41.5 ± 0.7	0.000	26	34.5 ± 0.7	0.310
29	0/0/0	27	45.9 ± 0.7		24	36.5 ± 1.0	
	240/120/240	22	41.7 ± 0.7	0.000	26	35.3 ± 0.7	0.368
30	0/0/0	27	46.2 ± 0.7		24	37.0 ± 1.0	
	240/120/240	22	41.6 ± 0.8	0.000	26	35.7 ± 0.8	0.370

[a] Mean ± standard error
[b] Probability of significant difference from 0/0/0 mg/kg group by a *t*-test using contrasts; significant at P≤0.05

TABLE D4
Tests of Main Effects and Interactions for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

Source of Variation	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	P Value[b]
Male				
Postnatal week	43	4,177	704.3	0.000
Dose	3	104	5.3	0.002
Postnatal week × dose	129	4,177	0.4	1.000
Female				
Postnatal week	43	4,222	495.1	0.000
Dose	3	103	3.7	0.014
Postnatal week × dose	129	4,222	0.5	1.000

[a] Statistical analysis by repeated measures ANOVA
[b] Probability of significant main effect (postnatal week and dose) or significant interaction (postnatal week × dose); significant at P≤0.05

TABLE D5
Overall Trend Tests for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

Contrasts of Interest	Estimate	Standard Error	Degrees of Freedom	t Value	P Value[b]
Male					
AZT dose; linear trend	–6.3	2.0	104	–3.2	0.002
AZT dose; quadratic trend	0.3	0.9	104	0.4	0.700
Female					
AZT dose; linear trend	–4.2	2.1	103	–2.0	0.049
AZT dose; quadratic trend	1.5	0.9	103	1.6	0.113

[a] Statistical analysis by repeated measures ANOVA
[b] Probability of significant linear or quadratic trend using contrasts; significant at P≤0.05

TABLE D6
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)[a]	P Value[b]	n	Body Weight (g)	P Value
2	0/0/0	27	8.0 ± 0.2		26	7.9 ± 0.2	
	80/40/80	27	7.6 ± 0.2	0.365	27	7.3 ± 0.2	0.071
	160/80/160	27	7.8 ± 0.2	0.773	27	7.7 ± 0.1	0.840
	240/120/240	27	7.3 ± 0.2	0.029	27	7.7 ± 0.2	0.797
3	0/0/0	27	10.3 ± 0.2		26	10.0 ± 0.3	
	80/40/80	27	9.8 ± 0.2	0.263	27	9.2 ± 0.3	0.051
	160/80/160	27	9.9 ± 0.2	0.535	27	9.6 ± 0.1	0.537
	240/120/240	27	9.5 ± 0.2	0.035	27	9.7 ± 0.2	0.699
4	0/0/0	27	15.2 ± 0.2		26	13.8 ± 0.3	
	80/40/80	27	14.4 ± 0.4	0.112	27	12.7 ± 0.3	0.007
	160/80/160	27	14.9 ± 0.3	0.769	27	13.5 ± 0.2	0.695
	240/120/240	27	13.8 ± 0.3	0.002	27	13.2 ± 0.2	0.253
5	0/0/0	27	21.1 ± 0.3		26	18.0 ± 0.3	
	80/40/80	27	20.2 ± 0.5	0.172	26	17.0 ± 0.3	0.043
	160/80/160	27	20.6 ± 0.3	0.667	27	17.6 ± 0.2	0.737
	240/120/240	27	19.8 ± 0.4	0.042	27	17.2 ± 0.3	0.182
6	0/0/0	27	24.1 ± 0.3		26	20.6 ± 0.3	
	80/40/80	25	23.3 ± 0.4	0.078	26	19.6 ± 0.3	0.029
	160/80/160	27	23.7 ± 0.3	0.800	27	20.2 ± 0.2	0.559
	240/120/240	27	22.8 ± 0.3	0.029	27	19.8 ± 0.3	0.086
7	0/0/0	27	25.8 ± 0.3		26	21.8 ± 0.3	
	80/40/80	25	25.0 ± 0.4	0.071	26	20.5 ± 0.3	0.005
	160/80/160	27	25.3 ± 0.3	0.715	27	21.2 ± 0.2	0.286
	240/120/240	27	24.4 ± 0.4	0.018	26	20.9 ± 0.3	0.050
8	0/0/0	27	27.5 ± 0.3		26	22.8 ± 0.3	
	80/40/80	25	26.4 ± 0.4	0.033	26	21.7 ± 0.3	0.030
	160/80/160	27	26.9 ± 0.4	0.505	27	22.1 ± 0.2	0.268
	240/120/240	27	26.0 ± 0.4	0.014	26	21.9 ± 0.3	0.089
9	0/0/0	27	28.7 ± 0.4		26	23.7 ± 0.4	
	80/40/80	25	27.7 ± 0.4	0.096	26	22.7 ± 0.4	0.131
	160/80/160	27	28.2 ± 0.4	0.757	27	23.1 ± 0.3	0.514
	240/120/240	27	27.1 ± 0.4	0.020	26	22.9 ± 0.4	0.237
10	0/0/0	27	29.8 ± 0.4		26	24.7 ± 0.4	
	80/40/80	25	28.8 ± 0.5	0.092	26	23.7 ± 0.4	0.120
	160/80/160	27	29.3 ± 0.4	0.677	27	24.2 ± 0.2	0.713
	240/120/240	27	27.8 ± 0.4	0.003	26	23.8 ± 0.4	0.156

TABLE D6
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
11	0/0/0	27	31.0 ± 0.5		26	25.8 ± 0.4	
	80/40/80	24	30.1 ± 0.5	0.191	26	24.6 ± 0.4	0.078
	160/80/160	27	30.3 ± 0.5	0.579	27	25.0 ± 0.2	0.303
	240/120/240	27	28.7 ± 0.4	0.002	26	25.0 ± 0.4	0.291
12	0/0/0	26	32.1 ± 0.5		26	26.6 ± 0.5	
	80/40/80	24	31.0 ± 0.6	0.242	26	25.6 ± 0.4	0.213
	160/80/160	27	31.5 ± 0.5	0.817	27	26.0 ± 0.3	0.593
	240/120/240	27	29.6 ± 0.5	0.003	26	25.6 ± 0.5	0.181
13	0/0/0	26	33.3 ± 0.5		26	27.5 ± 0.5	
	80/40/80	24	32.1 ± 0.6	0.250	26	26.0 ± 0.5	0.050
	160/80/160	27	32.6 ± 0.5	0.796	27	26.7 ± 0.3	0.409
	240/120/240	26	30.4 ± 0.6	0.002	26	26.4 ± 0.5	0.187
14	0/0/0	26	34.4 ± 0.5		26	28.4 ± 0.6	
	80/40/80	24	32.9 ± 0.6	0.152	26	26.8 ± 0.5	0.049
	160/80/160	27	33.6 ± 0.6	0.775	27	27.3 ± 0.3	0.229
	240/120/240	25	31.5 ± 0.6	0.002	26	27.3 ± 0.6	0.203
15	0/0/0	26	35.5 ± 0.6		26	29.6 ± 0.6	
	80/40/80	24	33.8 ± 0.7	0.129	26	27.5 ± 0.6	0.013
	160/80/160	27	34.6 ± 0.6	0.723	27	28.2 ± 0.4	0.157
	240/120/240	25	32.6 ± 0.6	0.003	26	28.0 ± 0.6	0.076
16	0/0/0	26	36.3 ± 0.6		26	30.3 ± 0.7	
	80/40/80	24	34.6 ± 0.7	0.161	26	28.4 ± 0.7	0.056
	160/80/160	27	35.4 ± 0.7	0.761	27	28.8 ± 0.4	0.167
	240/120/240	25	33.4 ± 0.7	0.006	26	28.6 ± 0.6	0.114
17	0/0/0	26	37.3 ± 0.6		26	31.1 ± 0.7	
	80/40/80	24	35.3 ± 0.8	0.101	26	29.0 ± 0.8	0.067
	160/80/160	27	36.4 ± 0.7	0.796	27	29.6 ± 0.4	0.250
	240/120/240	25	34.3 ± 0.7	0.006	26	29.3 ± 0.6	0.116
18	0/0/0	26	38.1 ± 0.6		26	31.8 ± 0.7	
	80/40/80	24	36.1 ± 0.8	0.102	26	29.8 ± 0.8	0.084
	160/80/160	27	37.3 ± 0.8	0.826	27	30.2 ± 0.5	0.194
	240/120/240	25	35.1 ± 0.7	0.009	26	29.9 ± 0.7	0.108
19	0/0/0	26	38.9 ± 0.6		26	32.7 ± 0.8	
	80/40/80	24	36.8 ± 0.8	0.098	26	30.5 ± 0.8	0.084
	160/80/160	27	38.0 ± 0.8	0.784	27	30.9 ± 0.5	0.175
	240/120/240	25	35.9 ± 0.8	0.014	26	30.6 ± 0.8	0.098

TABLE D6
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
20	0/0/0	26	39.8 ± 0.6		26	33.1 ± 0.8	
	80/40/80	24	37.2 ± 0.9	0.048	26	30.8 ± 0.8	0.076
	160/80/160	27	38.6 ± 0.8	0.630	27	31.5 ± 0.5	0.274
	240/120/240	25	36.6 ± 0.8	0.012	26	31.3 ± 0.8	0.198
21	0/0/0	26	40.5 ± 0.7		26	33.7 ± 0.8	
	80/40/80	24	37.9 ± 0.9	0.062	26	31.5 ± 0.9	0.112
	160/80/160	27	39.2 ± 0.9	0.592	27	32.2 ± 0.5	0.395
	240/120/240	25	37.6 ± 0.8	0.029	26	31.7 ± 0.8	0.169
22	0/0/0	26	40.9 ± 0.7		26	34.5 ± 0.8	
	80/40/80	24	38.8 ± 0.9	0.178	26	32.1 ± 1.0	0.081
	160/80/160	27	40.1 ± 0.9	0.890	27	32.8 ± 0.6	0.287
	240/120/240	25	38.2 ± 0.8	0.068	26	32.2 ± 0.8	0.109
23	0/0/0	25	41.8 ± 0.7		26	34.8 ± 0.9	
	80/40/80	24	39.4 ± 1.0	0.176	26	32.6 ± 1.0	0.146
	160/80/160	27	40.6 ± 0.9	0.828	27	33.1 ± 0.6	0.349
	240/120/240	25	38.8 ± 0.8	0.067	26	32.9 ± 0.8	0.255
24	0/0/0	25	42.2 ± 0.8		26	35.0 ± 0.9	
	80/40/80	24	40.0 ± 1.0	0.282	26	33.0 ± 1.0	0.197
	160/80/160	27	41.0 ± 1.0	0.828	26	34.1 ± 0.6	0.637
	240/120/240	25	39.4 ± 0.8	0.119	26	33.4 ± 0.9	0.374
25	0/0/0	25	42.8 ± 0.8		26	35.1 ± 1.0	
	80/40/80	24	40.6 ± 1.0	0.281	26	33.4 ± 1.0	0.413
	160/80/160	27	41.5 ± 1.0	0.785	26	34.4 ± 0.7	0.792
	240/120/240	25	40.1 ± 0.8	0.149	26	33.7 ± 0.9	0.556
26	0/0/0	25	43.4 ± 0.7		26	35.3 ± 1.1	
	80/40/80	24	41.2 ± 1.0	0.290	26	33.9 ± 1.0	0.583
	160/80/160	27	42.1 ± 1.0	0.772	26	34.6 ± 0.7	0.821
	240/120/240	25	40.8 ± 0.9	0.176	26	34.2 ± 0.9	0.737
27	0/0/0	25	44.0 ± 0.8		25	36.4 ± 1.0	
	80/40/80	24	41.7 ± 1.0	0.245	26	34.3 ± 1.0	0.446
	160/80/160	27	42.4 ± 1.0	0.593	25	35.3 ± 0.6	0.666
	240/120/240	25	41.1 ± 0.9	0.096	26	34.7 ± 1.0	0.652
28	0/0/0	25	44.4 ± 0.7		25	36.8 ± 1.0	
	80/40/80	24	42.3 ± 1.1	0.273	26	34.7 ± 1.1	0.405
	160/80/160	27	42.9 ± 1.0	0.601	25	35.7 ± 0.6	0.696
	240/120/240	25	41.5 ± 0.8	0.093	26	35.1 ± 1.0	0.617

TABLE D6
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
29	0/0/0	25	44.9 ± 0.7		25	37.0 ± 1.0	
	80/40/80	24	42.9 ± 1.0	0.343	26	35.2 ± 1.1	0.567
	160/80/160	27	43.3 ± 1.0	0.544	25	36.0 ± 0.6	0.759
	240/120/240	25	42.0 ± 0.8	0.089	26	35.3 ± 1.0	0.618
30	0/0/0	25	45.3 ± 0.7		25	37.9 ± 1.1	
	80/40/80	24	43.1 ± 1.0	0.255	26	35.5 ± 1.1	0.354
	160/80/160	27	43.6 ± 1.0	0.474	25	36.6 ± 0.6	0.687
	240/120/240	25	42.3 ± 0.9	0.083	26	35.8 ± 1.0	0.444
31	0/0/0	25	45.5 ± 0.7		25	38.1 ± 1.1	
	80/40/80	24	43.3 ± 1.1	0.268	25	35.8 ± 1.2	0.294
	160/80/160	27	43.9 ± 1.0	0.537	25	36.5 ± 0.6	0.504
	240/120/240	25	42.4 ± 0.8	0.062	26	36.1 ± 1.0	0.481
32	0/0/0	25	45.3 ± 0.7		25	38.6 ± 1.0	
	80/40/80	23	43.8 ± 1.1	0.495	25	36.3 ± 1.1	0.272
	160/80/160	27	44.1 ± 1.0	0.787	25	37.2 ± 0.6	0.576
	240/120/240	25	42.6 ± 0.9	0.135	26	36.6 ± 1.0	0.408
33	0/0/0	24	45.5 ± 0.7		25	38.6 ± 1.1	
	80/40/80	23	44.1 ± 1.1	0.708	25	36.7 ± 1.2	0.451
	160/80/160	27	44.5 ± 1.0	0.958	25	37.7 ± 0.6	0.837
	240/120/240	24	43.1 ± 0.9	0.242	26	36.6 ± 1.1	0.465
34	0/0/0	24	45.8 ± 0.7		25	39.2 ± 1.2	
	80/40/80	23	44.5 ± 1.1	0.675	25	36.4 ± 1.3	0.209
	160/80/160	27	44.7 ± 1.0	0.890	25	38.0 ± 0.6	0.759
	240/120/240	24	43.5 ± 0.9	0.231	26	36.8 ± 1.1	0.344
35	0/0/0	24	46.4 ± 0.7		25	39.6 ± 1.1	
	80/40/80	23	45.0 ± 1.1	0.654	25	36.7 ± 1.3	0.189
	160/80/160	27	45.2 ± 1.0	0.859	25	38.7 ± 0.6	0.881
	240/120/240	24	43.8 ± 0.9	0.151	25	37.1 ± 1.2	0.254
36	0/0/0	24	46.9 ± 0.6		25	39.9 ± 1.2	
	80/40/80	23	45.1 ± 1.1	0.429	25	37.0 ± 1.3	0.172
	160/80/160	27	45.4 ± 0.9	0.635	25	39.1 ± 0.7	0.900
	240/120/240	24	44.2 ± 0.8	0.105	25	37.5 ± 1.1	0.293
37	0/0/0	24	47.2 ± 0.6		25	40.2 ± 1.3	
	80/40/80	23	45.2 ± 1.1	0.288	25	37.2 ± 1.3	0.168
	160/80/160	27	45.7 ± 0.9	0.636	25	39.2 ± 0.6	0.864
	240/120/240	24	44.7 ± 0.8	0.153	25	37.7 ± 1.1	0.277

TABLE D6
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
38	0/0/0	24	47.5 ± 0.6		25	40.7 ± 1.3	
	80/40/80	22	45.5 ± 1.1	0.302	25	37.6 ± 1.3	0.162
	160/80/160	27	46.0 ± 0.9	0.637	25	39.4 ± 0.7	0.743
	240/120/240	24	44.9 ± 0.9	0.150	25	38.0 ± 1.2	0.221
39	0/0/0	24	47.9 ± 0.6		24	41.6 ± 1.3	
	80/40/80	22	45.9 ± 1.1	0.296	25	38.3 ± 1.3	0.191
	160/80/160	27	46.1 ± 0.9	0.448	25	39.8 ± 0.7	0.709
	240/120/240	24	45.3 ± 0.9	0.139	25	38.3 ± 1.2	0.174
40	0/0/0	24	48.0 ± 0.6		24	41.7 ± 1.1	
	80/40/80	22	46.3 ± 1.2	0.373	25	38.2 ± 1.4	0.115
	160/80/160	27	46.4 ± 0.9	0.497	25	40.1 ± 0.6	0.739
	240/120/240	24	45.6 ± 0.8	0.155	23	39.3 ± 1.2	0.195
41	0/0/0	24	48.4 ± 0.6		24	42.3 ± 1.2	
	80/40/80	22	46.3 ± 1.1	0.229	24	38.1 ± 1.3	0.047
	160/80/160	27	46.6 ± 0.9	0.376	25	40.8 ± 0.6	0.773
	240/120/240	22	45.9 ± 0.8	0.140	22	39.3 ± 1.3	0.109
42	0/0/0	24	48.5 ± 0.6		23	42.6 ± 1.3	
	80/40/80	22	46.4 ± 1.1	0.205	24	38.4 ± 1.5	0.091
	160/80/160	27	47.0 ± 0.9	0.499	25	40.9 ± 0.8	0.812
	240/120/240	22	45.9 ± 0.8	0.117	21	39.9 ± 1.2	0.191
43	0/0/0	24	48.9 ± 0.6		23	43.2 ± 1.3	
	80/40/80	22	46.5 ± 1.1	0.164	24	38.4 ± 1.5	0.039
	160/80/160	27	47.2 ± 0.9	0.465	24	40.6 ± 0.8	0.462
	240/120/240	22	46.2 ± 0.8	0.125	21	40.5 ± 1.3	0.202
44	0/0/0	24	49.2 ± 0.5		23	43.6 ± 1.3	
	80/40/80	22	46.5 ± 1.2	0.077	24	38.5 ± 1.5	0.019
	160/80/160	27	47.1 ± 0.9	0.240	24	40.9 ± 0.7	0.411
	240/120/240	22	46.4 ± 0.8	0.093	21	40.7 ± 1.3	0.167
45	0/0/0	24	49.2 ± 0.5		23	43.3 ± 1.3	
	80/40/80	22	46.7 ± 1.1	0.127	23	39.7 ± 1.6	0.105
	160/80/160	27	46.9 ± 1.0	0.199	24	41.2 ± 0.7	0.623
	240/120/240	22	46.3 ± 0.9	0.091	21	41.1 ± 1.3	0.385

[a] Mean ± standard error
[b] Probability of significant difference from 0/0/0 mg/kg group by a Dunnett's test using contrasts; significant at P≤0.05

TABLE D7
Tests of Main Effects and Interactions for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

Source of Variation	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	P Value[b]
Male				
Postnatal week	43	1,939	539.9	0.000
Dose	1	46	5.7	0.022
Postnatal week × dose	43	1,939	1.2	0.205
Female				
Postnatal week	43	2,095	232.8	0.000
Dose	1	50	2.9	0.097
Postnatal week × dose	43	2,095	1.3	0.071

[a] Statistical analysis by repeated measures ANOVA
[b] Probability of significant main effect (postnatal week and dose) or significant interaction (postnatal week × dose); significant at P≤0.05

TABLE D8
Overall Trend Tests for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

Contrast of Interest	Estimate	Standard Error	Degrees of Freedom	t Value	P Value[b]
Male					
AZT dose; linear trend	–1.3	0.55	46	–2.4	0.022
Female					
AZT dose; linear trend	–1.2	0.69	50	–1.7	0.097

[a] Statistical analysis by repeated measures ANOVA
[b] Probability of significant linear trend using contrasts; significant at P≤0.05

