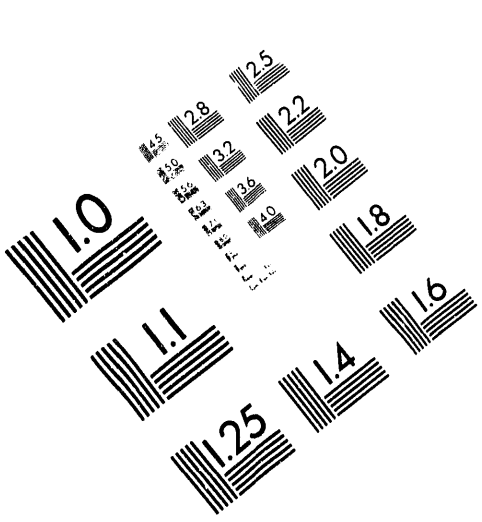

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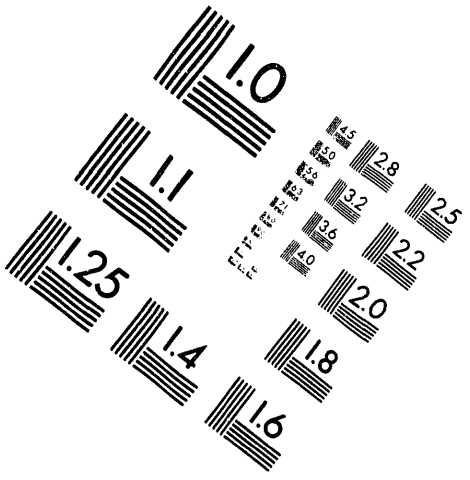

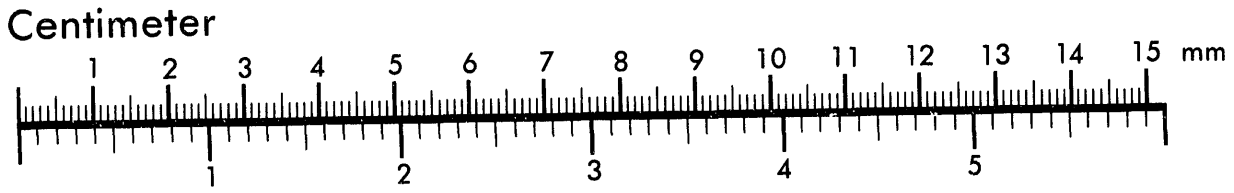

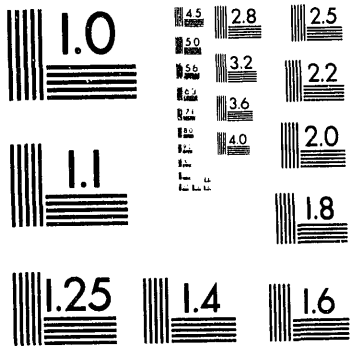

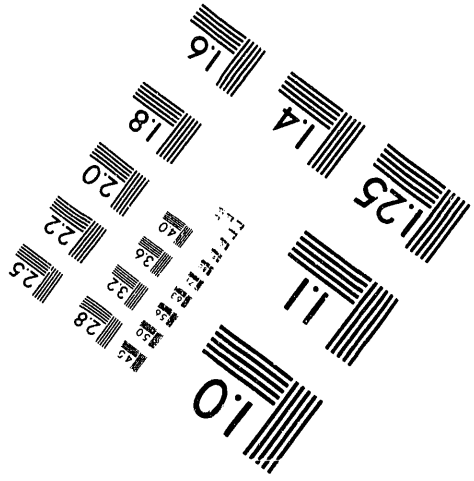

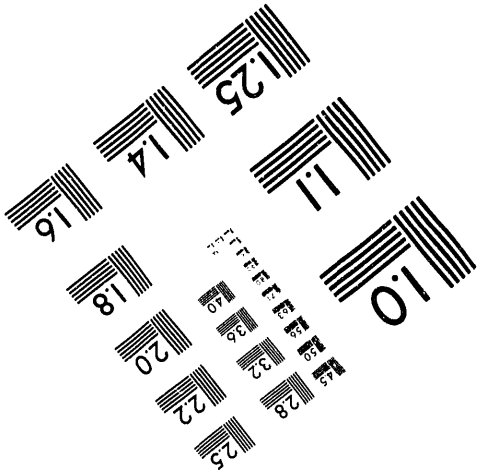

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CHEMOPREVENTION BY WR-2721

David J. Grdina,*†[1] Ph.D. and Bruce A. Carnes,* Ph.D.

*Biological and Medical Research Division, Argonne National Laboratory
Argonne, Illinois 60439, U.S.A.;
†Department of Radiation and Cellular Oncology,
University of Chicago, Chicago, Illinois 60637, U.S.A.

