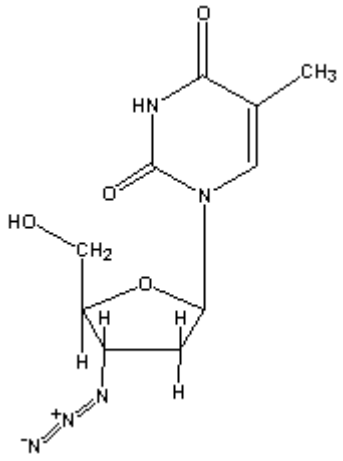
(This document has been reformatted unchanged from the original document except for the bold emphasis editing by Stuart Thomson, Director, the Gaia Research Institute. S.T.)

TR-469
Toxicology and Carcinogenesis Studies of AZT (CAS No. 30516-87-1) and AZT/-Interferon A/D B6C3F$_1$ Mice (Gavage Studies)



Chemical Formula: C10H13N5O4 –

3'-Azido-3'-deoxythymidine (AZT) is the most widely used and evaluated chemotherapeutic agent for the treatment of persons with acquired immune deficiency syndrome (AIDS) and persons seropositive for human immunodeficiency virus (HIV). ***The National Cancer Institute nominated AZT for toxicity and carcinogenicity studies because of the impending large-scale use of AZT in the treatment of adult patients with AIDS or AIDS-related complex.***

α-Interferon A/D, which displays antiviral activity in mice, is a hybrid molecule composed of the N-terminal portion of human α-interferon A and the C-terminal portion of human α-interferon D. AZT and α-interferon A/D combination studies were conducted because in vitro studies of AZT and α-interferon have demonstrated that the combination is more effective in blocking HIV infection than either agent alone. Male and female B6C3F$_1$ mice received AZT (approximately 98% pure) in 0.5% aqueous methylcellulose by gavage for 14 weeks or 2 years.

In addition, male and female B6C3F$_1$ mice received α-interferon A or α-interferon A/D by subcutaneous injection for 2 years, and male and female B6C3F$_1$ mice received AZT in 0.5% aqueous methylcellulose by gavage in combination with α-interferon A/D by subcutaneous injection for 2 years. Genetic toxicology studies were conducted in Salmonella typhimurium, cultured Chinese hamster ovary cells, mouse bone marrow erythrocytes, and mouse peripheral blood erythrocytes.

14-WEEK AZT STUDY

Groups of 10 male and 10 female mice received AZT in 0.5% methylcellulose by gavage at doses of 0, 50, 100, 200, 800, or 2,000 mg/kg daily for 14 weeks. Additional groups of 10 male and 10 female mice received AZT in 0.5% methylcellulose by gavage at doses of 0, 100, 800, or 2,000 mg/kg daily for 14 weeks and then were held without treatment for an additional 4 weeks before necropsy. One female receiving 100 mg/kg and two females receiving 200 mg/kg died during week 1 as a result of gavage trauma; one female receiving 2,000 mg/kg also died prior to the end of the 14-week dosing period. One female receiving 2,000 mg/kg in the recovery study also died from gavage trauma during week 1. The final mean body weights of dosed mice were similar to those of the vehicle control groups at the end of the dosing period and at the end of the recovery period. Female mice receiving 200, 800 or 2,000 mg/kg gained less weight than the vehicle controls during the 14-week dosing period.

Exposure to AZT was toxic to the bone marrow, resulting in significant changes in the peripheral blood (decreased hematocrit values, erythrocyte counts, and hemoglobin concentrations, and increased mean cell volume and mean cell hemoglobin) and bone marrow (erythroid hypoplasia) characteristic of a dose- and time-dependent, minimal to moderate, poorly regenerative macrocytic anemia. At the end of the 4-week recovery period, the hematology parameters had returned to normal, indicating that the hematotoxicity was reversible.

2-YEAR STUDIES

AZT

Groups of 95 male and 95 female mice received AZT in 0.5% methylcellulose by gavage at daily doses of 0, 30, 60, or 120 mg/kg body weight, administered as two equal doses at least 6 hours apart, 5 days per week for 105 weeks. Each group of 95 animals was composed of a core group of 50 animals for evaluation of carcinogenic response, a group of 30 animals for evaluation of hematology and bone marrow cellularity, and a group of 15 animals from which blood was drawn for determination of plasma AZT concentrations at week 54.