TABLE D9
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)[a]	P Value[b]	n	Body Weight (g)	P Value
2	0/0	24	8.1 ± 0.2		26	8.1 ± 0.2	
	240/40	24	7.6 ± 0.2	0.133	26	7.4 ± 0.3	0.033
3	0/0	24	10.2 ± 0.2		26	10.2 ± 0.2	
	240/40	24	9.6 ± 0.3	0.099	26	9.1 ± 0.3	0.009
4	0/0	24	15.1 ± 0.3		26	14.2 ± 0.2	
	240/40	24	14.4 ± 0.4	0.191	26	13.1 ± 0.4	0.009
5	0/0	24	21.3 ± 0.4		26	19.0 ± 0.3	
	240/40	24	21.0 ± 0.5	0.671	26	17.9 ± 0.4	0.033
6	0/0	24	24.4 ± 0.2		26	21.2 ± 0.3	
	240/40	24	23.7 ± 0.4	0.093	26	20.3 ± 0.4	0.058
7	0/0	24	26.8 ± 0.3		26	22.3 ± 0.2	
	240/40	24	25.8 ± 0.4	0.031	26	21.4 ± 0.4	0.040
8	0/0	24	28.4 ± 0.3		26	23.2 ± 0.3	
	240/40	24	27.4 ± 0.4	0.045	26	22.8 ± 0.4	0.346
9	0/0	24	30.2 ± 0.3		26	24.5 ± 0.3	
	240/40	24	28.8 ± 0.5	0.012	26	23.9 ± 0.5	0.293
10	0/0	24	30.5 ± 0.4		26	25.3 ± 0.4	
	240/40	24	29.7 ± 0.5	0.248	26	24.4 ± 0.5	0.147
11	0/0	24	31.3 ± 0.4		26	25.9 ± 0.3	
	240/40	24	30.6 ± 0.5	0.249	26	25.4 ± 0.5	0.404
12	0/0	24	32.4 ± 0.4		26	26.5 ± 0.4	
	240/40	24	30.9 ± 0.6	0.046	26	25.6 ± 0.6	0.224
13	0/0	24	32.8 ± 0.4		26	26.3 ± 0.3	
	240/40	24	32.2 ± 0.6	0.433	26	26.2 ± 0.6	1.000
14	0/0	24	34.6 ± 0.5		26	28.0 ± 0.4	
	240/40	24	33.5 ± 0.7	0.212	26	27.6 ± 0.6	0.515
15	0/0	24	36.2 ± 0.6		26	28.8 ± 0.5	
	240/40	24	34.8 ± 0.7	0.125	26	28.7 ± 0.6	1.000
16	0/0	24	36.7 ± 0.5		26	29.0 ± 0.6	
	240/40	24	35.8 ± 0.8	0.345	26	28.7 ± 0.7	0.794
17	0/0	24	37.8 ± 0.5		26	30.1 ± 0.7	
	240/40	24	37.1 ± 0.8	0.496	26	28.8 ± 0.7	0.181
18	0/0	24	39.0 ± 0.6		26	30.6 ± 0.7	
	240/40	24	37.6 ± 0.9	0.196	26	30.1 ± 0.8	0.597

TABLE D9
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
19	0/0	24	39.9 ± 0.5		26	31.1 ± 0.7	
	240/40	24	38.9 ± 0.9	0.350	26	30.8 ± 0.8	1.000
20	0/0	24	40.9 ± 0.5		26	32.0 ± 0.8	
	240/40	24	39.9 ± 0.9	0.356	26	31.6 ± 0.8	0.733
21	0/0	24	41.3 ± 0.6		26	32.7 ± 0.7	
	240/40	24	40.6 ± 0.9	0.502	26	32.1 ± 0.8	0.565
22	0/0	24	42.6 ± 0.5		26	33.3 ± 0.8	
	240/40	24	41.4 ± 1.0	0.294	26	32.9 ± 0.8	0.683
23	0/0	24	44.0 ± 0.6		26	33.9 ± 0.9	
	240/40	24	42.6 ± 0.9	0.223	26	33.2 ± 0.8	0.552
24	0/0	24	44.1 ± 0.7		26	35.1 ± 0.9	
	240/40	19	42.5 ± 1.1	0.253	21	33.3 ± 1.0	0.209
25	0/0	24	45.2 ± 0.6		26	35.3 ± 0.9	
	240/40	24	43.7 ± 1.0	0.193	26	34.4 ± 0.9	0.498
26	0/0	24	45.9 ± 0.6		26	35.1 ± 0.9	
	240/40	24	44.6 ± 0.9	0.237	26	35.4 ± 0.9	0.795
27	0/0	24	46.5 ± 0.6		26	35.9 ± 0.9	
	240/40	24	44.8 ± 1.0	0.138	26	36.0 ± 1.1	1.000
28	0/0	24	47.2 ± 0.5		26	36.7 ± 1.0	
	240/40	24	45.5 ± 0.9	0.118	26	35.4 ± 1.0	0.347
29	0/0	24	47.5 ± 0.6		26	36.8 ± 1.0	
	240/40	24	45.7 ± 0.9	0.113	26	35.9 ± 1.0	0.521
30	0/0	24	47.9 ± 0.7		26	37.7 ± 1.0	
	240/40	24	46.6 ± 0.8	0.232	26	37.1 ± 1.1	0.715
31	0/0	24	48.4 ± 0.8		26	38.6 ± 1.0	
	240/40	24	47.0 ± 0.8	0.222	26	37.5 ± 1.0	0.440
32	0/0	24	48.5 ± 0.9		26	39.6 ± 1.1	
	240/40	24	47.3 ± 0.8	0.324	26	37.7 ± 1.1	0.222
33	0/0	24	48.6 ± 1.1		26	39.8 ± 1.0	
	240/40	24	47.7 ± 0.8	0.495	25	38.8 ± 1.0	0.329
34	0/0	23	50.0 ± 0.5		26	40.6 ± 1.1	
	240/40	24	47.6 ± 0.8	0.055	25	39.6 ± 1.0	0.342
35	0/0	23	50.3 ± 0.5		26	41.3 ± 1.0	
	240/40	24	48.0 ± 0.8	0.062	25	39.6 ± 1.2	0.184

TABLE D9
Summary Statistics by Sex, Time, and Dose for Body Weights of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

		Male			Female		
Week	AZT Dose (mg/kg)	n	Body Weight (g)	P Value	n	Body Weight (g)	P Value
36	0/0	23	50.7 ± 0.5		26	41.6 ± 1.1	
	240/40	24	48.3 ± 0.9	0.060	25	39.6 ± 1.2	0.151
37	0/0	23	51.0 ± 0.4		25	41.7 ± 1.2	
	240/40	24	48.2 ± 0.8	0.013	24	41.0 ± 1.1	0.331
38	0/0	23	51.1 ± 0.4		25	42.0 ± 1.1	
	240/40	24	48.2 ± 0.9	0.011	24	40.1 ± 1.2	0.085
39	0/0	23	51.1 ± 0.5		25	42.0 ± 1.2	
	240/40	24	48.8 ± 0.9	0.069	24	40.7 ± 1.2	0.210
40	0/0	23	51.5 ± 0.5		25	43.0 ± 1.2	
	240/40	23	49.2 ± 0.9	0.075	24	40.9 ± 1.1	0.088
41	0/0	23	51.7 ± 0.5		25	43.1 ± 1.2	
	240/40	23	49.0 ± 0.9	0.027	24	40.9 ± 1.2	0.094
42	0/0	23	51.4 ± 0.5		25	43.6 ± 1.2	
	240/40	23	49.0 ± 0.8	0.037	23	41.1 ± 1.2	0.056
43	0/0	23	51.8 ± 0.5		25	43.9 ± 1.1	
	240/40	22	50.0 ± 0.9	0.142	23	41.9 ± 1.3	0.120
44	0/0	23	52.1 ± 0.6		25	44.2 ± 1.2	
	240/40	22	50.0 ± 0.9	0.065	23	42.4 ± 1.2	0.154
45	0/0	18	52.1 ± 0.7		17	45.4 ± 1.5	
	240/40	14	49.0 ± 1.1	0.106	16	41.3 ± 1.5	0.207

[a] Mean ± standard error. Statistical tests were performed on rounded data.
[b] Probability of significant difference from 0/0 mg/kg group by a *t*-test; significant at P≤0.05

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

TABLE E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	240/120/240 mg/kg
Male		
n	27	21
Necropsy body wt	42.3 ± 0.7	38.8 ± 1.0**
Heart		
Absolute	0.210 ± 0.007[b]	0.198 ± 0.006
Relative	4.799 ± 0.233[b]	5.107 ± 0.137
L. and R. Kidney		
Absolute	0.608 ± 0.012	0.552 ± 0.016**
Relative	14.404 ± 0.226	14.309 ± 0.522
Liver		
Absolute	1.562 ± 0.041	1.426 ± 0.038*
Relative	36.854 ± 0.539	36.668 ± 0.470
Lung		
Absolute	0.260 ± 0.010	0.237 ± 0.012
Relative	6.183 ± 0.224	6.143 ± 0.317
Female		
n	24	26
Necropsy body wt	33.8 ± 1.0	32.5 ± 0.8
Heart		
Absolute	0.172 ± 0.007	0.165 ± 0.004
Relative	5.229 ± 0.334	5.131 ± 0.160
L. and R. Kidney		
Absolute	0.402 ± 0.006	0.369 ± 0.007**
Relative	12.054 ± 0.298	11.463 ± 0.252
Liver		
Absolute	1.250 ± 0.030	1.253 ± 0.036
Relative	37.562 ± 1.254	38.790 ± 1.059
Lung		
Absolute	0.297 ± 0.043	0.232 ± 0.010
Relative	9.266 ± 1.717	7.248 ± 0.349

* Significantly different (P≤0.05) from the 0/0/0 mg/kg group by a *t*-test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight-ratios (relative weight are given as mg organ weight/g body weight (mean ± standard error).
[b] n=26

TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Male				
n	24	21	27	22
Necropsy body wt	44.4 ± 0.5	42.6 ± 1.0	42.3 ± 1.0	42.2 ± 0.8
Brain				
Absolute	0.494 ± 0.004	0.479 ± 0.004	0.486 ± 0.005	0.471 ± 0.006*
Relative	11.14 ± 0.10	11.37 ± 0.31	11.63 ± 0.29	11.21 ± 0.20
Heart				
Absolute	0.227 ± 0.007	0.209 ± 0.005	0.222 ± 0.007	0.222 ± 0.008
Relative	5.12 ± 0.15	4.93 ± 0.11	5.26 ± 0.13	5.24 ± 0.14
L. and R. Kidney				
Absolute	0.714 ± 0.012	0.666 ± 0.017	0.691 ± 0.018	0.657 ± 0.019
Relative	16.10 ± 0.23	15.67 ± 0.28	16.33 ± 0.25	15.53 ± 0.26
Liver				
Absolute	1.962 ± 0.054	1.987 ± 0.088	2.013 ± 0.086	2.022 ± 0.124
Relative	44.11 ± 0.92	46.22 ± 1.12	47.17 ± 1.25	47.49 ± 2.48
Female				
n	23	23	24	21
Necropsy body wt	40.1 ± 1.2	36.3 ± 1.5	37.9 ± 0.8	37.7 ± 1.3
Brain				
Absolute	0.515 ± 0.004	0.494 ± 0.006*	0.496 ± 0.003*	0.491 ± 0.004*
Relative	13.12 ± 0.43	14.09 ± 0.57	13.23 ± 0.29	13.32 ± 0.50
Heart				
Absolute	0.164 ± 0.004	0.168 ± 0.008	0.168 ± 0.006	0.162 ± 0.005
Relative	4.15 ± 0.13	4.78 ± 0.28	4.49 ± 0.20	4.36 ± 0.16
L. and R. Kidney				
Absolute	0.463 ± 0.008	0.441 ± 0.011	0.444 ± 0.007[b]	0.445 ± 0.008
Relative	11.70 ± 0.25	12.42 ± 0.41	11.79 ± 0.26[b]	11.98 ± 0.34
Liver				
Absolute	1.499 ± 0.041	1.441 ± 0.046	1.508 ± 0.028	1.496 ± 0.042
Relative	37.61 ± 0.67	40.29 ± 1.02	40.12 ± 0.95	40.03 ± 0.92

* Significantly different (P≤0.05) from the 0/0/0 mg/kg group by Dunnett's test

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight-ratios (relative weight are given as mg organ weight/g body weight (mean ± standard error).

[b] n=23

TABLE E3
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

	0/0 mg/kg	240/40 mg/kg
Male		
n	23	22
Necropsy body wt	46.9 ± 0.5	44.7 ± 0.8*
Brain		
Absolute	0.509 ± 0.004	0.489 ± 0.005**
Relative	10.886 ± 0.126	10.997 ± 0.188
Heart		
Absolute	0.228 ± 0.006	0.227 ± 0.009
Relative	4.871 ± 0.124	5.064 ± 0.171
L. and R. Kidney		
Absolute	0.772 ± 0.017	0.713 ± 0.019*
Relative	16.503 ± 0.401	15.954 ± 0.319
Liver		
Absolute	2.297 ± 0.066	2.362 ± 0.148
Relative	49.050 ± 1.399	52.401 ± 2.845
Female		
n	25	23
Necropsy body wt	40.464 ± 1.119	38.748 ± 1.216*
Brain		
Absolute	0.533 ± 0.006	0.502 ± 0.006**
Relative	13.439 ± 0.424	13.199 ± 0.387
Heart		
Absolute	0.186 ± 0.006	0.171 ± 0.005*
Relative	4.678 ± 0.181	4.467 ± 0.147
L. and R. Kidney		
Absolute	0.474 ± 0.008	0.451 ± 0.011
Relative	11.858 ± 0.268	11.765 ± 0.302
Liver		
Absolute	1.567 ± 0.042	1.687 ± 0.180
Relative	38.983 ± 0.834	44.225 ± 5.222

* Significantly different (P≤0.05) from the 0/0 mg/kg group by a *t*-test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight-ratios (relative weight are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

AZT

3′-Azido-3′-deoxythymidine (AZT) was obtained from Cipla Ltd., Mumbai Central (Mumbai, India) in one lot (FX4159) used in the 30- and 45-week studies and the 45-week stop-study. 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). Reports on analyses performed in support of the AZT studies are on file at the NCTR.

Lot FX4159 of the chemical, a white crystalline powder, was identified as AZT by the study laboratory using proton nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe-electron ionization mass spectrometry, and high-performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry. All spectra were consistent with literature spectra and the structure of AZT. A representative proton NMR spectrum is presented in Figure F1. The melting point range of lot FX4159 was determined to be 122.5° to 123.4° C by Galbraith Laboratories, Inc.; these values were consistent with those reported in the literature for AZT crystallized from water (*Merck*, 2006).

Karl Fischer titration and elemental analyses of lot FX4159 were performed by Galbraith Laboratories, Inc., and the study laboratory determined the purity of the bulk chemical by HPLC. HPLC was conducted with a Waters Millennium® system using photodiode array detection at 254 nm (Waters Corporation, Milford, MA). The analytical column was a Nova-Pak® (3.9 mm × 150 mm, 4 µm particle size) C18 column (Waters Corporation). The mobile phase (flow rate 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.

For lot FX4159, Karl Fischer titration indicated less than 0.46% water. Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were in agreement with the theoretical values for AZT. HPLC detected no impurity peaks by comparisons to spectra from previously characterized AZT standards, and the purity of lot FX4159 was determined to be 100% under the conditions of the assay.

Dosing Vehicle

The vehicle used for dose formulations in the 30- and 45-week studies and the 45-week stop-study was a 0.2% methylcellulose/0.1% Tween® 80 aqueous solution. Methylcellulose was obtained from Sigma-Aldrich® Corporation (St. Louis, MO) in one lot (014K0081). Identity studies of lot 014K0081 were performed by the study laboratory using proton and carbon-13 NMR spectroscopy; the results of these analyses were consistent with those obtained previously for a methylcellulose standard obtained from Fischer Scientific (Pittsburgh, PA). Tween® 80 was obtained from Sigma-Aldrich® Corporation in one lot (073K00641).

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing AZT with the dosing vehicle to give the required concentrations (Table F1). The dose formulations were stored at room temperature in sealed amber glass bottles for up to 29 days.

Stability studies of 0.05 and 0.20 mg/mL formulations were performed by the study laboratory with HPLC using the same Waters chromatography equipment utilized for determination of test chemical purity. For the stability studies however, two mobile phases were used: mobile phase A consisted of 5% methanol:95% water, and mobile phase B consisted of 90% methanol:10% water; both mobile phases contained 0.005 M sodium phosphate monobasic and 0.003 M sodium pentanesulfonic acid and were adjusted to pH 2.5 with phosphoric acid. The HPLC solvent program (flow rate 1.0 mL/minute) was a linear gradient from 100% A to 100% B in 3 minutes, followed by a 7.5-minute hold. Stability was confirmed for at least 29 days for formulations stored in sealed amber glass bottles at room temperature.

Periodic analyses of the dose formulations of AZT were conducted by the study laboratory using the HPLC system utilized for the dose formulation stability studies; accuracy of dose delivery from the dosing apparatus was also periodically determined using this HPLC system. Of the dose formulations analyzed and used during the studies, 209 of 211 were within 10% of the target concentrations (Table F2). For the dose accuracy of delivered doses, 17 of the 20 samples analyzed were within 10% of the target doses (Table F3).

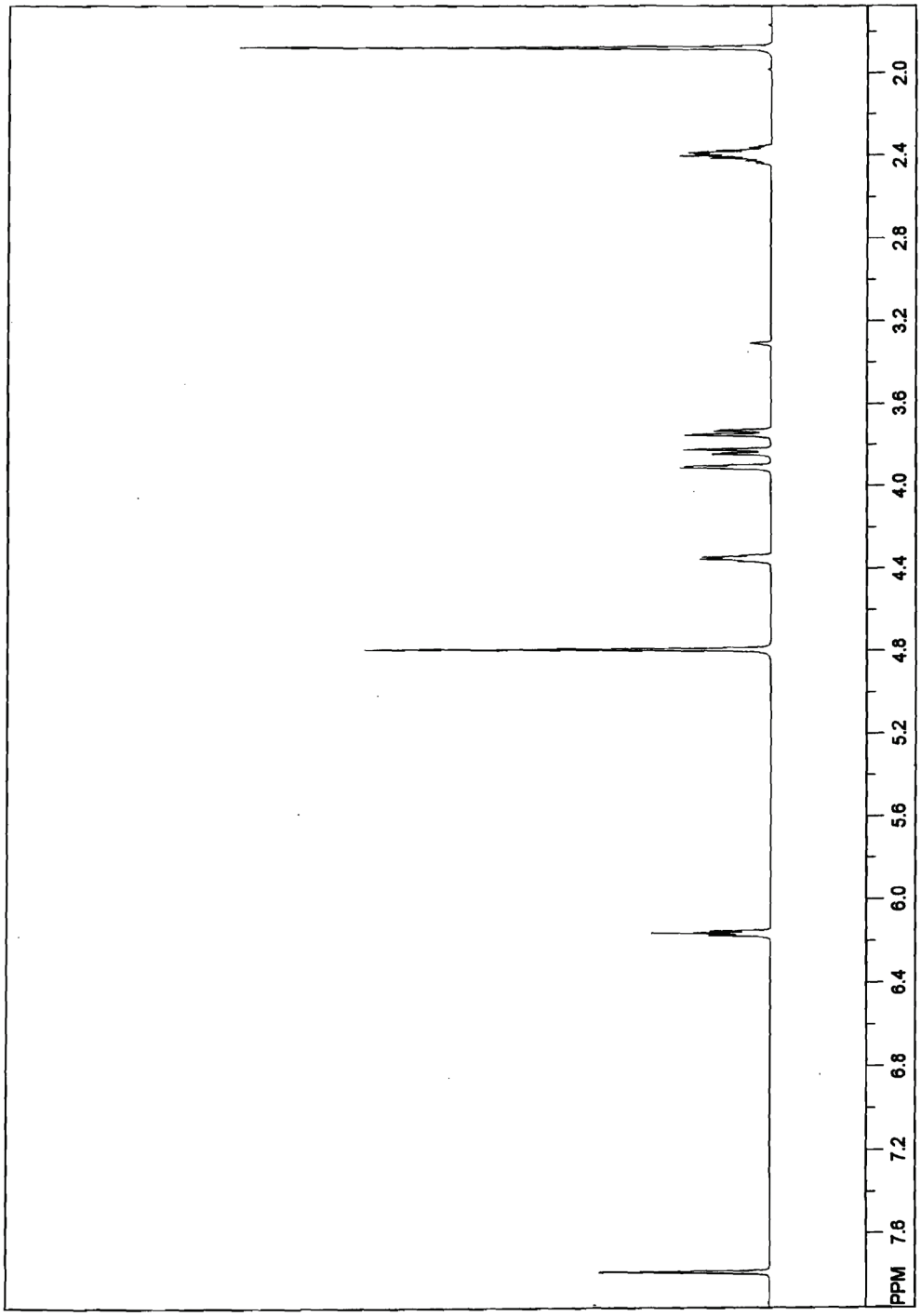

FIGURE F1
Proton Nuclear Magnetic Resonance Spectrum of AZT

TABLE F1
Preparation and Storage of Dose Formulations in the *In Utero*/Postnatal Gavage Studies of AZT

Preparation
AZT was added to an aqueous solution of 0.2% methylcellulose and 0.1% Tween® 80 and stirred with a magnetic stir bar to form a suspension; suspensions were then filtered through a 0.2 µm filter. The dose formulations were prepared approximately weekly.