[1]To whom all requests for reprints should be addressed at: Biological and Medical Research Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439-4833, U.S.A.

ABSTRACT

WR-2721 [*S*-2-(3-aminopropylamino)ethylphosphorothioic acid] is an effective chemopreventive agent. C57BL × BALB/c F_1 female mice, 200 per experimental group, were exposed to a single whole-body dose of 206 cGy from a ^{60}Co photon source. Animals were sham treated or irradiated at 110 days of age. Those groups treated with WR-2721 (400 mg/kg) were administered the agent i.p. 30 min prior to irradiation. Animals were housed five to a cage and were checked daily throughout life. All deceased animals were necropsied, and tissues were removed and fixed for histopathological analysis. Over 90% of deaths were determined to be due to tumor involvement. WR-2721 afforded significant protection against life shortening due to radiation-induced tumors of connective tissue (p = 0.0224) and epithelial tissue (p = 0.0250) origins. Subsequent survival time in WR-2721-treated and irradiated animals as compared to matched irradiated-only controls was extended up to 59 days. A single exposure of animals to WR-2721 did not affect the cumulative survival curves for unirradiated mice. WR-2721 possesses chemopreventive properties which can be clinically exploited to reduce the risk to therapy-induced secondary cancers in patients who otherwise would have an excellent prognosis for cure and long-term survival.

Key words: aminothiols, carcinogenesis, carcinomas, radiation, sarcomas

Running title: Chemoprevention by WR-2721

WR-2721 [*S*-2-(3-aminopropylamino)ethylphosphorothioic acid] is a phosphorothioate which, as early as 1984, was reported to possess anticarcinogenic properties (1). Since then, WR-2721 and its corresponding free thiol, WR-1065 (2-[(aminopropyl)amino]ethanethiol), have been found to exhibit antimutagenic (2–4), anticlastogenic (5), antitransforming (6–7), and anticarcinogenic (8–9) properties in rodent systems. A close analogue of WR-2721, WR-151327 [*S*-3-(3-methylamino-propylamino)propylphosphorothioic acid], was also observed to be effective in protecting against the carcinogenic effects of ionizing radiation (10,11). Its corresponding free thiol, WR-151326, was demonstrated to be protective against the mutagenic properties of AZT (3′-azido-3′-deoxythymidine) on HepG2 cells (12). Protection against both radiation- and chemical-induced mutagenesis could be demonstrated even when the aminothiols were administered up to 3 h following the exposure of animals or cells to these mutagens (2,4). These results, coupled with the current clinical interest in this phosphorothioate as both a radiation and chemical protector (13–16), make WR-2721 a strong candidate for use as a clinically efficacious chemopreventive agent for use in reducing the risk of therapy-induced secondary tumors in patients having potentially curable disease.

MATERIALS AND METHODS

A C57BL × BALB/c F_1 mouse system was used in these studies (8). Two hundred female animals at 100 ± 7 days of age were used in each experimental group. Experimental groups included a saline-injected, unirradiated control; a WR-2721-injected, unirradiated control; a saline-injected, 206-cGy irradiated group; and a

WR-2721-injected, 206-cGy irradiated group. Animals were exposed to whole-body irradiation by ^{60}Co γ rays. WR-2721 was dissolved in sterile saline just prior to use. Thirty minutes before irradiation, animals were injected i.p. with 400 mg/kg of WR-2721. This dosage approximates two-thirds the toxic 50% lethal dose. No WR-2721-related deaths were observed. Following treatment, animals were housed five per cage, maintained in animal rooms having a 200-cage capacity, and given access to food and water at all times. Temperature and humidity were controlled to 72 ± 4 °F and 50 ± 10% relative humidity. A 12-hour light/dark cycle was used. All operational logistics, cage assignments, and locations in the holding rooms were controlled by computer and randomized. Daily death checks were carried out, and obviously moribund animals were removed and sacrificed. Under these conditions, tissues from approximately 80% of the animals in each group were suitable for histopathological examination. Histopathological analysis was performed on a contract basis by pathology Associates, Inc. (IIT Research Institute, Chicago, IL). Patterns of mortality were determined by generating Kaplan and Meier (17) survival curves. In the tumor analyses, only neoplastic deaths were considered events. Mice dying from other causes (e.g., decomposed, cannibalized, escaped) were included in the analysis but were treated as censored observations. The generalized Wilcoxon test, distributed as an χ^2, was used to identify significant differences (heterogeneity) among survival curves (18). All analyses were performed on an IBM 3033 computer at the Argonne National Laboratory using the LIFETEST procedure in the SAS software package (19).