α-Interferon A/D and AZT/-Interferon A/D Studies

Groups of 80 male and 80 female mice received AZT in 0.5% aqueous methylcellulose by 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. Those groups receiving AZT also received sub-cutaneous 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, three times per week for 105 weeks.

Each group of 80 animals was composed of a core group of 50 animals for evaluation of carcinogenic response and a group of 30 animals for evaluation of hematology and bone marrow cellularity.

Because of the large number of animals involved, the 2-year studies were started in four phases and, for clarity, are presented as follows: the AZT study, the α-interferon A/D study, the AZT/500 U α-interferon A/D study, and the AZT/5,000 U α-interferon A/D study.

Design of the 2-year AZT, AZT/a-Interferon A/D, and a-Interferon A/D Studies				
AZT Dose	**AZT Study**	**AZT/500 U a-Interferon A/D Study**	**AZT/5,000 U a-Inerferon A/D Study**	**500 or 5,000 U a-Interferon A/D or 5,000 U a-Interferon A Study**
Vehicle Control	95 male and 95 female mice[a]	80 male and 80 female mice[b]	80 male and 80 female mice[b]	80 male and 80 female mice[b]
30 mg/kg AZT	95 male and 95 female mice	80 male and 80 female mice	80 male and 80 female mice	none
60 mg/kg AZT	95 male and 95 female mice	80 male and 80 female mice	80 male and 80 female mice	none
120 mg/kg AZT	95 male and 95 female mice	80 male and 80 female mice	80 male and 80 female mice	none

[a]For the AZT study, there were 95 male and 95 female mice; these were divided into 50 males and 50 females in the core groups, 30 males and 30 females in the clinical pathology groups (hematology and bone marrow analyses only), and 15 males and 15 females for plasma AZT concentration determinations.
[b]For the a-interferon A/D study and the AZT/a-interferon A/D studies, there were 80 male and 80 female mice for each study; these were divided into 50 males and 50 females in the core groups and 30 males and 30 females in the clinical pathology groups (hematology and bone marrow analyses only).

Survival and Body Weights

Survival and mean body weights of mice exposed to AZT, α-interferon A, α-interferon A/D, or AZT plus α-interferon A/D were generally similar to those of the vehicle control groups.

Hematology and Bone Marrow Analyses

All groups of male and female mice receiving AZT exhibited changes in peripheral blood and bone marrow characteristic of a dose- and time-dependent, minimal to mild, macrocytic, nonresponsive anemia. In females, these changes were evident throughout the study. In males, the macrocytic anemia had resolved by week 80 in the 30 mg/kg group; at study termination erythrocyte macrocytosis was present only in males receiving 60 or 120 mg/kg AZT or AZT plus α-interferon A/D. There were no treatment-related alterations in hematology or bone marrow parameters in groups that received only α-interferon A or A/D.

Pathology Findings

Incidences of squamous cell carcinoma and squamous cell papilloma or carcinoma (combined) of the vagina occurred with a positive trend and were significantly increased in groups of female mice receiving 60 or 120 mg/kg AZT alone or in combination with α-interferon A/D. Epithelial hyperplasia was observed in all dosed groups of females, and the incidence was significantly increased in the 120 mg/kg AZT group.

Three renal tubule adenomas and one renal tubule carcinoma were observed in male mice receiving 120 mg/kg AZT; the combined incidence in this group exceeded the range in historical controls. A renal tubule adenoma was observed in one male receiving 60 mg AZT/kg and 500 U α-interferon A/D; how ever, none were observed in other groups. Evaluation of step sections revealed a few more renal tubule hyperplasias but no additional neoplasms.

The incidence of harderian gland adenoma was increased in male mice receiving 120 mg/kg AZT and exceeded the range in historical controls. Harderian gland neoplasms were observed in other groups but did not follow a treatment-related pattern.