Chemical Lot Number
FX4159

Maximum Storage Time
29 days

Storage Conditions
Stored 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 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference From Target (%)
November 29, 2004	8	8.08 ± 0.10	+1
	16	15.9 ± 0.8	–1
	24	22.9 ± 0.2	–5
December 06, 2004	8	8.78 ± 0.11	+10
	16	17.4 ± 0.1	+9
	24	24.1 ± 0.2	0
December 20, 2004	8	7.92 ± 0.10	–1
	16	16.5 ± 0.3	+3
	24	23.7 ± 0.1	–1
December 27, 2004	8	7.98 ± 0.06	0
	8	7.96 ± 0.03	0
	16	16.7 ± 0.1	+4
	16	16.7 ± 0.2	+4
	24	23.6 ± 0.1	–2
December 29, 2004	8	7.96 ± 0.22	0
	16	16.5 ± 0.1	+3
	24	23.5 ± 0.3	–2
January 05, 2005	8	7.85 ± 0.19	–2
	16	16.0 ± 0.4	0
	24	23.4 ± 0.3	–2
January 11, 2005	8	7.72 ± 0.02	–3
	16	15.8 ± 0.3	–1
	24	22.9 ± 0.5	–5
January 12, 2005	8	7.83 ± 0.20	–2
	16	16.6 ± 0.1[b]	+4
	24	23.3 ± 0.1	–3
January 19, 2005	8	8.17 ± 0.04	+2
	16	16.5 ± 0.2	+3
	24	23.4 ± 0.3	–3
January 25, 2005	8	8.21 ± 0.07	+3
	16	16.6 ± 0.2	+4
	24	23.4 ± 0.1	–3
January 28, 2005	16	17.1 ± 0.3	+7
	24	23.9 ± 0.2	0
February 01, 2005	8	8.17 ± 0.12	+2
	16	16.6 ± 0.2[c]	+2
	24	22.1 ± 0.4	–8

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference From Target (%)
February 07, 2005	8	8.50 ± 0.13	+6
	16	16.1 ± 0.3	+1
	24	24.0 ± 1.2	0
February 14, 2005	8	8.16 ± 0.13	+2
	16	16.4 ± 0.1	+2
	24	24.4 ± 0.1	+2
February 18, 2005	8	8.21 ± 0.09	+3
	16	16.7 ± 0.3	+4
	24	24.7 ± 0.4	+3
February 23, 2005	8	8.11 ± 0.28	+1
	16	16.7 ± 0.1	+5
	24	24.7 ± 0.4	+3
March 01, 2005	8	8.38 ± 0.14	+5
	16	17.1 ± 0.3	+7
	24	24.9 ± 0.1	+4
March 03, 2005	8	8.30 ± 0.04	+4
	16	17.2 ± 0.2	+7
	24	24.2 ± 0.8	+1
March 08, 2005	8	8.08 ± 0.14	+1
	16	16.5 ± 0.6	+3
	24	24.9 ± 0.1	+4
March 14, 2005	8	8.32 ± 0.22	+4
	16	17.2 ± 0.6	+8
	24	24.7 ± 0.9	+3
March 18, 2005	8	8.22 ± 0.10	+3
	16	16.8 ± 0.2	+5
	24	24.6 ± 0.4	+2
March 22, 2005	8	8.49 ± 0.13	+6
	16	16.3 ± 0.1	+2
	24	22.6 ± 1.4	–6
March 29, 2005	24	23.2 ± 0.2	–2
March 31, 2005	8	8.28 ± 0.10	+3
	16	16.0 ± 0.2	0
	24	22.3 ± 0.4	–7
April 05, 2005	24	23.4 ± 0.0	–2
April 08, 2005	8	8.14 ± 0.16	+2
	16	16.2 ± 0.2	+1
	24	23.2 ± 0.3	–3

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference From Target (%)
April 13, 2005	8	8.51 ± 0.10	+6
	16	16.4 ± 0.1	+2
	24	23.3 ± 0.1	–3
April 18, 2005	8	8.27 ± 0.20	+3
	16	15.4 ± 0.8	–4
	24	23.0 ± 0.5	–4
April 26, 2005	8	8.53 ± 0.09	+7
	16	16.4 ± 0.2	+3
	24	23.3 ± 0.2	–3
	24	23.7 ± 0.2	–1
April 29, 2005	8	8.37 ± 0.06	+5
	16	16.4 ± 0.1	+2
May 04, 2005	8	8.49 ± 0.02	+6
	16	16.1 ± 0.2	+1
	24	23.1 ± 0.1	–4
May 10, 2005	8	8.51 ± 0.05	+6
	16	16.0 ± 0.2	0
	24	22.9 ± 0.4	–5
May 16, 2005	8	8.46 ± 0.18	+6
	16	13.9 ± 0.1	–13
	24	22.7 ± 0.0	–5
May 19, 2005	8	8.55 ± 0.29	+7
	16	16.7 ± 0.1	+4
	24	23.3 ± 0.3	–3
May 25, 2005	8	8.19 ± 0.05	+2
	16	16.1 ± 0.1	+1
	24	23.0 ± 0.1	–4
June 01, 2005	8	7.90 ± 0.15	–1
	16	16.2 ± 0.2	+1
	24	23.1 ± 0.1	–4
June 08, 2005	8	8.48 ± 0.08	+6
	16	16.6 ± 0.1	+4
	24	23.8 ± 0.3	–1
June 14, 2005	8	7.38 ± 0.04	–8
	16	14.3 ± 0.1	–10
	24	21.0 ± 0.0	–13
June 20, 2005	8	8.19 ± 0.08	+2
	16	16.4 ± 0.1	+2
	24	23.3 ± 0.1	–3

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference From Target (%)
June 22, 2005	8	7.84 ± 0.04	–2
	16	15.8 ± 0.1	–1
	24	22.5 ± 0.2	–6
June 30, 2005	8	8.44 ± 0.09	+6
	16	13.6 ± 0.2[b]	–15
	24	22.4 ± 0.4	–7
July 01, 2005	16	16.3 ± 0.5	+2
July 06, 2005	8	8.26 ± 0.02	+3
	16	15.5 ± 0.1	–3
	24	22.8 ± 0.2	–5
July 13, 2005	8	8.23 ± 0.06	+3
	16	15.5 ± 0.2	–3
	24	22.8 ± 0.1	–5
July 20, 2005	8	8.36 ± 0.09	+5
	16	15.5 ± 0.1	–3
	24	22.8 ± 0.2	–5
July 27, 2005	8	8.15 ± 0.06	+2
	16	14.8 ± 0.6	–8
	24	22.3 ± 0.9	–7
August 01, 2005	8	8.49 ± 0.13	+6
	16	16.1 ± 0.1	+1
	24	23.9 ± 0.3	0
August 02, 2005	16	15.6 ± 0.2	–3
	24	23.2 ± 0.2	–3
August 04, 2005	8	8.12 ± 0.06	+2
	16	15.6 ± 0.2	–2
	24	22.8 ± 0.4	–5
August 09, 2005	8	8.24 ± 0.04	+3
	16	15.5 ± 0.0	–3
	24	22.9 ± 0.1	–5
	24	22.9 ± 0.3	–5
	24	22.9 ± 0.2	–5
August 18, 2005	8	8.33 ± 0.11	+4
	16	15.4 ± 0.1	–3
	24	22.5 ± 0.3	–6
August 25, 2005	8	8.01 ± 0.13	0
	16	14.8 ± 0.4	–8
	24	22.9 ± 0.2	–5
August 31, 2005	8	8.02 ± 0.06	0
	16	15.3 ± 0.1	–4
	24	24.0 ± 0.2	0

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference From Target (%)
September 08, 2005	8	8.00 ± 0.11	0
	16	16.7 ± 0.1	+5
	16	16.8 ± 0.3	+5
	24	24.9 ± 0.2	+4
September 15, 2005	8	7.89 ± 0.22	–1
	16	16.8 ± 0.1	+5
	24	24.9 ± 0.2	+4
September 22, 2005	8	8.03 ± 0.16	0
	16	16.4 ± 1.1	+2
	24	24.2 ± 1.1	+1
September 30, 2005	8	8.27 ± 0.08	+3
	16	15.8 ± 0.0	–1
	24	23.1 ± 0.2	–4
October 03, 2005	8	8.49 ± 0.28	+6
	16	16.4 ± 0.0	+3
	24	24.3 ± 0.1	+1
October 06, 2005	8	8.57 ± 0.11	+7
	16	16.3 ± 0.1	+2
	24	23.7 ± 0.1	–1
October 12, 2005	8	8.05 ± 0.08	+1
	16	15.5 ± 0.1	–3
	24	22.7 ± 0.3	–5
October 19, 2005	8	8.38 ± 0.08	+5
	16	15.8 ± 0.1	–1
	24	23.3 ± 0.1	–3
October 26, 2005	8	8.10 ± 0.26	+1
	16	16.2 ± 0.4	+1
	24	23.4 ± 0.5	–2
November 02, 2005	8	8.25 ± 0.08	+3
	16	15.8 ± 0.3	–2
	24	22.8 ± 0.3	–5
November 09, 2005	8	8.10 ± 0.41	+1
	16	16.1 ± 0.2	+1
	24	23.5 ± 0.3	–2
November 16, 2005	8	8.36 ± 0.04	+5
	16	15.9 ± 0.1	0
	24	23.0 ± 0.4	–4
December 05, 2005	8	8.41 ± 0.17	+5
	16	16.3 ± 0.1	+2
	24	24.1 ± 0.1	0

TABLE F2
Results of Analyses of Dose Formulations Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference From Target (%)
December 12, 2005	8	8.29 ± 0.18	+4
	16	16.4 ± 0.2	+3
	24	23.9 ± 0.4	–1
December 15, 2005	8	8.38 ± 0.28	+5
	16	16.4 ± 0.3	+3
	24	23.5 ± 0.2	–2
December 21, 2005	8	8.35 ± 0.02	+4
	16	16.2 ± 0.4	+1
	24	21.8 ± 0.4	–9
December 26, 2005	8	8.25 ± 0.07	+3
	16	15.8 ± 0.3	–1
	24	23.2 ± 0.0	–3
January 10, 2006	16	15.8 ± 0.1	–1
January 18, 2006	16	16.8 ± 0.4	+5
	24	24.9 ± 0.6	+4
February 01, 2006	16	16.1 ± 0.2	0
February 15, 2006	16	14.6 ± 0.3	–9
February 21, 2006	16	15.5 ± 0.4	–3

[a] Results of triplicate analyses (mean ± standard deviation). For pups [postnatal days (PND) 1 to 10], dosing volume=5 mL/kg; 8 mg/mL=40 mg/kg, 16 mg/mL=80 mg/kg, 24 mg/mL=120 mg/kg. For dams (gestational days 12 to 18) and pups after PND 10, dosing volume=10 mL/kg; 8 mg/mL=80 mg/kg, 16 mg/mL=160 mg/kg, 24 mg/mL=240 mg/kg.
[b] Not used in the animal studies
[c] n=2

TABLE F3
Accuracy of Doses Administered to Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week, 45-Week, and 45-Week Stop-Exposure *In Utero*/Postnatal Gavage Studies of AZT

Date Analyzed	Target Dose (mg)	Actual Dose (mg)[a]	Difference From Target (%)
November 29, 2004	4.87	4.44 ± 0.09	–9
	9.67	8.92 ± 0.33	–8
December 06, 2004	0.48	0.47 ± 0.01	–2
	3.248	3.05 ± 0.10	–6
December 09, 2004	1.44	1.43 ± 0.03	–1
	7.20	6.89 ± 0.25	–4
January 11, 2005	1.44	1.36 ± 0.02	–5
	7.20	6.69 ± 0.10	–7
March 03, 2005	1.44	1.40 ± 0.05	–3
	7.20	6.75 ± 0.09	–6
March 16, 2005	1.44	1.44 ± 0.04	0
	7.20	6.98 ± 0.23	–3
April 29, 2005	1.44	1.37 ± 0.02	–5
	7.20	6.62 ± 0.07	–8
July 07, 2005	1.44	1.34 ± 0.04	–7
	7.20	6.43 ± 0.24	–11
September 14, 2005	7.20	6.20 ± 0.16	–14
September 21, 2005	7.20	6.80 ± 0.08	–5
December 09, 2005	7.20	6.44 ± 0.07	–10
January 25, 2006	7.20	7.02 ± 0.12	–2

[a] Results of triplicate analyses (mean ± standard deviation).

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

LITTER EFFECTS ON SURVIVAL, BODY WEIGHT, AND PATHOLOGY

BACKGROUND

The study design consisted of three different dosing regimens: (1) 30-week continuous dosing consisting of two dose groups (0/0/0 and 240/120/240 mg AZT/kg body weight per day); (2) 45-week continuous dosing consisting of four dose groups (0/0/0, 80/40/80, 160/80/160 and 240/120/240 mg/kg per day); and (3) 45-week stop-study (0/0 and 240/40 mg/kg per day). There were 24 to 27 animals per sex per 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 six females. The pregnant females were randomized to the treatment groups and heterozygous F1 p53$^{+/-}$ mice 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, except in the 45-week stop-study where pups received one-sixth of the maternal dose in half the dose volume. Pups from the same litters could be utilized for the 30- and 45-week continuous dosing studies, because they used the same dosing regimen. Different litters were used for the 45-week stop-study. Pups were weighed and dosed as a litter (i.e. each pup received a dose calculated from the total weight of the litter divided by the number of surviving pups in that litter) from PND 1 through PND 10 for the 30-week and 45-week continuous dosing studies and from PND 1 through PND 8 (with either vehicle or 40 mg/kg) for the 45-week stop-study. For the continuous dosing studies, the pups were individually weighed and administered the same adult dose as their mother from PND 11 onwards. Pups used for the 45-week stop-study were not dosed after PND 8.

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 evaluations (see Appendix B). In general, either one or two mice of each sex from the same litter were assigned to each study; however, in certain cases three or four pups had to be assigned in order to provide sufficient mice to complete the dose group. The litter and sire assignments of all pups assigned to the three studies 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 outcome of the survival, body weight, and pathology evaluations in the three studies.

METHODS

Survival

A proportional hazards model (Cox, 1972) was run by Sex and Group using Days-On-Study, the censor flag, and AZT dose as the predictor. For the 45-week continuous dosing 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 NTP convention of appending an "N" for negative dose effects.

Three sex × group × dose combinations had no dead/moribund events: 0/0/0 mg/kg males in the 30-week study, 240/120/240 mg/kg females in the 30-week study, and 160/80/160 mg/kg males in the 45-week study. To facilitate convergence for the analyses involving these combinations, an augmented dataset was created by adding a dummy event at the study termination time for each combination and weighting the observation as one-tenth of an observation. Actual observations were weighted as a full observation.

Body Weight

A repeated measures model with a heterogeneous autoregressive covariance structure was used for the analyses of body weight unadjusted for litter or sire (Appendix D). The repeated measures model refers to the fact that multiple observations were taken on the same animal over time and that the observations for this animal are likely to be more alike than those between two different animals. The design within dosing regimen and sex was a one-way repeated measures model with AZT dose as the fixed effect and time as the repeated measures variable. Testing for an overall linear or quadratic dose trend was accomplished using contrasts. Dunnett's tests (Dunnett, 1955) on AZT dose were computed for each time point. These tests compared each dosed group with the vehicle control group and made an adjustment for the fact that several comparisons were being carried out concurrently. Summary statistics for the animals' body weights (in grams) are presented by sex, time (in weeks), and AZT dose (in order to compare dose effects) for each dosing regimen.

The body weight data for each animal were rasterized to evenly-spaced time points (every week) via 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.

This study needed to correct for possible correlation among littermates and it 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 curves: early growth rate (slope between 2 and 5 weeks for all studies) and late growth rate (slope between 38 and 44 weeks for the 45-week studies and slope between 23 and 29 weeks for the 30-week study). These analyses determined whether there were any treatment-related effects on early growth or late growth.

Dunnett's 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 incidence. For liver neoplasms in male mice, a k=5 analysis was run in addition to the standard k=3 analysis because comparison between the neoplasm incidences at 30 weeks and 45 weeks suggested a steeper tumor progression curve for heterozygous F1 $p53^{+/-}$ mice than for B6C3F1 mice that are used in 2-year carcinogenicity studies.

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. Beginning with a regression paradigm with binomial weights, this framework can be generalized and the Cochran-Armitage test can be viewed as a generalized linear model (GLIM) with binomial variation and an identity link function. If this analysis is performed with Poly-k

weights, 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 GLIM described above with estimation using generalized estimating equations (GEE) (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 appended 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 incidences and the nonzero severity score averages were computed. The cluster-adjusted models were 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 the vehicle controls (JT/SW). No attempt was made to adjust JT/SW for correlation among clusters.

RESULTS

Survival

In the 30-week study, dosed male mice exhibited significantly greater hazard compared to the 0/0/0 mg/kg group, indicated by five early deaths or moribund animals in the 240/120/240 mg/kg group relative to no early deaths or moribund animals in the 0/0/0 mg/kg group. Malignant lymphoma was present in three of the five early removals. Cluster analyses for litter or sire effects did not alter the empirical variance or its significance (Table G3).

There is no indication that AZT significantly increased the hazard in female mice dosed continuously with 240/120/240 mg/kg until 30 weeks, in male or female mice dosed continuously with 80/40/80, 160/80/160, or 240/120/240 mg/kg until 45 weeks, or in male or female mice dosed with 240/40 mg/kg until PND 8 and killed at 45 weeks. Cluster analyses for litter or sire effects did not alter the empirical variance or significance of any of these dose groups (Tables G3, G4, and G5).

Two AZT dose groups demonstrated significantly smaller hazard than the vehicle control group: 30-week study females administered 240/120/240 mg/kg (Table G3) and 45-week study males administered 160/80/160 mg/kg (Table G4). As before, this was due to the lack of failures in these AZT-dosed groups in this study and not to any other distinction. It seems unlikely that AZT serves any protective function and the result is more likely due to chance.

The linear trend tests performed for the 45-week study males were significant (Table G4). This was due to the zero failures in the 160/80/160 mg/kg males; if this cell is ignored then no significant trends are apparent. For the 45-week stop-study, there was no significant dose effect on survival in either male or female mice with or without adjustment for litter or sire clustering (Table G5).

The results tend to indicate that intralitter correlation with regards to survival is either not present or is negative. In any case, merely using the empirical variance estimator caused far more impact than the actual aggregation over dams (litters) or sires.

Body Weight

The unadjusted body weight tables are shown in Appendix D. The two adjusted analyses (data not shown) were generally identical to the unadjusted analysis (Appendix D).

Pathology

The following results refer to the litter-adjusted analyses unless otherwise stated. Liver hepatocellular adenoma was the only non-systemic neoplasm found to occur within male mice in sufficient quantity to justify analysis. Hepatocellular carcinoma was 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 neoplasm found within female mice in sufficient quantity to justify analysis was the systemic lesion malignant lymphoma.

Neoplasms

Generally, the adverse dose-response effects in males were not very notable with the exception of incidences of hepatocellular adenoma.

For males in the 45-week study, significant positive trends occurred in the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined), and the incidences of these lesions in the 240/120/240 mg/kg group were significantly increased relative to the 0/0/0 mg/kg group (Table G7). Using k=5 rather than k=3 for the Poly-k survival adjustment did not alter the significance of the tumor incidence increases. Incidences of benign neoplasms (in all organs) showed a significant positive dose trend but no individual dose group incidence differed significantly from that in the 0/0/0 mg/kg group. Adjusting the data for intralitter correlation did not substantially change the conclusions, and in these tumor pools, the estimated correlation was negative or nearly zero. P values were decreased slightly for the litter-adjusted and sire-adjusted Poly-3 tests relative to those of the naive Poly-3 test.

In the 30-week study, incidences (in all organs) of malignant lymphoma, malignant neoplasms, and benign and malignant neoplasms (combined) were significantly increased in 240/120/240 mg/kg males compared to those in the 0/0/0 mg/kg group (Table G6). The naive analysis did not find the incidence of malignant lymphoma to be significantly increased.

No significant dose effects were found in 45-week stop-study males (Table G8).

Generally, the adverse dose-response effects in females were not very notable. For females in the 45-week study, significant positive trends occurred in the incidences (in all organs) of malignant lymphoma and malignant neoplasms found (Table G10), and the incidence of malignant lymphoma in the 240/120/240 mg/kg group was significantly increased compared to that in the 0/0/0 mg/kg group. Using the naive analysis, there was no significant trend in the incidences of malignant neoplasms and, although there was a positive trend in the incidences of malignant lymphoma, the incidence in the 240/120/240 mg/kg group did not differ from that in the 0/0/0 mg/kg group.

No significant dose effects were found in females in the 30-week study (Table G9) or 45-week stop-study (Table G11).

Nonneoplastic Lesions

In general, AZT administration significantly increased the incidence or severity of few nonneoplastic lesions when analyzed by naive Poly-3 tests not adjusting for litter or sire correlation. Adjusting for either litter or sire increased the number of significantly increased nonneoplastic lesion incidences.

In males, positive dose effects were found in the 45-week study in the incidences of preputial gland degeneration and hyperplasia of the cecum when adjusted for litter or sire effects but not with the naive Poly-3 analysis (Table G13). The sire-adjusted analysis also showed the incidence of cellular infiltration of the kidney in 45-week 80/40/80 mg/kg males to be significantly increased, although this result was inconsistent with the (nonsignificant) dose trend in this study and with the significantly decreased incidence in the 30-week study (Table G12). There

were several other decreases in the incidences of nonneoplastic lesions, which differed in significance between the naive and adjusted evaluations; details appear in Tables G12 through G14. Generally, the adverse dose responses were not very notable.

In females, the only positive dose effects found were in the 45-week study, including increased incidences of cerebral mineralization, myeloid cell bone marrow hyperplasia, cellular infiltration of the pancreas, and tension lipidosis of the liver (Table G16). These increases were significant when adjusted for litter or sire effects but not with the naive Poly-3 analysis. The only notable positive dose effect seen in 30-week study females was a significant increase in the litter-adjusted incidence of cecum hyperplasia (Table G15). There were several other decreases in the incidences of nonneoplastic lesions, which differed in significance between the naive and adjusted evaluations; details appear in Tables G15 through G17. Generally, the adverse dose responses were not very notable.

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. There appeared to be a slightly negative, rather than positive, correlation between littermates and pathologic 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 *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN[a]	Sire UIN[b]	Litter UIN[b]	Lesion[c]	Duration[d]	Dose[e]
426	7F000000316	7E000000019	7G000001650	Pc	1	0/0
578	7F000000316	7E000000019	7G000001650	L	1	0/0
598	7F000000315	7E000000019	7G000001700	LgA	1	0/0
597	7F000000315	7E000000019	7G000001700		1	0/0
625	7F000000346	7E000000019	7G000001782		1	0/0
618	7F000000346	7E000000019	7G000001782		1	0/0
575	7F000000309	7E000000033	7G000001635		1	0/0
574	7F000000309	7E000000033	7G000001635		1	0/0
599	7F000000310	7E000000033	7G000001721	L	1	0/0
600	7F000000310	7E000000033	7G000001721		1	0/0
629	7F000000351	7E000000033	7G000001818		1	0/0
628	7F000000351	7E000000033	7G000001818		1	0/0
587	7F000000327	7E000000034	7G000001666		1	0/0
588	7F000000327	7E000000034	7G000001666	LgA	1	0/0
576	7F000000320	7E000000037	7G000001642		1	0/0
577	7F000000320	7E000000037	7G000001642		1	0/0
626	7F000000326	7E000000046	7G000001796		1	0/0
585	7F000000325	7E000000049	7G000001659	L	1	0/0
586	7F000000325	7E000000049	7G000001659		1	0/0
635	7F000000345	7E000000052	7G000001849		1	0/0
647	7F000000322	7E000000053	7G000001856		1	0/0
648	7F000000322	7E000000053	7G000001856		1	0/0
652	7F000000360	7E000000055	7G000001880		1	0/0
653	7F000000360	7E000000055	7G000001880	HA	1	0/0
611	7F000000324	7E000000019	7G000001738		1	240/40
612	7F000000324	7E000000019	7G000001738		1	240/40
646	7F000000336	7E000000019	7G000001871		1	240/40
645	7F000000336	7E000000019	7G000001871	L	1	240/40
488	7F000000199	7E000000022	7G000001393		1	240/40
565	7F000000323	7E000000028	7G000001673	L	1	240/40
564	7F000000323	7E000000028	7G000001673		1	240/40
593	7F000000306	7E000000031	7G000001707	L	1	240/40
634	7F000000352	7E000000033	7G000001852	Lym	1	240/40
643	7F000000352	7E000000033	7G000001852	Lym	1	240/40
566	7F000000319	7E000000037	7G000001679		1	240/40
567	7F000000319	7E000000037	7G000001679		1	240/40
581	7F000000312	7E000000039	7G000001695		1	240/40
582	7F000000312	7E000000039	7G000001695		1	240/40
568	7F000000321	7E000000041	7G000001686	Lc	1	240/40
580	7F000000321	7E000000041	7G000001686	Lc	1	240/40
605	7F000000317	7E000000048	7G000001728	L	1	240/40
549	7F000000317	7E000000048	7G000001728	S	1	240/40
644	7F000000341	7E000000052	7G000001865		1	240/40
656	7F000000356	7E000000053	7G000001893	L	1	240/40
657	7F000000356	7E000000053	7G000001893		1	240/40
651	7F000000357	7E000000056	7G000001886		1	240/40
655	7F000000357	7E000000056	7G000001886		1	240/40
664	7F000000338	7E000000056	7G000001899		1	240/40
665	7F000000338	7E000000056	7G000001899		1	240/40
269	7F000000194	7E000000017	7G000001150		2	0/0/0
432	7F000000194	7E000000017	7G000001150		3	0/0/0
517	7F000000177	7E000000017	7G000001446		3	0/0/0
516	7F000000177	7E000000017	7G000001446		3	0/0/0
295	7F000000116	7E000000019	7G000000539		3	0/0/0
394	7F000000347	7E000000019	7G000001752		2	0/0/0
393	7F000000347	7E000000019	7G000001752		2	0/0/0

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
332	7F000000161	7E000000022	7G000000609		3	0/0/0
210	7F000000123	7E000000023	7G000000478		2	0/0/0
493	7F000000123	7E000000023	7G000000478		3	0/0/0
294	7F000000125	7E000000024	7G000000522	OS	3	0/0/0
212	7F000000125	7E000000024	7G000000522		2	0/0/0
279	7F000000231	7E000000025	7G000001563		2	0/0/0
515	7F000000171	7E000000026	7G000001461	HA	3	0/0/0
514	7F000000171	7E000000026	7G000001461	L	3	0/0/0
469	7F000000135	7E000000029	7G000000542	Lym	3	0/0/0
213	7F000000135	7E000000029	7G000000542		2	0/0/0
348	7F000000225	7E000000029	7G000000636		3	0/0/0
230	7F000000225	7E000000029	7G000000636		2	0/0/0
293	7F000000144	7E000000033	7G000000484		3	0/0/0
211	7F000000144	7E000000033	7G000000484		2	0/0/0
395	7F000000350	7E000000033	7G000001765		2	0/0/0
396	7F000000350	7E000000033	7G000001765		2	0/0/0
350	7F000000120	7E000000036	7G000000672		3	0/0/0
386	7F000000197	7E000000037	7G000000791		3	0/0/0
256	7F000000197	7E000000037	7G000000791		2	0/0/0
349	7F000000132	7E000000038	7G000000642		3	0/0/0
231	7F000000132	7E000000038	7G000000642		2	0/0/0
415	7F000000261	7E000000038	7G000000796	OS	3	0/0/0
355	7F000000142	7E000000041	7G000000680		3	0/0/0
232	7F000000142	7E000000041	7G000000680	L	2	0/0/0
278	7F000000229	7E000000041	7G000001546		2	0/0/0
539	7F000000229	7E000000041	7G000001546		3	0/0/0
277	7F000000229	7E000000041	7G000001546		2	0/0/0
254	7F000000147	7E000000042	7G000000768		2	0/0/0
375	7F000000147	7E000000042	7G000000768	S	3	0/0/0
284	7F000000223	7E000000042	7G000001571		2	0/0/0
280	7F000000223	7E000000042	7G000001571		2	0/0/0
457	7F000000152	7E000000044	7G000000627		3	0/0/0
226	7F000000152	7E000000044	7G000000627	L	2	0/0/0
374	7F000000153	7E000000045	7G000000731		3	0/0/0
255	7F000000153	7E000000045	7G000000731		2	0/0/0
417	7F000000170	7E000000045	7G000000853		3	0/0/0
263	7F000000170	7E000000045	7G000000853		2	0/0/0
356	7F000000156	7E000000046	7G000000694	Lf	3	0/0/0
233	7F000000156	7E000000046	7G000000694		2	0/0/0
416	7F000000146	7E000000047	7G000000804		3	0/0/0
257	7F000000146	7E000000047	7G000000804		2	0/0/0
357	7F000000166	7E000000049	7G000000701		3	0/0/0
234	7F000000166	7E000000049	7G000000701		2	0/0/0
630	7F000000339	7E000000053	7G000001745	L	3	0/0/0
631	7F000000339	7E000000053	7G000001745		3	0/0/0
557	7F000000364	7E000000056	7G000001945		2	0/0/0
558	7F000000364	7E000000056	7G000001945		2	0/0/0
323	7F000000158	7E000000019	7G000000587		3	80/40/80
324	7F000000158	7E000000019	7G000000587		3	80/40/80
547	7F000000208	7E000000019	7G000001556	L	3	80/40/80
306	7F000000118	7E000000020	7G000000490		3	80/40/80
305	7F000000118	7E000000020	7G000000490		3	80/40/80
494	7F000000149	7E000000022	7G000001198		3	80/40/80
428	7F000000149	7E000000022	7G000001198	Lf	3	80/40/80
427	7F000000149	7E000000022	7G000001198	Lc	3	80/40/80

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
316	7F000000126	7E000000024	7G000000566		3	80/40/80
309	7F000000126	7E000000024	7G000000566		3	80/40/80
307	7F000000127	7E000000025	7G000000529		3	80/40/80
308	7F000000127	7E000000025	7G000000529		3	80/40/80
543	7F000000205	7E000000034	7G000001518		3	80/40/80
480	7F000000205	7E000000034	7G000001518		3	80/40/80
402	7F000000244	7E000000035	7G000000812		3	80/40/80
401	7F000000244	7E000000035	7G000000812		3	80/40/80
450	7F000000201	7E000000038	7G000001579	Lym	3	80/40/80
362	7F000000137	7E000000039	7G000000777	LgA	3	80/40/80
361	7F000000137	7E000000039	7G000000777		3	80/40/80
171	7F000000243	7E000000041	7G000001512		3	80/40/80
541	7F000000196	7E000000042	7G000001498		3	80/40/80
542	7F000000196	7E000000042	7G000001498		3	80/40/80
511	7F000000220	7E000000043	7G000001476		3	80/40/80
512	7F000000220	7E000000043	7G000001476		3	80/40/80
510	7F000000220	7E000000043	7G000001476	StA	3	80/40/80
444	7F000000246	7E000000048	7G000001437	Nb	3	80/40/80
509	7F000000246	7E000000048	7G000001437	L Lym	3	80/40/80
328	7F000000157	7E000000019	7G000000595	L	3	160/80/160
330	7F000000157	7E000000019	7G000000595		3	160/80/160
320	7F000000145	7E000000020	7G000000572		3	160/80/160
321	7F000000145	7E000000020	7G000000572		3	160/80/160
298	7F000000121	7E000000022	7G000000498	L	3	160/80/160
299	7F000000121	7E000000022	7G000000498		3	160/80/160
410	7F000000232	7E000000022	7G000000836	L	3	160/80/160
409	7F000000232	7E000000022	7G000000836		3	160/80/160
412	7F000000210	7E000000025	7G000000848		3	160/80/160
411	7F000000210	7E000000025	7G000000848		3	160/80/160
379	7F000000224	7E000000028	7G000000752		3	160/80/160
380	7F000000224	7E000000028	7G000000752		3	160/80/160
551	7F000000200	7E000000028	7G000001595		3	160/80/160
358	7F000000190	7E000000029	7G000000666	Lym	3	160/80/160
337	7F000000190	7E000000029	7G000000666	L	3	160/80/160
531	7F000000215	7E000000036	7G000001454		3	160/80/160
530	7F000000215	7E000000036	7G000001454		3	160/80/160
433	7F000000233	7E000000039	7G000001158	L	3	160/80/160
434	7F000000233	7E000000039	7G000001158		3	160/80/160
335	7F000000162	7E000000047	7G000000653		3	160/80/160
334	7F000000162	7E000000047	7G000000653		3	160/80/160
381	7F000000164	7E000000048	7G000000740		3	160/80/160
382	7F000000164	7E000000048	7G000000740		3	160/80/160
424	7F000000173	7E000000048	7G000000862		3	160/80/160
413	7F000000173	7E000000048	7G000000862	L	3	160/80/160
336	7F000000165	7E000000049	7G000000664		3	160/80/160
633	7F000000343	7E000000055	7G000001810		3	160/80/160
344	7F000000216	7E000000019	7G000000689	L	3	240/120/120
314	7F000000117	7E000000020	7G000000554	L	3	240/120/120
219	7F000000117	7E000000020	7G000000554	OS	2	240/120/120
482	7F000000160	7E000000020	7G000000602		3	240/120/120
223	7F000000160	7E000000020	7G000000602		2	240/120/120
675	7F000000218	7E000000022	7G000000658		3	240/120/120
342	7F000000184	7E000000023	7G000000616		3	240/120/120
227	7F000000184	7E000000023	7G000000616		2	240/120/120
241	7F000000185	7E000000024	7G000000784		2	240/120/120
389	7F000000185	7E000000024	7G000000784		3	240/120/120

TABLE G1
Distribution of Male Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
322	7F000000167	7E000000025	7G000000581		3	240/120/120
368	7F000000186	7E000000025	7G000000746		3	240/120/120
315	7F000000130	7E000000026	7G000000557		3	240/120/120
220	7F000000130	7E000000026	7G000000557		2	240/120/120
343	7F000000222	7E000000026	7G000000619	L	3	240/120/120
240	7F000000222	7E000000026	7G000000619		2	240/120/120
262	7F000000203	7E000000026	7G000000820		2	240/120/120
421	7F000000203	7E000000026	7G000000820	Lf	3	240/120/120
313	7F000000134	7E000000028	7G000000514	SfS	3	240/120/120
218	7F000000134	7E000000028	7G000000514		2	240/120/120
312	7F000000136	7E000000029	7G000000505		3	240/120/120
285	7F000000193	7E000000033	7G000001613	Nb	2	240/120/120
243	7F000000112	7E000000034	7G000000717	Ac	2	240/120/120
365	7F000000112	7E000000034	7G000000717		3	240/120/120
367	7F000000113	7E000000035	7G000000773		3	240/120/120
463	7F000000211	7E000000035	7G000000843	Lym	2	240/120/120
422	7F000000211	7E000000035	7G000000843	L	3	240/120/120
366	7F000000119	7E000000036	7G000000706	Nb	3	240/120/120
481	7F000000119	7E000000036	7G000000706	Lym	2	240/120/120
456	7F000000122	7E000000037	7G000000723	L	3	240/120/120
242	7F000000122	7E000000037	7G000000723		2	240/120/120
271	7F000000175	7E000000037	7G000001470		2	240/120/120
272	7F000000175	7E000000037	7G000001470		2	240/120/120
465	7F000000175	7E000000037	7G000001470	Lym	2	240/120/120
273	7F000000175	7E000000037	7G000001470		2	240/120/120
524	7F000000139	7E000000038	7G000001421		3	240/120/120
523	7F000000139	7E000000038	7G000001421		3	240/120/120
270	7F000000236	7E000000039	7G000001428		2	240/120/120
522	7F000000236	7E000000039	7G000001428	L	3	240/120/120
521	7F000000236	7E000000039	7G000001428		3	240/120/120
288	7F000000245	7E000000039	7G000001618	L	2	240/120/120
553	7F000000245	7E000000039	7G000001618	L	3	240/120/120
476	7F000000150	7E000000043	7G000000765	Lym	3	240/120/120
265	7F000000179	7E000000043	7G000000829		2	240/120/120
423	7F000000179	7E000000043	7G000000829	L	3	240/120/120
504	7F000000230	7E000000043	7G000001625	L	3	240/120/120
261	7F000000151	7E000000044	7G000000758		2	240/120/120
388	7F000000151	7E000000044	7G000000758		3	240/120/120
441	7F000000340	7E000000053	7G000001834		2	240/120/120
442	7F000000340	7E000000053	7G000001834		2	240/120/120
439	7F000000342	7E000000055	7G000001825		2	240/120/120
452	7F000000342	7E000000055	7G000001825		2	240/120/120
501	7F000000354	7E000000056	7G000001841		2	240/120/120

[a] UIN = unique identification number
[b] Adjacent cells within a column with the same shading share the same UIN number.
[c] Ac = Astrocytoma; HA = Harderian gland adenoma; L = Liver, hepatocellular adenoma; Lc = Liver, hepatocellular carcinoma; LgA = Lung, adenoma; Lf = Liver, basophilic focus; Lym = Malignant lymphoma; L Lym= Liver, hepatocellular adenoma + Malignant lymphoma; Nb = Neuroblastoma; OS = Osteosarcoma; Pc = Pancreas, carcinoma; S = Sarcoma; SfS = Skin, fibrosarcoma; StA = Stomach, adenocarcinoma
[d] Duration 1, 2, and 3, designate the 45-week stop-study, 30-week study, and 45-week study, respectively; shading highlights nonconsecutive durations
[e] AZT dose is presented as mg/kg body weight per day

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN[a]	Sire UIN[b]	Litter UIN[b]	Lesion[c]	Duration[d]	Dose[e]
569	7F000000316	7E000000019	7G000001650		1	0/0
570	7F000000316	7E000000019	7G000001650		1	0/0
571	7F000000315	7E000000019	7G000001700	Up	1	0/0
572	7F000000315	7E000000019	7G000001700	OTm	1	0/0
573	7F000000346	7E000000019	7G000001782		1	0/0
579	7F000000346	7E000000019	7G000001782		1	0/0
589	7F000000309	7E000000033	7G000001635		1	0/0
590	7F000000309	7E000000033	7G000001635		1	0/0
591	7F000000310	7E000000033	7G000001721		1	0/0
592	7F000000310	7E000000033	7G000001721		1	0/0
601	7F000000351	7E000000033	7G000001818		1	0/0
602	7F000000351	7E000000033	7G000001818		1	0/0
603	7F000000327	7E000000034	7G000001666		1	0/0
604	7F000000327	7E000000034	7G000001666		1	0/0
608	7F000000320	7E000000037	7G000001642	Oc	1	0/0
619	7F000000320	7E000000037	7G000001642		1	0/0
620	7F000000326	7E000000046	7G000001796	MA	1	0/0
621	7F000000326	7E000000046	7G000001796		1	0/0
622	7F000000326	7E000000046	7G000001796		1	0/0
623	7F000000325	7E000000049	7G000001659		1	0/0
624	7F000000325	7E000000049	7G000001659		1	0/0
627	7F000000345	7E000000052	7G000001849		1	0/0
636	7F000000345	7E000000052	7G000001849		1	0/0
637	7F000000322	7E000000053	7G000001856		1	0/0
649	7F000000360	7E000000055	7G000001880		1	0/0
654	7F000000360	7E000000055	7G000001880		1	0/0
390	7F000000324	7E000000019	7G000001738		1	240/40
556	7F000000324	7E000000019	7G000001738		1	240/40
559	7F000000336	7E000000019	7G000001871		1	240/40
560	7F000000336	7E000000019	7G000001871		1	240/40
561	7F000000323	7E000000028	7G000001673		1	240/40
562	7F000000323	7E000000028	7G000001673		1	240/40
563	7F000000306	7E000000031	7G000001707		1	240/40
583	7F000000306	7E000000031	7G000001707		1	240/40
584	7F000000306	7E000000031	7G000001707	Lk	1	240/40
594	7F000000352	7E000000033	7G000001852		1	240/40
595	7F000000319	7E000000037	7G000001679		1	240/40
596	7F000000319	7E000000037	7G000001679		1	240/40
606	7F000000308	7E000000038	7G000001714	Up	1	240/40
607	7F000000308	7E000000038	7G000001714	US	1	240/40
610	7F000000312	7E000000039	7G000001695	Ac	1	240/40
613	7F000000321	7E000000041	7G000001686		1	240/40
638	7F000000321	7E000000041	7G000001686		1	240/40
639	7F000000317	7E000000048	7G000001728		1	240/40
640	7F000000341	7E000000052	7G000001865		1	240/40
641	7F000000341	7E000000052	7G000001865		1	240/40
642	7F000000356	7E000000053	7G000001893		1	240/40
650	7F000000356	7E000000053	7G000001893		1	240/40
658	7F000000357	7E000000056	7G000001886		1	240/40
659	7F000000357	7E000000056	7G000001886		1	240/40
666	7F000000338	7E000000056	7G000001899		1	240/40
667	7F000000338	7E000000056	7G000001899		1	240/40
173	7F000000194	7E000000017	7G000001150		2	0/0/0
209	7F000000194	7E000000017	7G000001150	S	3	0/0/0
289	7F000000177	7E000000017	7G000001446		3	0/0/0
290	7F000000177	7E000000017	7G000001446		3	0/0/0