Overall Incidences of Vaginal Neoplasms and Hyperplasia of the Vaginal Epithelium in Female Mice in the 2-Year Gavage Studies of AZT and AZT/α-Interferon A/D[a]

	Vehicle Control	30 mg AZT/kg	60 mg AZT/kg	120 mg AZT/kg
AZT alone	2/197 (1%)b 1/197	0/49 (0%) 3/49	5/45 (11%) 4/45	11/49 (22%) 11/49
500 U α-Interferon A/D	0/49 (0%) 0/49	0/44 (0%) 4/44	5/48 (10%) 8/48	6/48 (13%) 12/48
5,000 U α-Interferon A/D	1/50 (2%) 1/50	1/48 (2%) 4/48	5/48 (10%) 8/48	4/50 (8%) 15/50

[a] Data are presented as number of vaginal neoplasms/number of animals microscopically examined (first line) and number of vaginal hyperplasias/number of animals microscopically examined (second line)
[b] Combined incidences of controls from the AZT alone study and the AZT/-interferon A/D studies; incidences in the vehicle control group from the AZT alone study are 0/50 (0%) (neoplasms) and 0/50 (hyperplasia)

Overall Incidence of Harderian Gland Neoplasms in Male Mice in the 2-Year Gavage Studies of AZT and AZT/-Interferon A/D[a]

	Vehicle Control	30 mg AZT/kg	60 mg AZT/kg	120 mg AZT/kg
AZT alone	13/200 (6%)[b]	5/50 (10%)	2/50 (4%)	10/50 (20%)
500 U α-Interferon A/D	3/50 (6%)	3/50 (6%)	1/50 (2%)	4/50 (8%)
5,000 U α-Interferon A/D	3/50 (6%)	9/50 (18%)	4/50 (8%)	4/50 (8%)

[a] Data are presented as number of harderian gland neoplasms/number of animals necropsied
[b] Combined incidences of controls from the AZT alone study and the AZT/-interferon A/D studies; incidence in the vehicle control group from the AZT alone study is 3/50 (6%)

Male mice had a pattern of nonneoplastic liver lesions along with silver-staining helical organisms within the liver consistent with an infection with Helicobacter hepaticus. An organism compatible with H. hepaticus was confirmed by polymerase chain reaction-restriction fragment length polymorphism-based assays. Detection of dose-related differences in neoplasm incidences in these studies was not considered to have been significantly impacted by the infection with H. hepaticus or its associated hepatitis.

GENETIC TOXICOLOGY

AZT is mutagenic in vitro and in vivo. It induced gene mutations in Salmonella typhimurium strain TA102, with and without S9; no increases in mutations were noted in the other tested strains of S. typhimurium. ***AZT induced sister chromatid exchanges***, but not chromosomal aberrations, in cultured Chinese hamster ovary cells, with and without S9. ***In vivo studies with male mice administered AZT by gavage showed highly significant increases in micronucleated erythrocytes in bone marrow and peripheral blood after exposure periods that ranged from 72 hours to 14 weeks.***

CONCLUSIONS

Under the conditions of these 2-year gavage studies there was equivocal evidence of carcinogenic activity of AZT in male mice based on increased incidences of renal tubule and harderian gland neoplasms in groups receiving AZT alone. There was clear evidence of carcinogenic activity of AZT in female mice based on increased incidences of squamous cell neoplasms of the vagina in groups that received AZT alone or in combination with -interferon A/D.

Hematotoxicity occurred in all groups that received AZT.

Treatment with AZT alone and AZT in combination with -interferon A/D resulted in increased incidences of epithelial hyperplasia of the vagina in all dosed groups of females.

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®

Pathology Tables, Survival and Growth Curves from NTP 2-year Studies
Summary Data from 2-year Studies
NTIS# PB99-145807
Report Date: February 1999

(Editor's Comment: Preliminary data for several years indicated that AZT represented unacceptable risks. Responsible scientists accordingly called for comprehensive testing such as that reported above, which is mandatory prior to the approval of all other drugs before licensing. AZT (as is Fluoride) is unique in that it alone was approved for "human experimentation" prior to such animal studies and this for the treatment of a disease of scientifically disputed etiology. Existing pro-AZT studies are fraudulent in that they were subject to serious methodological flaws and or were strategically terminated prematurely, prior to the drug revealing it's true unacceptable risk / benefit profile and the paradoxical inevitable suppression of immunity, not similarly experiment termination protected in the real-life applications of this radical drug.)