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
291	7F000000116	7E000000019	7G000000539		3	0/0/0
215	7F000000347	7E000000019	7G000001752		2	0/0/0
214	7F000000347	7E000000019	7G000001752		2	0/0/0
216	7F000000161	7E000000022	7G000000609		2	0/0/0
292	7F000000161	7E000000022	7G000000609		3	0/0/0
333	7F000000123	7E000000023	7G000000478		3	0/0/0
217	7F000000123	7E000000023	7G000000478		2	0/0/0
351	7F000000125	7E000000024	7G000000522		3	0/0/0
222	7F000000125	7E000000024	7G000000522		2	0/0/0
235	7F000000128	7E000000025	7G000000548		2	0/0/0
236	7F000000231	7E000000025	7G000001563		2	0/0/0
238	7F000000231	7E000000025	7G000001563		2	0/0/0
237	7F000000231	7E000000025	7G000001563		2	0/0/0
353	7F000000171	7E000000026	7G000001461		3	0/0/0
352	7F000000171	7E000000026	7G000001461		3	0/0/0
354	7F000000225	7E000000029	7G000000636		3	0/0/0
239	7F000000144	7E000000033	7G000000484		2	0/0/0
360	7F000000144	7E000000033	7G000000484		3	0/0/0
249	7F000000350	7E000000033	7G000001765		2	0/0/0
376	7F000000120	7E000000036	7G000000672	OL	3	0/0/0
250	7F000000120	7E000000036	7G000000672		2	0/0/0
377	7F000000197	7E000000037	7G000000791		3	0/0/0
251	7F000000197	7E000000037	7G000000791	Usp	2	0/0/0
252	7F000000261	7E000000038	7G000000796		2	0/0/0
378	7F000000261	7E000000038	7G000000796		3	0/0/0
253	7F000000142	7E000000041	7G000000680		2	0/0/0
400	7F000000142	7E000000041	7G000000680		3	0/0/0
414	7F000000229	7E000000041	7G000001546		3	0/0/0
268	7F000000229	7E000000041	7G000001546		2	0/0/0
266	7F000000229	7E000000041	7G000001546		2	0/0/0
431	7F000000147	7E000000042	7G000000768		3	0/0/0
274	7F000000147	7E000000042	7G000000768		2	0/0/0
446	7F000000192	7E000000042	7G000001497	Mth	3	0/0/0
276	7F000000223	7E000000042	7G000001571		2	0/0/0
275	7F000000223	7E000000042	7G000001571	MA	2	0/0/0
281	7F000000152	7E000000044	7G000000627		2	0/0/0
513	7F000000152	7E000000044	7G000000627		3	0/0/0
282	7F000000153	7E000000045	7G000000731		2	0/0/0
518	7F000000153	7E000000045	7G000000731		3	0/0/0
519	7F000000170	7E000000045	7G000000853		3	0/0/0
283	7F000000170	7E000000045	7G000000853	Ip	2	0/0/0
399	7F000000156	7E000000046	7G000000694		2	0/0/0
520	7F000000156	7E000000046	7G000000694		3	0/0/0
443	7F000000146	7E000000047	7G000000804	Lym	2	0/0/0
538	7F000000146	7E000000047	7G000000804		3	0/0/0
464	7F000000166	7E000000049	7G000000701		2	0/0/0
540	7F000000166	7E000000049	7G000000701		3	0/0/0
552	7F000000339	7E000000053	7G000001745	OS	3	0/0/0
632	7F000000339	7E000000053	7G000001745		3	0/0/0
300	7F000000158	7E000000019	7G000000587		3	80/40/80
301	7F000000158	7E000000019	7G000000587		3	80/40/80
302	7F000000208	7E000000019	7G000001556	LgA	3	80/40/80
303	7F000000118	7E000000020	7G000000490		3	80/40/80
304	7F000000118	7E000000020	7G000000490		3	80/40/80

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
326	7F000000149	7E000000022	7G000001198		3	80/40/80
317	7F000000149	7E000000022	7G000001198		3	80/40/80
325	7F000000149	7E000000022	7G000001198		3	80/40/80
363	7F000000126	7E000000024	7G000000566		3	80/40/80
364	7F000000126	7E000000024	7G000000566	Usp	3	80/40/80
403	7F000000127	7E000000025	7G000000529		3	80/40/80
429	7F000000127	7E000000025	7G000000529		3	80/40/80
445	7F000000244	7E000000035	7G000000812	OS	3	80/40/80
430	7F000000244	7E000000035	7G000000812	OS	3	80/40/80
451	7F000000201	7E000000038	7G000001579	Sh	3	80/40/80
483	7F000000137	7E000000039	7G000000777		3	80/40/80
505	7F000000137	7E000000039	7G000000777		3	80/40/80
506	7F000000243	7E000000041	7G000001512		3	80/40/80
507	7F000000243	7E000000041	7G000001512		3	80/40/80
508	7F000000196	7E000000042	7G000001498		3	80/40/80
529	7F000000196	7E000000042	7G000001498		3	80/40/80
544	7F000000220	7E000000043	7G000001476		3	80/40/80
545	7F000000220	7E000000043	7G000001476	Sp	3	80/40/80
546	7F000000246	7E000000048	7G000001437		3	80/40/80
548	7F000000246	7E000000048	7G000001437	KA	3	80/40/80
550	7F000000246	7E000000048	7G000001437		3	80/40/80
673	7F000000246	7E000000048	7G000001437		3	80/40/80
296	7F000000157	7E000000019	7G000000595		3	160/80/160
297	7F000000157	7E000000019	7G000000595		3	160/80/160
318	7F000000145	7E000000020	7G000000572	OS	3	160/80/160
319	7F000000145	7E000000020	7G000000572	HA	3	160/80/160
327	7F000000121	7E000000022	7G000000498		3	160/80/160
331	7F000000121	7E000000022	7G000000498		3	160/80/160
338	7F000000232	7E000000022	7G000000836		3	160/80/160
339	7F000000232	7E000000022	7G000000836		3	160/80/160
340	7F000000210	7E000000025	7G000000848	AA	3	160/80/160
341	7F000000210	7E000000025	7G000000848		3	160/80/160
359	7F000000224	7E000000028	7G000000752		3	160/80/160
383	7F000000224	7E000000028	7G000000752		3	160/80/160
384	7F000000200	7E000000028	7G000001595		3	160/80/160
385	7F000000190	7E000000029	7G000000666	ScC	3	160/80/160
404	7F000000190	7E000000029	7G000000666		3	160/80/160
405	7F000000215	7E000000036	7G000001454		3	160/80/160
406	7F000000215	7E000000036	7G000001454		3	160/80/160
407	7F000000233	7E000000039	7G000001158	OS Lym	3	160/80/160
408	7F000000233	7E000000039	7G000001158		3	160/80/160
435	7F000000162	7E000000047	7G000000653		3	160/80/160
425	7F000000162	7E000000047	7G000000653		3	160/80/160
436	7F000000164	7E000000048	7G000000740	MA	3	160/80/160
458	7F000000164	7E000000048	7G000000740		3	160/80/160
462	7F000000173	7E000000048	7G000000862		3	160/80/160
532	7F000000173	7E000000048	7G000000862		3	160/80/160
533	7F000000165	7E000000049	7G000000664		3	160/80/160
609	7F000000343	7E000000055	7G000001810	MA	3	160/80/160
221	7F000000216	7E000000019	7G000000689		2	240/120/120
310	7F000000216	7E000000019	7G000000689		3	240/120/120
311	7F000000117	7E000000020	7G000000554	MA	3	240/120/120
224	7F000000160	7E000000020	7G000000602		2	240/120/120
329	7F000000160	7E000000020	7G000000602	Lym	3	240/120/120
345	7F000000218	7E000000022	7G000000658		3	240/120/120
225	7F000000218	7E000000022	7G000000658		2	240/120/120

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
346	7F000000184	7E000000023	7G000000616		3	240/120/120
347	7F000000185	7E000000024	7G000000784		3	240/120/120
228	7F000000185	7E000000024	7G000000784		2	240/120/120
369	7F000000167	7E000000025	7G000000581	Lym	3	240/120/120
229	7F000000167	7E000000025	7G000000581		2	240/120/120
244	7F000000186	7E000000025	7G000000746		2	240/120/120
370	7F000000186	7E000000025	7G000000746		3	240/120/120
371	7F000000130	7E000000026	7G000000557		3	240/120/120
245	7F000000130	7E000000026	7G000000557		2	240/120/120
372	7F000000222	7E000000026	7G000000619		3	240/120/120
246	7F000000222	7E000000026	7G000000619		2	240/120/120
373	7F000000203	7E000000026	7G000000820		3	240/120/120
247	7F000000203	7E000000026	7G000000820		2	240/120/120
387	7F000000134	7E000000028	7G000000514		3	240/120/120
418	7F000000136	7E000000029	7G000000505		3	240/120/120
248	7F000000349	7E000000031	7G000001757		2	240/120/120
419	7F000000112	7E000000034	7G000000717		3	240/120/120
258	7F000000112	7E000000034	7G000000717		2	240/120/120
259	7F000000113	7E000000035	7G000000773		2	240/120/120
420	7F000000113	7E000000035	7G000000773		3	240/120/120
447	7F000000211	7E000000035	7G000000843		3	240/120/120
260	7F000000211	7E000000035	7G000000843		2	240/120/120
448	7F000000119	7E000000036	7G000000706	OS	3	240/120/120
264	7F000000119	7E000000036	7G000000706	SbC	2	240/120/120
449	7F000000122	7E000000037	7G000000723	SfS	3	240/120/120
267	7F000000122	7E000000037	7G000000723		2	240/120/120
453	7F000000175	7E000000037	7G000001470	Lym	3	240/120/120
525	7F000000139	7E000000038	7G000001421		3	240/120/120
497	7F000000139	7E000000038	7G000001421		3	240/120/120
527	7F000000236	7E000000039	7G000001428	Ost	3	240/120/120
526	7F000000236	7E000000039	7G000001428		3	240/120/120
528	7F000000245	7E000000039	7G000001618		3	240/120/120
286	7F000000245	7E000000039	7G000001618		2	240/120/120
287	7F000000318	7E000000041	7G000001802	Lym	2	240/120/120
391	7F000000318	7E000000041	7G000001802		2	240/120/120

TABLE G2
Distribution of Female Heterozygous F1 p53$^{+/-}$ Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

Animal Number	Dam UIN	Sire UIN	Litter UIN	Lesion	Duration	Dose
554	7F000000150	7E000000043	7G000000765		3	240/120/120
555	7F000000179	7E000000043	7G000000829		3	240/120/120
392	7F000000179	7E000000043	7G000000829		2	240/120/120
674	7F000000230	7E000000043	7G000001625	Sh	3	240/120/120
397	7F000000230	7E000000043	7G000001625		2	240/120/120
398	7F000000311	7E000000049	7G000001769		2	240/120/120
437	7F000000340	7E000000053	7G000001834		2	240/120/120
438	7F000000342	7E000000055	7G000001825		2	240/120/120
440	7F000000342	7E000000055	7G000001825		2	240/120/120
503	7F000000354	7E000000056	7G000001841		2	240/120/120
502	7F000000354	7E000000056	7G000001841	S	2	240/120/120

[a] UIN = unique identification number
[b] Adjacent cells within a column with the same shading share the same UIN number.
[c] AA = Adrenal cortex adenoma; Ac = Astrocytoma; HA = Harderian gland adenoma; Ip = Intestine, polyp; KA = Renal tubule adenoma; LgA = Lung, adenoma; Lk = Leukemia, granulocytic; Lym = Malignant lymphoma; MA = Mammary gland adenocarcinoma; Mth = Malignant mesothelioma; Oc = Ovary, carcinoma; OL = Ovary, luteoma; OS = Osteosarcoma; Ost = Osteoma; OS Lym = Osteosarcoma + Malignant lymphoma; OTm = Ovary, malignant teratoma; S = Sarcoma; SbC = Skin, basal cell carcinoma; ScC = Skin, squamous cell carcinoma; SfS = Skin, fibrosarcoma; Sh = Sarcoma, histiocytic; Sp = Stomach, papilloma; Up = Uterus, polyp; Usp = Uterus, stomal polyp; US = Uterus, sarcoma
[d] Duration 1, 2, and 3, designate the 45-week stop-study, 30-week study, and 45-week study, respectively; shading highlights nonconsecutive durations
[e] AZT dose is presented as mg/kg body weight per day

TABLE G3
Effect of AZT Dose on Survival of Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg vs. 240/120/240 mg/kg
Male	
Hazard Ratio[b]	6174.4%
Independence	4.1 ± 3.2 P=0.097
Empirical Variance - Naive	4.1 ± 1.1 P≤0.001***
Correlation within Dam/Litter	4.1 ± 1.0 P≤0.001***
Correlation within Sire	4.1 ± 1.1 P≤0.001***
Female	
Hazard Ratio	3.3%
Independence	–3.4 ± 3.2 P=0.143N
Empirical Variance - Naive	–3.4 ± 1.2 P=0.002N**
Correlation within Dam/Litter	–3.4 ± 1.2 P=0.002N**
Correlation within Sire	–3.4 ± 1.2 P=0.002N**

** Significantly different (P≤0.01) by the Cox (1972) proportional hazard model
***P≤0.001
[a] The results are presented for the naive model assuming pup independence, an empirical variance model, an empirical variance model aggregated over litters, and an empirical variance model aggregated over sires. A lower P value in a dosed group is indicated by **N**.
[b] The Hazard Ratio represents the treatment group hazard relative to 0/0/0 mg/kg group. The ratio for the 0/0/0 mg/kg group is always 100%.

TABLE G4
Effect of AZT Dose on Survival of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT[a]

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Male				
Hazard Ratio[b]	–	217.1%	3.1%	169.4%
Independence	–3.2 ± 3.5 P=0.180N[c]	0.8 ± 0.7 P=0.136	–3.5 ± 3.2 P=0.139N	0.5 ± 0.7 P=0.234
Empirical Variance - Naive	–3.2 ± 1.8 P=0.039N*	0.8 ± 0.7 P=0.137	–3.5 ± 1.2 P=0.001N**	0.5 ± 0.7 P=0.233
Correlation within Dam/Litter	–3.2 ± 1.4 P=0.013N*	0.8 ± 0.7 P=0.128	–3.5 ± 0.9 P≤0.001N***	0.5 ± 0.7 P=0.235
Correlation within Sire	–3.2 ± 1.4 P=0.013N*	0.8 ± 0.7 P=0.135	–3.5 ± 0.9 P≤0.001N***	0.5 ± 0.8 P=0.252
Female				
Hazard Ratio	–	130.3%	96.5%	202.0%
Independence	1.1 ± 1.6 P=0.246	0.3 ± 0.8 P=0.364	0.0 ± 0.8 P=0.483N	0.7 ± 0.7 P=0.160
Empirical Variance - Naive	1.1 ± 1.6 P=0.244	0.3 ± 0.8 P=0.364	0.0 ± 0.8 P=0.482N	0.7 ± 0.7 P=0.158
Correlation within Dam/Litter	1.1 ± 1.5 P=0.235	0.3 ± 0.7 P=0.349	0.0 ± 0.8 P=0.482N	0.7 ± 0.7 P=0.151
Correlation within Sire	1.1 ± 1.3 P=0.196	0.3 ± 0.7 P=0.350	0.0 ± 0.7 P=0.480N	0.7 ± 0.6 P=0.135

* Significantly different (P≤0.05) by the Cox (1972) proportional hazard model
** P≤0.01
***P≤0.001

[a] The results are presented for the naive model assuming pup independence, an empirical variance model, an empirical variance model aggregated over litters, and an empirical variance model aggregated over sires.

[b] The Hazard Ratio represents the treatment group hazard relative to the 0/0/0 mg/kg. The ratio for the 0/0/0 mg/kg group is always 100%.

[c] P values for the 0/0/0 mg/kg group represent linear dose trends and not the Cox estimate which is implicitly zero for this group. A negative trend or a lower P value in a dosed group is indicated by **N**.

TABLE G5
Effect of AZT Dose on Survival of Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT[a]

	0/0 mg/kg vs. 240/40 mg/kg
Male	
Hazard Ratio[b]	293.8%
Independence	1.1 ± 1.2 P=0.175
Empirical Variance - Naive	1.1 ± 1.1 P=0.173
Correlation within Dam/Litter	1.1 ± 1.1 P=0.167
Correlation within Sire	1.1 ± 1.2 P=0.178
Female	
Hazard Ratio	306.1%
Independence	1.1 ± 1.2 P=0.166
Empirical Variance - Naive	1.1 ± 1.1 P=0.164
Correlation within Dam/Litter	1.1 ± 1.1 P=0.157
Correlation within Sire	1.1 ± 1.1 P=0.159

[a] The results are presented for the naive model assuming pup independence, an empirical variance model, an empirical variance model aggregated over litters, and an empirical variance model aggregated over sires.

[b] The Hazard Ratio represents the treatment group hazard relative to the 0/0 mg/kg group. The ratio for the 0/0 mg/kg group is always 100%.

TABLE G6
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Liver: Hepatocellular Adenoma		
Overall rate[a]	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate[b]	1/27.0 (3.7%)	1/22.8 (4.4%)
Terminal rate[c]	1/27 (3.7%)	1/21 (4.8%)
First incidence (days)	217 (T)	213 (T)
Poly-3 test[d]		0.722
Litter-adjusted correlation	0.04	
Litter-adjusted Poly-3 rate	3.8%	4.5%
Litter-adjusted Poly-3 test		0.451
Sire-adjusted correlation	–0.01	
Sire-adjusted Poly-3 rate	3.6%	4.4%
Sire-adjusted Poly-3 test		0.446
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate	1/27.0 (3.7%)	1/22.8 (4.4%)
Terminal rate	1/27 (3.7%)	1/21 (4.8%)
First incidence (days)	217 (T)	213 (T)
Poly-3 test		0.722
Litter-adjusted correlation	0.04	
Litter-adjusted Poly-3 rate	3.8%	4.5%
Litter-adjusted Poly-3 test		0.451
Sire-adjusted correlation	–0.01	
Sire-adjusted Poly-3 rate	3.6%	4.4%
Sire-adjusted Poly-3 test		0.446
All Organs: Malignant Lymphoma		
Overall rate	0/27 (0.0%)	3/26 (11.5%)
Adjusted rate	0/27.0 (0.0%)	3/25.2 (11.9%)
Terminal rate	0/27 (0.0%)	0/21 (0.0%)
First incidence (days)	—[e]	84
Poly-3 test		0.102
Litter-adjusted correlation	–0.21	
Litter-adjusted Poly-3 rate	0.0%	14.4%
Litter-adjusted Poly-3 test		0.005**
Sire-adjusted correlation	–0.05	
Sire-adjusted Poly-3 rate	0.0%	12.0%
Sire-adjusted Poly-3 test		0.016*
All Organs: Osteosarcoma		
Overall rate	0/27 (0.0%)	1/26 (3.8%)
Adjusted rate	0/27.0 (0.0%)	1/22.8 (4.4%)
Terminal rate	0/27 (0.0%)	1/21 (4.8%)
First incidence (days)	—	213 (T)
Poly-3 test		0.467
Litter-adjusted correlation	0.03	
Litter-adjusted Poly-3 rate	0.0%	4.5%
Litter-adjusted Poly-3 test		0.155
Sire-adjusted correlation	–0.01	
Sire-adjusted Poly-3 rate	0.0%	4.4%
Sire-adjusted Poly-3 test		0.148

TABLE G6
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
All Organs: Benign Neoplasms		
Overall rate	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate	1/27.0 (3.7%)	1/22.8 (4.4%)
Terminal rate	1/27 (3.7%)	1/21 (4.8%)
First incidence (days)	217 (T)	213 (T)
Poly-3 test		0.722
Litter-adjusted correlation	0.04	
Litter-adjusted Poly-3 rate	3.8%	4.5%
Litter-adjusted Poly-3 test		0.451
Sire-adjusted correlation	–0.01	
Sire-adjusted Poly-3 rate	3.6%	4.4%
Sire-adjusted Poly-3 test		0.446
All Organs: Malignant Neoplasms		
Overall rate	0/27 (0.0%)	6/26 (23.1%)
Adjusted rate	0/27.0 (0.0%)	6/25.2 (23.8%)
Terminal rate	0/27 (0.0%)	2/21 (9.5%)
First incidence (days)	—	–84
Poly-3 test		0.008**
Litter-adjusted correlation	–0.15	
Litter-adjusted Poly-3 rate	0.0%	23.5%
Litter-adjusted Poly-3 test		<0.001***
Sire-adjusted correlation	–0.04	
Sire-adjusted Poly-3 rate	0.0%	23.3%
Sire-adjusted Poly-3 test		0.001**
All Organs: Benign or Malignant Neoplasms		
Overall rate	1/27 (3.7%)	7/26 (26.9%)
Adjusted rate	1/27.0 (3.7%)	7/25.2 (27.8%)
Terminal rate	1/27 (3.7%)	3/21 (14.3%)
First incidence (days)	217 (T)	84
Poly-3 test		0.017*
Litter-adjusted correlation	–0.10	
Litter-adjusted Poly-3 rate	3.6%	27.3%
Litter-adjusted Poly-3 test		0.003**
Sire-adjusted correlation	–0.12	
Sire-adjusted Poly-3 rate	3.4%	25.9%
Sire-adjusted Poly-3 test		0.002**

(T) Terminal kill
* Significantly different (P≤0.05) from the 0/0/0 mg/kg group by the Poly-3 test
** P≤0.01
***P≤0.001
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[e] Not applicable; no neoplasms in animal group

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Liver: Hepatocellular Adenoma				
Overall rate[a]	3/26 (11.5%)	2/27 (7.4%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate[b]	3/24.4 (12.3%)	2/22.8 (8.8%)	6/27.0 (22.2%)	9/24.7 (36.5%)
Terminal rate[c]	3/24 (12.5%)	2/21 (9.5%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test[d]	0.013*	0.531N	0.288	0.048*
Poly-5 test	0.013*	0.536N	0.293	0.047*
Litter-adjusted correlation	–0.23			
Litter-adjusted Poly-3 rate	13.8%	7.2%	22.8%	35.9%
Litter-adjusted Poly-3 test	0.010*	0.214N	0.168	0.027*
Sire-adjusted correlation	0.04			
Sire-adjusted Poly-3 rate	12.4%	7.9%	21.2%	36.1%
Sire-adjusted Poly-3 test	0.009**	0.305N	0.119	0.022*
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/26 (11.5%)	3/27 (11.1%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate	3/24.4 (12.3%)	3/22.8 (13.2%)	6/27.0 (22.2%)	9/24.7 (36.5%)
Terminal rate	3/24 (12.5%)	3/21 (14.3%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test	0.019*	0.634	0.288	0.048*
Poly-5 test	0.019*	0.629	0.293	0.047*
Litter-adjusted correlation	–0.26			
Litter-adjusted Poly-3 rate	14.0%	12.0%	22.9%	35.8%
Litter-adjusted Poly-3 test	0.016*	0.417N	0.174	0.029*
Sire-adjusted correlation	0.10			
Sire-adjusted Poly-3 rate	12.4%	10.9%	19.5%	35.7%
Sire-adjusted Poly-3 test	0.018*	0.421N	0.167	0.024*
All Organs: Malignant Lymphoma				
Overall rate	1/27 (3.7%)	2/27 (7.4%)	1/27 (3.7%)	1/27 (3.7%)
Adjusted rate	1/25.4 (3.9%)	2/23.5 (8.5%)	1/27.0 (3.7%)	1/24.7 (4.1%)
Terminal rate	0/24 (0.0%)	1/21 (4.8%)	1/27 (3.7%)	0/22 (0.0%)
First incidence (days)	159	217	321 (T)	94
Poly-3 test	0.502N	0.473	0.746N	0.754
Litter-adjusted correlation	0.00			
Litter-adjusted Poly-3 rate	3.9%	8.5%	3.7%	4.1%
Litter-adjusted Poly-3 test	0.402N	0.258	0.482N	0.492
Sire-adjusted correlation	0.09			
Sire-adjusted Poly-3 rate	3.5%	9.0%	3.5%	3.8%
Sire-adjusted Poly-3 test	0.388N	0.221	0.494N	0.472
All Organs: Osteosarcoma				
Overall rate	2/27 (7.4%)	0/27 (0.0%)	0/27 (0.0%)	0/27 (0.0%)
Adjusted rate	2/24.5 (8.2%)	0/22.8 (0.0%)	0/27.0 (0.0%)	0/23.7 (0.0%)
Terminal rate	2/24 (8.3%)	0/21 (0.0%)	0/27 (0.0%)	0/22 (0.0%)
First incidence (days)	319 (T)	—[e]	—	—
Poly-3 test	0.050N*	0.251N	0.214N	0.242N
Litter-adjusted correlation	0.01			
Litter-adjusted Poly-3 rate	8.2%	0.0%	0.0%	0.0%
Litter-adjusted Poly-3 test	0.072N	0.072N	0.072N	0.072N
Sire-adjusted correlation	0.00			
Sire-adjusted Poly-3 rate	8.2%	0.0%	0.0%	0.0%
Sire-adjusted Poly-3 test	0.067N	0.067N	0.067N	0.067N

TABLE G7
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
All Organs: Benign Neoplasms				
Overall rate	4/27 (14.8%)	2/27 (7.4%)	6/27 (22.2%)	9/27 (33.3%)
Adjusted rate	4/24.5 (16.3%)	2/22.8 (8.8%)	6/27.0 (22.2%)	9/24.7 (36.5%)
Terminal rate	4/24 (16.7%)	2/21 (9.5%)	6/27 (22.2%)	7/22 (31.8%)
First incidence (days)	321 (T)	319 (T)	320 (T)	228
Poly-3 test	0.031*	0.367N	0.430	0.099
Litter-adjusted correlation	0.00			
Litter-adjusted Poly-3 rate	16.4%	8.8%	22.2%	36.5%
Litter-adjusted Poly-3 test	0.031*	0.236N	0.295	0.059
Sire-adjusted correlation	–0.07			
Sire-adjusted Poly-3 rate	16.4%	7.4%	20.7%	35.2%
Sire-adjusted Poly-3 test	0.046*	0.202N	0.328	0.087
All Organs: Malignant Neoplasms				
Overall rate	4/27 (14.8%)	6/27 (22.2%)	1/27 (3.7%)	3/27 (11.1%)
Adjusted rate	4/25.4 (15.8%)	6/24.0 (25.0%)	1/27.0 (3.7%)	3/24.7 (12.2%)
Terminal rate	3/24 (12.5%)	3/21 (14.3%)	1/27 (3.7%)	2/22 (9.1%)
First incidence (days)	159	217	321 (T)	94
Poly-3 test	0.176N	0.328	0.155N	0.516N
Litter-adjusted correlation	0.06			
Litter-adjusted Poly-3 rate	16.0%	25.0%	3.7%	12.3%
Litter-adjusted Poly-3 test	0.153N	0.226	0.068N	0.356N
Sire-adjusted correlation	0.01			
Sire-adjusted Poly-3 rate	15.7%	25.1%	3.6%	12.1%
Sire-adjusted Poly-3 test	0.140N	0.223	0.031N*	0.361N
All Organs: Benign or Malignant Neoplasms				
Overall rate	8/27 (29.6%)	7/27 (25.9%)	7/27 (25.9%)	12/27 (44.4%)
Adjusted rate	8/25.4 (31.5%)	7/24.0 (29.1%)	7/27.0 (25.9%)	12/25.7 (46.8%)
Terminal rate	7/24 (29.2%)	4/21 (19.0%)	7/27 (25.9%)	9/22 (40.9%)
First incidence (days)	159	217	320 (T)	94
Poly-3 test	0.185	0.550N	0.444N	0.205
Litter-adjusted correlation	0.10			
Litter-adjusted Poly-3 rate	31.1%	29.6%	25.6%	47.1%
Litter-adjusted Poly-3 test	0.155	0.457N	0.334N	0.123
Sire-adjusted correlation	0.02			
Sire-adjusted Poly-3 rate	31.5%	28.3%	25.1%	46.0%
Sire-adjusted Poly-3 test	0.200	0.407N	0.285N	0.188

(T) Terminal kill
* Significantly different (P≤0.05) by the Poly-3 test
** P≤0.01
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group and that dosed group. The Poly-k test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE G8
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Liver: Hepatocellular Adenoma		
Overall rate[a]	3/24 (12.5%)	5/25 (20.0%)
Adjusted rate[b]	3/23.4 (12.8%)	5/23.5 (21.3%)
Terminal rate[c]	3/23 (13.0%)	5/22 (22.7%)
First incidence (days)	317 (T)	317 (T)
Poly-3 test[d]		0.352
Poly-5 test		0.350
Litter-adjusted correlation	–0.17	
Litter-adjusted Poly-3 rate	12.8%	20.8%
Litter-adjusted Poly-3 test		0.212
Sire-adjusted correlation	–0.10	
Sire-adjusted Poly-3 rate	15.2%	22.3%
Sire-adjusted Poly-3 test		0.218
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	3/24 (12.5%)	7/25 (28.0%)
Adjusted rate	3/23.4 (12.8%)	7/23.5 (29.8%)
Terminal rate	3/23 (13.0%)	7/22 (31.8%)
First incidence (days)	317 (T)	317 (T)
Poly-3 test		0.143
Poly-5 test		0.141
Litter-adjusted correlation	–0.02	
Litter-adjusted Poly-3 rate	12.8%	29.8%
Litter-adjusted Poly-3 test		0.076
Sire-adjusted correlation	–0.07	
Sire-adjusted Poly-3 rate	12.6%	29.1%
Sire-adjusted Poly-3 test		0.077
All Organs: Malignant Lymphoma		
Overall rate	0/24 (0.0%)	2/25 (8.0%)
Adjusted rate	0/23.4 (0.0%)	2/23.7 (8.5%)
Terminal rate	0/23 (0.0%)	1/22 (4.5%)
First incidence (days)	—[e]	300
Poly-3 test		0.237
Litter-adjusted correlation	0.77	
Litter-adjusted Poly-3 rate	0.0%	7.9%
Litter-adjusted Poly-3 test		0.148
Sire-adjusted correlation	0.13	
Sire-adjusted Poly-3 rate		—[f]
Sire-adjusted Poly-3 test		—
All Organs: Benign Neoplasms		
Overall rate	6/24 (25.0%)	5/25 (20.0%)
Adjusted rate	6/23.4 (25.6%)	5/23.5 (21.3%)
Terminal rate	6/23 (26.1%)	5/22 (22.7%)
First incidence (days)	317 (T)	317 (T)
Poly-3 test		0.498N
Litter-adjusted correlation	–0.29	
Litter-adjusted Poly-3 rate	26.0%	20.4%
Litter-adjusted Poly-3 test		0.298N
Sire-adjusted correlation	–0.08	
Sire-adjusted Poly-3 rate	25.8%	21.1%
Sire-adjusted Poly-3 test		0.317N

TABLE G8
Litter-adjusted Statistical Analysis of Primary Neoplasms in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
All Organs: Malignant Neoplasms		
Overall rate	1/24 (4.2%)	5/25 (20.0%)
Adjusted rate	1/24.0 (4.2%)	5/24.0 (20.8%)
Terminal rate	0/23 (0.0%)	3/22 (13.6%)
First incidence (days)	240	281
Poly-3 test		0.093
Litter-adjusted correlation	0.40	
Litter-adjusted Poly-3 rate	4.0%	20.2%
Litter-adjusted Poly-3 test		0.076
Sire-adjusted correlation	–0.04	
Sire-adjusted Poly-3 rate	4.9%	21.1%
Sire-adjusted Poly-3 test		0.104
All Organs: Benign and Malignant Neoplasms		
Overall rate	7/24 (29.2%)	10/25 (40.0%)
Adjusted rate	7/24.0 (29.2%)	10/24.0 (41.7%)
Terminal rate	6/23 (26.1%)	8/22 (36.4%)
First incidence (days)	240	281
Poly-3 test		0.276
Litter-adjusted correlation	0.21	
Litter-adjusted Poly-3 rate	28.7%	41.8%
Litter-adjusted Poly-3 test		0.189
Sire-adjusted correlation	–0.02	
Sire-adjusted Poly-3 rate	29.5%	41.4%
Sire-adjusted Poly-3 test		0.233

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group. The Poly-k test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Sire-adjusted analysis produced invalid results

TABLE G9
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
All Organs: Malignant Lymphoma		
Overall rate[a]	1/27 (3.7%)	1/26 (3.8%)
Adjusted rate[b]	1/25.2 (4.0%)	1/26.0 (3.8%)
Terminal rate[c]	0/24 (0.0%)	1/26 (3.8%)
First incidence (days)	192	213 (T)
Poly-3 test[d]		0.753N
Litter-adjusted correlation	0.04	
Litter-adjusted Poly-3 rate	4.0%	3.9%
Litter-adjusted Poly-3 test		0.490N
Sire-adjusted correlation	–0.01	
Sire-adjusted Poly-3 rate	3.9%	3.8%
Sire-adjusted Poly-3 test		0.492N
All Organs: Benign Neoplasms		
Overall rate	2/27 (7.4%)	0/26 (0.0%)
Adjusted rate	2/24.9 (8.0%)	0/26.0 (0.0%)
Terminal rate	2/24 (8.3%)	0/26 (0.0%)
First incidence (days)	211 (T)	—[e]
Poly-3 test		0.225N
Litter-adjusted correlation	–0.11	
Litter-adjusted Poly-3 rate	8.2%	0.0%
Litter-adjusted Poly-3 test		0.063N
Sire-adjusted correlation	–0.02	
Sire-adjusted Poly-3 rate	8.0%	0.0%
Sire-adjusted Poly-3 test		0.060N
All Organs: Malignant Neoplasms		
Overall rate	2/27 (7.4%)	3/26 (11.5%)
Adjusted rate	2/25.2 (7.9%)	3/26.0 (11.5%)
Terminal rate	1/24 (4.2%)	3/26 (11.5%)
First incidence (days)	192	213 (T)
Poly-3 test		0.515
Litter-adjusted correlation	–0.21	
Litter-adjusted Poly-3 rate	8.2%	11.8%
Litter-adjusted Poly-3 test		0.327
Sire-adjusted correlation	–0.12	
Sire-adjusted Poly-3 rate	7.3%	12.1%
Sire-adjusted Poly-3 test		0.279

TABLE G9
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
All Organs: Benign and Malignant Neoplasms		
Overall rate	4/27 (14.8%)	3/26 (11.5%)
Adjusted rate	4/25.2 (15.9%)	3/26.0 (11.5%)
Terminal rate	3/24 (12.5%)	3/26 (11.5%)
First incidence (days)	192	213 (T)
Poly-3 test		0.482N
Litter-adjusted correlation	–0.34	
Litter-adjusted Poly-3 rate	15.9%	12.1%
Litter-adjusted Poly-3 test		0.344N
Sire-adjusted correlation	–0.12	
Sire-adjusted Poly-3 rate	14.5%	11.1%
Sire-adjusted Poly-3 test		0.353N

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE G10
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
All Organs: Histiocytic Sarcoma				
Overall rate[a]	0/26 (0.0%)	1/27 (3.7%)	0/27 (0.0%)	1/27 (3.7%)
Adjusted rate[b]	0/24.5 (0.0%)	1/25.5 (3.9%)	0/25.1 (0.0%)	1/24.4 (4.1%)
Terminal rate[c]	0/23 (0.0%)	0/23 (0.0%)	0/24 (0.0%)	0/21 (0.0%)
First incidence (days)	—[e]	212	—[f]	278
Poly-3 test[d]	0.378	0.509	—	0.500
Litter-adjusted correlation	–0.12			
Litter-adjusted Poly-3 rate	0.0%	5.0%	0.0%	4.6%
Litter-adjusted Poly-3 test	0.260	0.116	—	0.141
Sire-adjusted correlation	–0.02			
Sire-adjusted Poly-3 rate	0.0%	4.2%	0.1%	4.1%
Sire-adjusted Poly-3 test	0.254	0.123	—	0.144
All Organs: Malignant Lymphoma				
Overall rate	0/26 (0.0%)	0/27 (0.0%)	1/27 (3.7%)	3/27 (11.1%)
Adjusted rate	0/24.5 (0.0%)	0/24.8 (0.0%)	1/25.1 (4.0%)	3/24.7 (12.2%)
Terminal rate	0/23 (0.0%)	0/23 (0.0%)	1/24 (4.2%)	2/21 (9.5%)
First incidence (days)	—[e]	—	322 (T)	241
Poly-3 test	0.020*	—	0.505	0.115
Litter-adjusted correlation	–0.01			
Litter-adjusted Poly-3 rate	0.0%	0.0%	4.0%	12.2%
Litter-adjusted Poly-3 test	0.023*	—	0.148	0.033*
Sire-adjusted correlation	–0.03			
Sire-adjusted Poly-3 rate	0.0%	0.0%	4.1%	12.1%
Sire-adjusted Poly-3 test	0.018*	—	0.137	0.032*
All Organs: Mesothelioma				
Overall rate	1/26 (3.8%)	0/27 (0.0%)	0/27 (0.0%)	0/27 (0.0%)
Adjusted rate	1/24.7 (4.0%)	0/24.8 (0.0%)	0/25.1 (0.0%)	0/24.1 (0.0%)
Terminal rate	0/23 (0.0%)	0/23 (0.0%)	0/24 (0.0%)	0/21 (0.0%)
First incidence (days)	291	—	—	—
Poly-3 test	0.186N	0.499N	0.497N	0.506N
Litter-adjusted correlation	0.00			
Litter-adjusted Poly-3 rate	4.1%	0.0%	0.0%	0.0%
Litter-adjusted Poly-3 test	0.155N	0.155N	0.155N	0.155N
Sire-adjusted correlation	0.00			
Sire-adjusted Poly-3 rate	4.1%	0.0%	0.0%	0.0%
Sire-adjusted Poly-3 test	0.156N	0.156N	0.156N	0.156N
All Organs: Osteosarcoma				
Overall rate	1/26 (3.8%)	2/27 (7.4%)	3/27 (11.1%)	1/27 (3.7%)
Adjusted rate	1/24.9 (4.0%)	2/25.2 (7.9%)	3/25.1 (12.0%)	1/24.4 (4.1%)
Terminal rate	0/23 (0.0%)	1/23 (4.3%)	3/24 (12.5%)	0/21 (0.0%)
First incidence (days)	269	282	320 (T)	287
Poly-3 test	0.486	0.504	0.305	0.756
Litter-adjusted correlation	0.32			
Litter-adjusted Poly-3 rate	3.2%	6.5%	11.6%	4.3%
Litter-adjusted Poly-3 test	0.323	0.317	0.102	0.423
Sire-adjusted correlation	0.07			
Sire-adjusted Poly-3 rate	4.0%	7.4%	11.4%	4.0%
Sire-adjusted Poly-3 test	0.406	0.339	0.120	0.498N

TABLE G10
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
All Organs: Benign Neoplasms				
Overall rate	1/26 (3.8%)	4/27 (14.8%)	2/27 (7.4%)	1/27 (3.7%)
Adjusted rate	1/24.5 (4.1%)	4/24.8 (16.1%)	2/25.1 (8.0%)	1/24.1 (4.2%)
Terminal rate	1/23 (4.3%)	4/23 (17.4%)	2/24 (8.3%)	1/21 (4.8%)
First incidence (days)	320 (T)	319 (T)	322 (T)	321 (T)
Poly-3 test	0.452N	0.177	0.509	0.757
Litter-adjusted correlation	–0.06			
Litter-adjusted Poly-3 rate	4.0%	15.6%	7.8%	4.4%
Litter-adjusted Poly-3 test	0.361N	0.074	0.284	0.476
Sire-adjusted correlation	–0.05			
Sire-adjusted Poly-3 rate	4.2%	15.1%	6.9%	4.2%
Sire-adjusted Poly-3 test	0.339N	0.079	0.342	0.496
All Organs: Malignant Neoplasms				
Overall rate	3/26 (11.5%)	3/27 (11.1%)	6/27 (22.2%)	7/27 (25.9%)
Adjusted rate	3/26.0 (11.5%)	3/25.9 (11.6%)	6/25.3 (23.7%)	7/25.6 (27.3%)
Terminal rate	0/23 (0.0%)	1/23 (4.3%)	5/24 (20.8%)	3/21 (14.3%)
First incidence (days)	184	212	298	241
Poly-3 test	0.053	0.663	0.220	0.139
Litter-adjusted correlation	0.02			
Litter-adjusted Poly-3 rate	11.5%	11.4%	23.8%	27.3%
Litter-adjusted Poly-3 test	0.036*	0.494N	0.107	0.066
Sire-adjusted correlation	0.09			
Sire-adjusted Poly-3 rate	12.5%	10.4%	21.3%	28.3%
Sire-adjusted Poly-3 test	0.028*	0.410N	0.127	0.053
All Organs: Benign or Malignant Neoplasms				
Overall rate	4/26 (15.4%)	7/27 (25.9%)	8/27 (29.6%)	8/27 (29.6%)
Adjusted rate	4/26.0 (15.4%)	7/25.9 (27.0%)	8/25.3 (31.6%)	8/25.6 (31.2%)
Terminal rate	1/23 (4.3%)	5/23 (21.7%)	7/24 (29.2%)	4/21 (19.0%)
First incidence (days)	184	212	298	241
Poly-3 test	0.105	0.248	0.149	0.154
Litter-adjusted correlation	–0.10			
Litter-adjusted Poly-3 rate	15.4%	27.4%	30.9%	31.6%
Litter-adjusted Poly-3 test	0.067	0.123	0.099	0.067
Sire-adjusted correlation	0.06			
Sire-adjusted Poly-3 rate	15.8%	26.5%	30.9%	31.8%
Sire-adjusted Poly-3 test	0.080	0.163	0.094	0.074

(T) Terminal kill
* Significantly different (P≤0.05) from by the Poly-3 test
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group 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**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE G11
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
All Organs: Granulocytic Leukemia		
Overall rate[a]	0/26 (0.0%)	1/26 (3.8%)
Adjusted rate[b]	0/25.5 (0.0%)	1/24.7 (4.1%)
Terminal rate[c]	0/25 (0.0%)	1/23 (4.3%)
First incidence (days)	—[e]	320 (T)
Poly-3 test[d]		0.493
Litter-adjusted correlation	0.02	
Litter-adjusted Poly-3 rate	0.0%	4.2%
Litter-adjusted Poly-3 test		0.158
Sire-adjusted correlation	0.01	
Sire-adjusted Poly-3 rate	0.0%	4.2%
Sire-adjusted Poly-3 test		0.160
All Organs: Benign Neoplasms		
Overall rate	2/26 (7.7%)	1/26 (3.8%)
Adjusted rate	2/25.5 (7.8%)	1/24.7 (4.1%)
Terminal rate	2/25 (8.0%)	1/23 (4.3%)
First incidence (days)	320 (T)	318 (T)
Poly-3 test		0.512N
Litter-adjusted correlation	–0.11	
Litter-adjusted Poly-3 rate	7.7%	4.6%
Litter-adjusted Poly-3 test		0.314N
Sire-adjusted correlation	–0.08	
Sire-adjusted Poly-3 rate	7.7%	5.5%
Sire-adjusted Poly-3 test		0.368N
All Organs: Malignant Neoplasms		
Overall rate	2/26 (7.7%)	3/26 (11.5%)
Adjusted rate	2/26.0 (7.7%)	3/25.1 (11.9%)
Terminal rate	1/25 (4.0%)	2/23 (8.7%)
First incidence (days)	259	260
Poly-3 test		0.484
Litter-adjusted correlation	–0.02	
Litter-adjusted Poly-3 rate	7.7%	11.8%
Litter-adjusted Poly-3 test		0.309
Sire-adjusted correlation	0.03	
Sire-adjusted Poly-3 rate	8.4%	12.2%
Sire-adjusted Poly-3 test		0.319

TABLE G11
Litter-adjusted Statistical Analysis of Primary Neoplasms in Female Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
All Organs: Benign and Malignant Neoplasms		
Overall rate	4/26 (15.4%)	4/26 (15.4%)
Adjusted rate	4/26.0 (15.4%)	4/25.1 (15.9%)
Terminal rate	3/25 (12.0%)	3/23 (13.0%)
First incidence (days)	259	260
Poly-3 test		0.628
Litter-adjusted correlation	0.17	
Litter-adjusted Poly-3 rate	15.5%	16.7%
Litter-adjusted Poly-3 test		0.459
Sire-adjusted correlation	0.01	
Sire-adjusted Poly-3 rate	15.8%	15.9%
Sire-adjusted Poly-3 test		0.495

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE G12
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Kidney: Cellular Infiltration		
Overall rate[a]	7/27 (25.9%)	2/26 (7.7%)
Adjusted rate[b]	7/27.0 (25.9%)	2/22.8 (8.8%)
Terminal rate[c]	7/27 (25.9%)	2/21 (9.5%)
First incidence (days)	212 (T)	213 (T)
Average severity	1.0	1.0
Poly-3 test[d]		0.115N
Litter-adjusted correlation	0.34	
Litter-adjusted Poly-3 rate	25.5%	9.6%
Litter-adjusted Poly-3 test		0.076N
Sire-adjusted correlation	–0.17	
Sire-adjusted Poly-3 rate	30.0%	3.7%
Sire-adjusted Poly-3 test		0.003N**
SW Test[e]		0.040N*
Lymph Node (Mesenteric): Hyperplasia		
Overall rate	19/27 (70.4%)	16/25 (64.0%)
Adjusted rate	19/27.0 (70.4%)	16/23.5 (68.1%)
Terminal rate	19/27 (70.4%)	15/21 (71.4%)
First incidence (days)	212 (T)	125
Average severity	1.9	1.4
Poly-3 test		0.551N
Litter-adjusted correlation	0.04	
Litter-adjusted Poly-3 rate	70.2%	68.2%
Litter-adjusted Poly-3 test		0.439N
Sire-adjusted correlation	0.02	
Sire-adjusted Poly-3 rate	70.2%	68.3%
Sire-adjusted Poly-3 test		0.431N
SW Test		0.045N*

TABLE G12
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 $p53^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	240/120/240 mg/kg
Salivary Gland: Cellular Infiltration		
Overall rate	5/27 (18.5%)	0/26 (0.0%)
Adjusted rate	5/27.0 (18.5%)	0/22.8 (0.0%)
Terminal rate	5/27 (18.5%)	0/21 (0.0%)
First incidence (days)	212 (T)	—[f]
Average severity	1.0	—[f]
Poly-3 test		0.042N*
Litter-adjusted correlation	–0.12	
Litter-adjusted Poly-3 rate	18.6%	0.0%
Litter-adjusted Poly-3 test		0.004N**
Sire-adjusted correlation	–0.05	
Sire-adjusted Poly-3 rate	18.4%	0.0%
Sire-adjusted Poly-3 test		0.003N**
SW Test		0.011N*

(T) Terminal kill
* Significantly different ($P \leq 0.05$) by the Poly-3 test or the SW test.
** $P \leq 0.01$
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group by William's modified Shirley's test. A lower incidence in the dosed group is indicated by **N**.
[f] Not applicable; no lesions in animal group

TABLE G13
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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Brain (Cerebrum): Mineralization				
Overall rate[a]	4/27 (14.8%)	1/27 (3.7%)	2/27 (7.4%)	0/27 (0.0%)
Adjusted rate[b]	4/24.5 (16.3%)	1/22.8 (4.4%)	2/27.0 (7.4%)	0/23.7 (0.0%)
Terminal rate[c]	4/24 (16.7%)	1/21 (4.8%)	2/27 (7.4%)	0/22 (0.0%)
First incidence (days)	320 (T)	324 (T)	322 (T)	—[f]
Average severity	1.0	1.0	1.0	—[f]
Poly-3 test[d]	0.037N*	0.195N	0.289N	0.058N
Litter-adjusted correlation	0.00			
Litter-adjusted Poly-3 rate	16.3%	4.4%	7.4%	0.0%
Litter-adjusted Poly-3 test	0.027N*	0.085N	0.164N	0.016N*
Sire-adjusted correlation	0.02			
Sire-adjusted Poly-3 rate	16.3%	4.5%	7.5%	0.0%
Sire-adjusted Poly-3 test	0.045N*	0.105N	0.192N	0.029N*
JT/SW Test[e]	0.028N*	0.081N	0.136N	0.016N*
Large Intestine (Cecum): Hyperplasia				
Overall rate	0/26 (0.0%)	2/26 (7.7%)	2/27 (7.4%)	3/25 (12.0%)
Adjusted rate	0/24.4 (0.0%)	2/22.8 (8.8%)	2/27.0 (7.4%)	3/23.0 (13.0%)
Terminal rate	0/24 (0.0%)	2/21 (9.5%)	2/27 (7.4%)	3/22 (13.6%)
First incidence (days)	–	319 (T)	321 (T)	321 (T)
Average severity	–	2.5	2.0	2.0
Poly-3 test	0.080	0.220	0.259	0.103
Litter-adjusted correlation	–0.08			
Litter-adjusted Poly-3 rate	0.0%	8.8%	7.5%	12.9%
Litter-adjusted Poly-3 test	0.048*	0.060	0.062	0.034*
Sire-adjusted correlation	–0.03			
Sire-adjusted Poly-3 rate	0.0%	8.7%	7.5%	13.6%
Sire-adjusted Poly-3 test	0.029*	0.062	0.062	0.019*
JT/SW Test	0.059	0.077	0.127	0.056
Kidney: Cellular Infiltration				
Overall rate	6/26 (23.1%)	11/27 (40.7%)	6/27 (22.2%)	4/26 (15.4%)
Adjusted rate	6/24.4 (24.6%)	11/23.5 (46.8%)	6/27.0 (22.2%)	4/23.7 (16.9%)
Terminal rate	6/24 (25.0%)	10/21 (47.6%)	6/27 (22.2%)	4/22 (18.2%)
First incidence (days)	318 (T)	217	320 (T)	318 (T)
Average severity	1.0	1.0	1.0	1.3
Poly-3 test	0.151N	0.095	0.550N	0.382N
Litter-adjusted correlation	–0.14			
Litter-adjusted Poly-3 rate	24.3%	46.7%	22.6%	17.1%
Litter-adjusted Poly-3 test	0.103N	0.050	0.435N	0.261N
Sire-adjusted correlation	–0.01			
Sire-adjusted Poly-3 rate	24.6%	47.0%	22.5%	17.2%
Sire-adjusted Poly-3 test	0.130N	0.034*	0.422N	0.275N
JT/SW Test	0.155N	0.914N	0.554N	0.373N

TABLE G13
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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Liver: Cytoplasmic Vacuolization				
Overall rate	20/26 (76.9%)	17/27 (63.0%)	23/27 (85.2%)	13/27 (48.1%)
Adjusted rate	20/24.4 (82.1%)	17/22.8 (74.6%)	23/27.0 (85.2%)	13/23.7 (54.9%)
Terminal rate	20/24 (83.3%)	17/21 (81.0%)	23/27 (85.2%)	13/22 (59.1%)
First incidence (days)	318 (T)	317 (T)	318 (T)	318 (T)
Average severity	1.6	1.8	1.5	1.5
Poly-3 test	0.052N	0.392N	0.530	0.036N*
Litter-adjusted correlation	0.10			
Litter-adjusted Poly-3 rate	82.0%	75.0%	85.3%	55.3%
Litter-adjusted Poly-3 test	0.039N*	0.297N	0.366	0.017N*
Sire-adjusted correlation	–0.04			
Sire-adjusted Poly-3 rate	81.5%	73.5%	85.5%	54.8%
Sire-adjusted Poly-3 test	0.042N*	0.245N	0.341	0.014N*
JT/SW Test	0.022N*	0.343N	0.525N	0.012N*
Preputial Gland: Degeneration				
Overall rate	0/27 (0.0%)	0/26 (0.0%)	0/27 (0.0%)	3/26 (11.5%)
Adjusted rate	0/24.5 (0.0%)	0/22.8 (0.0%)	0/27.0 (0.0%)	3/24.7 (12.2%)
Terminal rate	0/24 (0.0%)	0/21 (0.0%)	0/27 (0.0%)	2/22 (9.1%)
First incidence (days)	—	—	—	94
Average severity	—	—	—	3.0
Poly-3 test	0.017*	—[g]	—	0.115
Litter-adjusted correlation	0.01			
Litter-adjusted Poly-3 rate	0.0%	0.0%	0.0%	12.2%
Litter-adjusted Poly-3 test	0.033*	—	—	0.033*
Sire-adjusted correlation	0.04			
Sire-adjusted Poly-3 rate	—	—	—	—
Sire-adjusted Poly-3 test	—	—	—	—
JT/SW Test	0.009**	0.500	0.583	0.007**

(T) Terminal kill
* Significantly different (P≤0.05) by the Poly-3 test or the JT/SW test
** P≤0.01
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group 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**.
[e] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group and that dosed group by William's modified Shirley's test. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no lesions in animal group
[g] Value of statistic cannot be computed.

TABLE G14
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Male Heterozygous F1 p53$^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Liver: Cytoplasmic Vacuolization		
Overall rate[a]	23/24 (95.8%)	18/25 (72.0%)
Adjusted rate[b]	23/23.4 (98.2%)	18/23.5 (76.7%)
Terminal rate[c]	23/23 (100.0%)	18/22 (81.8%)
First incidence (days)	294 (T)	317 (T)
Average severity	1.7	1.6
Poly-3 test[d]		0.026N*
Litter-adjusted correlation	0.27	
Litter-adjusted Poly-3 rate	98.6%	77.4%
Litter-adjusted Poly-3 test		0.022N*
Sire-adjusted correlation	–0.10	
Sire-adjusted Poly-3 rate	96.3%	74.4%
Sire-adjusted Poly-3 test		0.009N**
SW Test[e]		0.043N*

(T) Terminal kill
* Significantly different (P≤0.05) by the Poly-3 test or SW test
** P≤0.01
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group by William's modified Shirley's test. A lower incidence in the dosed group is indicated by **N**.

TABLE G15
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	
Large Intestine (Cecum): Hyperplasia		
Overall rate[a]	0/26 (0.0%)	2/26 (7.7%)
Adjusted rate[b]	0/24.7 (0.0%)	2/26.0 (7.7%)
Terminal rate[c]	0/24 (0.0%)	2/26 (7.7%)
First incidence (days)	—[f]	214 (T)
Average severity	—[f]	2.0
Poly-3 test[d]		0.247
Litter-adjusted correlation	–0.41	
Litter-adjusted Poly-3 rate	0.0%	11.2%
Litter-adjusted Poly-3 test		0.042*
Sire-adjusted correlation	–0.06	
Sire-adjusted Poly-3 rate	0.1%	7.9%
Sire-adjusted Poly-3 test		0.060
SW Test[e]		0.077
Kidney: Cellular Infiltration		
Overall rate	8/26 (30.8%)	3/26 (11.5%)
Adjusted rate	8/24.7 (32.4%)	3/26.0 (11.5%)
Terminal rate	8/24 (33.3%)	3/26 (11.5%)
First incidence (days)	212 (T)	213 (T)
Average severity	1.1	1.0
Poly-3 test		0.070N
Litter-adjusted correlation	0.12	
Litter-adjusted Poly-3 rate	31.5%	11.8%
Litter-adjusted Poly-3 test		0.042N*
Sire-adjusted correlation	–0.04	
Sire-adjusted Poly-3 rate	33.3%	11.6%
Sire-adjusted Poly-3 test		0.004N**
SW Test		0.043N*

TABLE G15
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 p53$^{+/-}$ Mice in the 30-Week *In Utero*/Postnatal Gavage Study of AZT

	0/0/0 mg/kg	
Salivary Gland: Cellular Infiltration		
Overall rate	14/26 (53.8%)	7/26 (26.9%)
Adjusted rate	14/24.7 (56.7%)	7/26.0 (26.9%)
Terminal rate	14/24 (58.3%)	7/26 (26.9%)
First incidence (days)	211 (T)	213 (T)
Average severity	1.1	1.1
Poly-3 test		0.028N*
Litter-adjusted correlation	–0.29	
Litter-adjusted Poly-3 rate	57.3%	26.0%
Litter-adjusted Poly-3 test		0.006N**
Sire-adjusted correlation	–0.10	
Sire-adjusted Poly-3 rate	55.5%	26.8%
Sire-adjusted Poly-3 test		0.016N*
SW Test		0.031N*

(T) Terminal kill
* Significantly different (P≤0.05) by the Poly-3 test or SW test
** P≤0.01
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0/0 mg/kg group and that dosed group by William's modified Shirley's test. A lower incidence in the dosed group is indicated by **N**.
[f] Not applicable, no lesions in animal group

TABLE G16
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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Adrenal Cortex: Subscapular Hyperplasia				
Overall rate[a]	25/26 (96.2%)	25/27 (92.6%)	27/27 (100.0%)	24/26 (92.3%)
Adjusted rate[b]	25/26.0 (96.2%)	25/25.7 (97.5%)	27/27.0 (100.0%)	24/24.7 (97.2%)
Terminal rate[c]	22/23 (95.7%)	23/23 (100.0%)	24/24 (100.0%)	21/21 (100.0%)
First incidence (days)	184	212	162	274
Average severity	1.0	1.2	1.2	1.2
Poly-3 test[d]	0.432	0.710	0.492	0.727
Litter-adjusted correlation	–0.07			
Litter-adjusted Poly-3 rate	95.9%	97.2%	100%	97.2%
Litter-adjusted Poly-3 test	0.322	0.390	0.144	0.392
Sire-adjusted correlation	–0.03			
Sire-adjusted Poly-3 rate	96.1%	97.3%	100%	97.2%
Sire-adjusted Poly-3 test	0.332	0.389	0.146	0.402
JT/SW Test[e]	0.108	0.153	0.030*[f]	0.096
Bone Marrow: Myeloid Cell Hyperplasia				
Overall rate	0/26 (0.0%)	0/26 (0.0%)	4/26 (15.4%)	1/26 (3.8%)
Adjusted rate	0/24.5 (0.0%)	0/24.8 (0.0%)	4/25.8 (15.5%)	1/24.6 (4.1%)
Terminal rate	0/23 (0.0%)	0/23 (0.0%)	3/24 (12.5%)	0/21 (0.0%)
First incidence (days)	—[g]	—	185	50
Average severity	—[g]	—	2.5	2.0
Poly-3 test	0.103	—[h]	0.061	0.501
Litter-adjusted correlation	–0.08			
Litter-adjusted Poly-3 rate	0.0%	0.0%	15.6%	4.0%
Litter-adjusted Poly-3 test	0.020*	—	0.008**	0.154
Sire-adjusted correlation	–0.01			
Sire-adjusted Poly-3 rate	0.0%	0.0%	15.6%	4.1%
Sire-adjusted Poly-3 test	0.019*	—	0.013*	0.139
JT/SW Test	0.079	0.500	0.007**[f]	0.066
Brain (Cerebrum): Mineralization				
Overall rate	0/26 (0.0%)	1/27 (3.7%)	4/27 (14.8%)	2/27 (7.4%)
Adjusted rate	0/24.5 (0.0%)	1/24.8 (4.0%)	4/25.1 (15.9%)	2/24.1 (8.3%)
Terminal rate	0/23 (0.0%)	1/23 (4.3%)	4/24 (16.7%)	2/21 (9.5%)
First incidence (days)	—	322 (T)	321 (T)	321 (T)
Average severity	—	1.0	1.0	1.0
Poly-3 test	0.073	0.503	0.058	0.231
Litter-adjusted correlation	–0.13			
Litter-adjusted Poly-3 rate	0.0%	3.8%	16.1%	8.6%
Litter-adjusted Poly-3 test	0.020*	0.151	0.007**	0.064
Sire-adjusted correlation	–0.07			
Sire-adjusted Poly-3 rate	0.0%	4.6%	15.6%	7.9%
Sire-adjusted Poly-3 test	0.021*	0.109	0.002**	0.065
JT/SW Test	0.062	0.163	0.015*[f]	0.064

TABLE G16
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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Kidney: Cellular Infiltration				
Overall rate	9/26 (34.6%)	6/27 (22.2%)	5/27 (18.5%)	4/27 (14.8%)
Adjusted rate	9/24.5 (36.8%)	6/24.8 (24.2%)	5/25.1 (19.9%)	4/24.5 (16.4%)
Terminal rate	9/23 (39.1%)	6/23 (26.1%)	5/24 (20.8%)	3/21 (14.3%)
First incidence (days)	320 (T)	319 (T)	320 (T)	274
Average severity	1.1	1.2	1.0	1.0
Poly-3 test	0.056N	0.258N	0.158N	0.095N
Litter-adjusted correlation	–0.04			
Litter-adjusted Poly-3 rate	36.9%	23.5%	20.0%	16.4%
Litter-adjusted Poly-3 test	0.034N*	0.129N	0.084N	0.033N*
Sire-adjusted correlation	0.06			
Sire-adjusted Poly-3 rate	36.7%	26.8%	20.5%	16.4%
Sire-adjusted Poly-3 test	0.014N*	0.175N	0.085N	0.026N*
JT/SW Test	0.038N*	0.171N	0.098N	0.048N*
Liver: Tension Lipidosis				
Overall rate	3/26 (11.5%)	5/27 (18.5%)	7/27 (25.9%)	4/27 (14.8%)
Adjusted rate	3/24.5 (12.3%)	5/25.2 (19.9%)	7/25.1 (27.9%)	4/24.1 (16.6%)
Terminal rate	3/23 (13.0%)	4/23 (17.4%)	7/24 (29.2%)	4/21 (19.0%)
First incidence (days)	319 (T)	282	320 (T)	318 (T)
Average severity	1.0	1.6	1.1	1.0
Poly-3 test	0.307	0.367	0.154	0.491
Litter-adjusted correlation	–0.17			
Litter-adjusted Poly-3 rate	11.7%	17.9%	28.0%	16.1%
Litter-adjusted Poly-3 test	0.229	0.260	0.047*	0.328
Sire-adjusted correlation	–0.07			
Sire-adjusted Poly-3 rate	11.8%	21.9%	27.3%	16.3%
Sire-adjusted Poly-3 test	0.294	0.149	0.070	0.335
JT/SW Test	0.339	0.203	0.110	0.258
Liver: Cytoplasmic Vacuolization				
Overall rate	6/26 (23.1%)	4/27 (14.8%)	3/27 (11.1%)	10/27 (37.0%)
Adjusted rate	6/24.5 (24.5%)	4/24.8 (16.1%)	3/25.3 (11.9%)	10/24.5 (40.9%)
Terminal rate	6/23 (26.1%)	4/23 (17.4%)	2/24 (8.3%)	9/21 (42.9%)
First incidence (days)	320 (T)	317 (T)	298	274
Average severity	1.2	1.0	1.3	1.0
Poly-3 test	0.150	0.354N	0.215N	0.179
Litter-adjusted correlation	–0.11			
Litter-adjusted Poly-3 rate	24.8%	15.5%	11.5%	40.2%
Litter-adjusted Poly-3 test	0.147	0.184N	0.092N	0.119
Sire-adjusted correlation	–0.00			
Sire-adjusted Poly-3 rate	24.5%	16.0%	11.8%	40.9%
Sire-adjusted Poly-3 test	0.121	0.213N	0.102N	0.104
JT/SW Test	0.152	0.792	0.906	0.163

TABLE G16
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

	0/0/0 mg/kg	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 mg/kg
Pancreas: Cellular Infiltration				
Overall rate	0/26 (0.0%)	0/27 (0.0%)	1/27 (3.7%)	3/26 (11.5%)
Adjusted rate	0/24.5 (0.0%)	0/24.8 (0.0%)	1/25.1 (4.0%)	3/23.4 (12.8%)
Terminal rate	0/23 (0.0%)	0/23 (0.0%)	1/24 (4.2%)	3/21 (14.3%)
First incidence (days)	—	—	322 (T)	321 (T)
Average severity	—	—	1.0	1.0
Poly-3 test	0.018*	—	0.505	0.106
Litter-adjusted correlation	–0.06			
Litter-adjusted Poly-3 rate	0.0%	0.0%	4.0%	13.0%
Litter-adjusted Poly-3 test	0.017*	—	0.148	0.025*
Sire-adjusted correlation	–0.02			
Sire-adjusted Poly-3 rate	0.0%	0.0%	3.9%	12.9%
Sire-adjusted Poly-3 test	0.015*	—	0.154	0.025*
JT/SW Test	0.011*	0.500	0.135	0.017*
Salivary Gland: Cellular Infiltration				
Overall rate	15/26 (57.7%)	14/27 (51.9%)	10/26 (38.5%)	10/27 (37.0%)
Adjusted rate	15/24.9 (60.2%)	14/24.9 (56.2%)	10/24.3 (41.1%)	10/24.7 (40.4%)
Terminal rate	14/23 (60.9%)	13/23 (56.5%)	10/24 (41.7%)	8/21 (38.1%)
First incidence (days)	269	312	320 (T)	274
Average severity	1.2	1.1	1.3	1.0
Poly-3 test	0.058N	0.498N	0.145N	0.131N
Litter-adjusted correlation	0.07			
Litter-adjusted Poly-3 rate	59.9%	56.6%	41.0%	40.8%
Litter-adjusted Poly-3 test	0.052N	0.410N	0.084N	0.089N
Sire-adjusted correlation	0.01			
Sire-adjusted Poly-3 rate	60.2%	56.3%	41.3%	40.5%
Sire-adjusted Poly-3 test	0.080N	0.399N	0.091N	0.124N
JT/SW Test	0.032N*	0.250N	0.134N	0.049N*

(T) Terminal kill
* Significantly different (P≤0.05) by the Poly-3 test or the JT/SW test
** P≤0.01
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group 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**.
[e] Beneath the 0/0/0 mg/kg group 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 0/0/0 mg/kg group 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**.
[f] The P value from the William's modified Shirley's test was significant, but the test violated the monotonic constraint.
[g] Not applicable, no lesions in animal group
[h] Value of statistic cannot be computed.

TABLE G17
Litter-adjusted Statistical Analysis of Selected Nonneoplastic Lesions in Female Heterozygous F1 $p53^{+/-}$ Mice in the 45-Week *In Utero*/Postnatal Gavage Stop-Study of AZT

	0/0 mg/kg	240/40 mg/kg
Large Intestine (Cecum): Hyperplasia		
Overall rate[a]	3/25 (12.0%)	0/26 (0.0%)
Adjusted rate[b]	3/25.0 (12.0%)	0/24.7 (0.0%)
Terminal rate[c]	3/25 (12.0%)	0/23 (0.0%)
First incidence (days)	318 (T)	—[f]
Average severity	2.0	—[f]
Poly-3 test[d]		0.116N
Litter-adjusted correlation	–0.07	
Litter-adjusted Poly-3 rate	12.0%	0.0%
Litter-adjusted Poly-3 test		0.024N*
Sire-adjusted correlation	–0.11	
Sire-adjusted Poly-3 rate	15.1%	0.4%
Sire-adjusted Poly-3 test		<0.001N***
SW Test[e]		0.036N*
Liver: Cytoplasmic Vacuolization		
Overall rate	12/25 (48.0%)	6/26 (23.1%)
Adjusted rate	12/25.0 (48.0%)	6/25.3 (23.7%)
Terminal rate	12/25 (48.0%)	5/23 (21.7%)
First incidence (days)	317 (T)	231
Average severity	1.0	1.0
Poly-3 test		0.064N
Litter-adjusted correlation	0.04	
Litter-adjusted Poly-3 rate	48.4%	23.6%
Litter-adjusted Poly-3 test		0.031N*
Sire-adjusted correlation	0.21	
Sire-adjusted Poly-3 rate	54.2%	22.6%
Sire-adjusted Poly-3 test		0.002N**
SW Test		0.033N*

(T) Terminal kill
* Significantly different ($P \leq 0.05$) by the Poly-3 test or SW test
** $P \leq 0.01$
*** $P \leq 0.01$
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in the dosed group is indicated by **N**.
[e] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the 0/0 mg/kg group and that dosed group by William's modified Shirley's test. A lower incidence in the dosed group is indicated by **N**.
[f] Not applicable; no lesions in animal group

APPENDIX H
LITTER SUCCESS AND SURVIVAL

LITTER SUCCESS AND SURVIVAL

METHODS AND RESULTS

Plug-positive dams were provisionally assigned to dose groups on gestational day (GD) 0 and monitored for body weight gain from GD 1 to GD 10 to determine whether they were pregnant. Non-pregnant dams were returned to the breeding pool. Dosing of pregnant dams began on GD 12. More dams were assigned to the 0/0/0 (0/0) mg/kg groups and 240/120/240 (240/40) mg/kg groups because more pups were required to populate the 45-week stop-study and the 30-week continuous dosing study, which only used these doses. Litters of more than six live pups were culled to six pups (three male and three female where possible) on postnatal day (PND) 1 prior to the first postnatal dose.

Litter success and pup survival were high across all dose groups with pup losses due mainly to gavage errors rather than dose effects (Table H1). There was no significant treatment-related effect on maternal body weight during gestation (Figure H1). Although the dams in the 80 mg/kg group exhibited higher mean body weight values, this was due to heavier dams being assigned to this dose group. The body weight curves for the treated pups between PND 1 and PND 10 for the litters used for the 30- and 45-week continuous dosing studies and from PND 1 though PND 8 for the 45-week stop-study litters are given in Figures H2 and H3 respectively. These growth curves are presented separately from the main growth curves for the heterozygous F1 $p53^{+/-}$ mice because during this period the daily pup weights were derived from the total weight of each litter divided by the number of surviving pups in the litter and not all pups were loaded onto the studies. Therefore, there is not a natural continuation between these growth curves and the main growth curves, which are derived from the individual mice that were loaded onto the studies. There was no significant dose effect on neonatal body weight gain in the litters used for the continuous dosing studies (Figure H2), but neonatal stop-study mice administered 240/40 mg/kg did exhibit a slower weight gain than the corresponding pups in the 0/0 mg/kg group (Figure H3).

TABLE H1
Litter Parameters and Pup Survival for Mouse Pups in the *In Utero*/Postnatal Gavage Studies of AZT

	0/0/0 (0/0) mg/kg[a]	80/40/80 mg/kg	160/80/160 mg/kg	240/120/240 (240/40) mg/kg[a]
Total dams pregnant	50	22	19	53
Dams which did not litter	4	4	3	3
Total litters	46	18	16	50
Total pups born	314	141	105	351
Average born per litter	6.8	7.8	6.6	7.0
Number of males born[b]	152 (48.4)	69 (48.9)	56 (53.3)	174 (49.6)
Sex ratio[c]	1:0.97	1:1.01	1:0.88	1:0.94
Pups born dead (%)	14 (4.5)	2 (1.4)	0 (0)	13 (3.7)
% Survival PND 1 – PND 10[d]	92.8	98.1	97.8	95.3
% Survival PND 11 – PND 28[d]	96.8	91.1	98.9	93.8

[a] Litters dosed to provide stop-study pups are included in the litter and pup birth data but are excluded from the pup survival estimates. Postnatal survival for stop-study pups was 98.6% and 96.5% between PND 1 and PND 10 for the 0/0 mg/kg and 240/40 mg/kg groups, respectively, and 98.6% and 100% between PND 11 and PND 28 for the 0/0 mg/kg and 240/40 mg/kg groups, respectively.

[b] Excludes pups born dead, percent of total live pups given in parentheses

[c] Male:female

[d] Excludes pups culled on PND 1 and stop-study litters

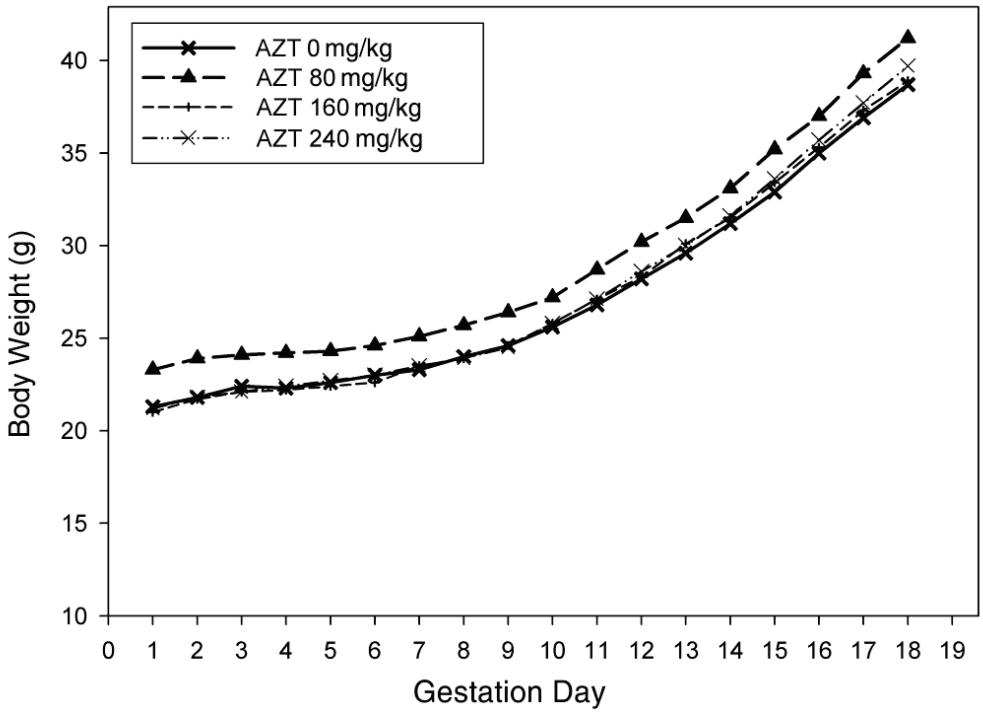

FIGURE H1
Growth Curves for Pregnant Dams Administered AZT by Gavage in the *In Utero*/Postnatal Studies of AZT

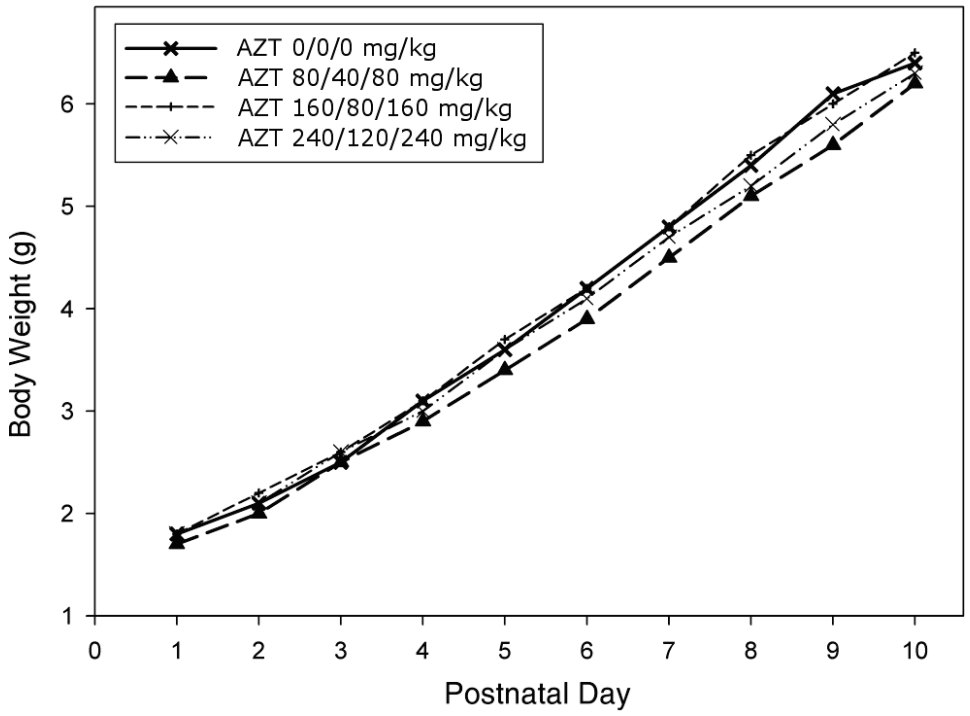

FIGURE H2
Growth Curves for Mice Administered AZT by Gavage in the 30- and 45-Week *In Utero*/Postnatal Studies of AZT

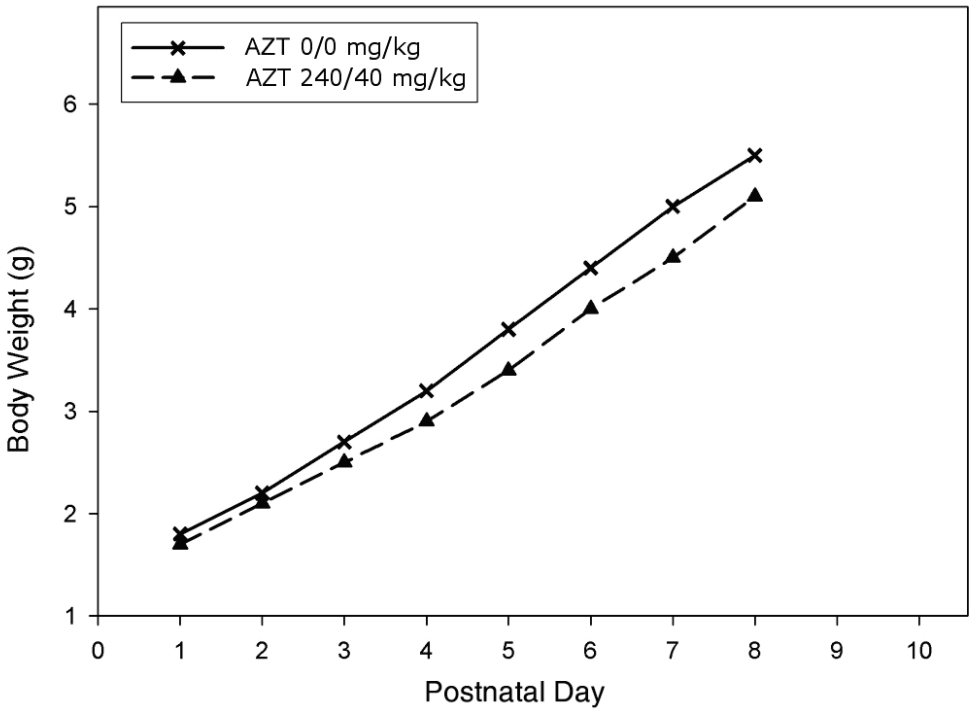

FIGURE H3
Growth Curves for Mice Administered AZT by Gavage in the 45-Week *In Utero*/Postnatal Stop-Study of AZT

APPENDIX I
HISTORICAL CONTROL INCIDENCES

TABLE I1
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 (30 Weeks)[b]	AZT (45 Weeks)[b]	AZT (45-Week Stop Study)[c]	AZT/3TC /NVP (45 Weeks)[d]	Senna (40 Weeks)[e]	Overall[f]
Bone						
Osteosarcoma[g]	0/27	0/27	0/24	1/25	0/25	1/101
Humerus, osteosarcoma	0/27	1/27	0/24	1/25	0/25	2/101
Femur, osteosarcoma	0/27	1/27	0/24	0/25	0/25	1/101
Tibia, osteosarcoma	0/27	0/27	0/24	1/25	0/25	1/101
Any location, osteosarcoma	0/27	2/27	0/24	3/25	0/25	5/101
Harderian gland						
Adenoma	0/27	1/27	1/24	0/25	0/25	2/101
Liver						
Hepatocellular adenoma	1/27	3/26	3/24	1/25	1/25	8/100
Lung						
Alveolar/bronchiolar adenoma	0/27	0/27	2/24	0/25	1/25	3/101
Mesentery						
Sarcoma	0/27	0/27	0/24	1/25	0/25	1/101
Pancreas						
Acinar cell, Carcinoma	0/27	0/27	1/23	0/24	0/25	1/99
Small intestine,						
Duodenum, leiomyosarcoma	0/27	0/26	0/24	1/23	0/25	1/98
Jejunum, adenocarcinoma	0/27	0/26	0/24	1/23	0/25	1/98
Tissue NOS,						
Sarcoma	0/27	1/26	0/24	0/25	0/25	1/100
Abdominal sarcoma	0/27	0/27	0/24	1/25	0/25	1/101
All organs						
Lymphoma, malignant	0/27	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 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] Includes 40- and 45-week studies only

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

TABLE I2
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 (30 Weeks)[b]	AZT (45 Weeks)[b]	AZT (45-Week Stop Study)[c]	AZT/3TC /NVP (45 Weeks)[d]	Senna (40 Weeks)[e]	Overall[f]
Adrenal medulla						
Pheochromocytoma, benign[g]	0/26	0/25	0/26	0/23	1/25	1/99
Bone						
Femur, osteosarcoma	0/27	1/26	0/26	0/25	0/25	1/102
Rib, osteosarcoma	0/27	0/26	0/26	0/25	1/25	1/102
Vertebra, osteosarcoma	0/27	0/26	0/26	0/25	1/25	1/102
Any location, osteosarcoma	0/27	1/26	0/26	0/25	2/25	3/102
Bone marrow						
Sarcoma	0/26	1/26	0/25	0/25	0/25	1/101
Brain						
Cerebrum, sarcoma	0/26	1/26	0/26	0/25	0/25	1/102
Large intestine						
Cecum polyp	1/26	0/25	0/25	0/25	0/25	0/100
Liver						
Sarcoma	0/26	1/26	0/25	0/25	0/25	1/101
Lung						
Alveolar/bronchiolar adenoma	0/26	0/26	0/26	1/25	0/25	1/102
Mediastium, sarcoma	0/26	1/26	0/26	0/25	0/25	1/102
Lymph node						
Mandibular, sarcoma	0/26	1/26	0/25	0/25	0/25	1/101
Mesenteric, sarcoma	0/24	1/26	0/25	0/25	0/23	1/99
Mammary gland						
Adenocarcinoma	1/24	0/25	0/26	1/25	0/25	1/101
Adenoma	0/24	0/25	1/26	0/25	0/25	1/101
Nose						
Sarcoma	0/26	1/26	0/26	0/25	0/25	1/102
Ovary						
Cystadenoma	0/26	0/26	0/26	0/25	1/25	1/102
Granuloma cell tumor, malignant	0/26	0/26	0/26	0/25	1/25	1/102
Luteoma	0/26	1/26	0/26	0/25	0/25	1/102
Teratoma malignant	0/26	0/26	1/26	0/25	0/25	1/102
Yolk sac carcinoma	0/26	0/26	1/26	0/25	0/25	1/102
Spleen						
Sarcoma	0/26	1/26	0/26	0/25	0/25	1/102
Uterus						
Polyp stromal	0/26	0/26	1/26	0/25	0/25	1/102
Endometrium, polyp stromal	1/26	0/26	0/26	0/25	0/25	0/102

TABLE I2
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 (30 Weeks)	AZT (45 Weeks)	AZT (45-Week Stop Study)	AZT/3TC /NVP (45 Weeks)	Senna (40 Weeks)	Overall
All organs						
Lymphoma, malignant	1/27	0/26	0/26	2/25	1/25	3/102
Mesothelioma, malignant	0/27	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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[f] Includes 40- and 45-week studies only

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

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-05
Durham, NC 27709
Tel: 984-287-3211
ntpwebrequest@niehs.nih.gov

https://ntp.niehs.nih.gov

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