RESULTS

Cumulative survival curves representing death from tumors of connective tissue origin (using tumor designations confirmed by histopathological examination) are presented in Figure 1. Each data point represents a separate event (i.e., an individual animal death). A listing of the tumor types used in this classification is contained in Table 1. The onset of death due to connective tissue tumors induced by irradiation was significantly delayed in the WR-2721-treated animals ($p = 0.022$). At the median survival time, animals given WR-2721 died from these tumors 51 days later than the group not injected with this chemopreventive agent.

Fig. 1 →

Table 1 →

Presented in Figure 2 are cumulative survival curves representing death from tumors of epithelial origin. A listing of these tumor types is presented in Table 2. Again, there was a significant extension of life span mediated by WR-2721 for animals dying from this class of radiation-induced tumors ($p = 0.025$). At the median survival times, the two curves were separated by 29 days.

Fig. 2 →

Table 2 →

For both general classes of tumor-related deaths, the maximum protection attributed to WR-2721 was observed during the first 800 days of age of the animals. The incidence of nontumor-associated life shortening was not found to be influenced by the radiation dose used. Finally, the primary long-term effect we observed following exposure of this mouse system to ionizing radiation was carcinogenesis.

DISCUSSION

WR-2721 is a phosphorothioate which is currently receiving considerable clinical attention for use in radiation and chemoprotection (13–16). In addition to its

protective properties against cell killing by radiation and chemicals, WR-2721 exhibits significant effectiveness as an anticarcinogenic and antimutagenic agent. We previously described these properties using a variety of systems and endpoints (2,4,8–11). In this communication, we have extended these observations to include the evaluation of WR-2721 as an agent to affect radiation-induced carcinogenesis related specifically to both connective- and epithelial-tissue-derived neoplasms. While significant protection was evidenced for both classes of tumors, the effect was more evident for connective tissue tumors. The reasons for this are at present unclear. In the mouse system used, the frequency of death attributed to connective tissue tumors is much greater than that observed for epithelial tissue tumors. The larger number of animals falling in the former group allows for better statistics when comparisons are made and may in part account for this perceived difference.

The data presented in Figures 1 and 2 do not suggest that WR-2721 is preventing the incidence of tumors due to radiation, but rather it is delaying the onset of these malignancies to the time of life at which they normally occur in untreated animals. This is not an unreasonable finding since these animals do not suffer from competing risks such as heart disease or accidents. Thus, deaths in untreated control animals are attributable to neoplastic disease in over 90% of all deaths (8,20).

The chemopreventive effectiveness of WR-2721 is also dependent upon its distribution throughout the animal at the time of radiation. WR-1065, the free thiol and principal metabolite of WR-2721, is found at maximal levels in tissues such as kidney, lung, heart, muscle, brain, spleen, and salivary gland within 5 to 15 minutes following injection with WR-2721 (21). Presumably, these tissues can be expected to

be afforded the greatest protection by WR-2721 against radiation-induced carcinogenesis.

The chemopreventive properties of WR-2721 and its analogue WR-151327 offer a significantly new clinical application for these agents. The ability of these agents to protect against radiation-induced carcinogenesis in rodent models has been clearly demonstrated (8–11). Of considerable interest, however, have been the observations that these agents have antimutational properties even when they are administered up to 3 h following radiation or chemical exposure (2,4), and they remain effective even if administered at concentrations 8- to 12-fold lower than those required to afford classical radiation- and chemoprotection against cell killing (4). These results suggest that clinical trials designed to test the efficacy of these phosphorothioates as chemopreventive drugs should include evaluations of (a) lower concentrations of WR-2721 than are currently being used in protection studies and (b) the efficacy of adding this agent immediately following each course of chemotherapy and/or radiation treatment. In this manner, the effectiveness of these therapies would not be compromised (i.e., low concentrations of WR-2721 added following therapy would have no effect on cell survival, and the probability of therapy-induced secondary tumors would be reduced).

Thus, through the judicious use of aminothiols as adjuvants in well-designed therapy protocols, it is anticipated that not only can therapeutic gain be improved, but it may also be possible to significantly reduce the risk of therapy-induced secondary cancers in patients who have an excellent prognosis for cure and long-term survival.

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TABLE 1

Primary Connective Tissue Tumors <CT_T>	
Histiocytic Leukemia	Histiocytic Lymphoma (Reticulum Cell Tumor, Type A)
Lymphocytic-Lymphoblastic Leukemia	Lymphocytic-Lymphoblastic Lymphoma
Myelogenous Leukemia	Plasma Cell Tumor
Undifferentiated Leukemia	Undifferentiated Lymphoma
Unclassified Lymphoma	Mixed Histiocytic-Lymphocytic Leukemia
Mixed Histiocytic-Lymphocytic Lymphoma (RCT, Type B)	Hemangioma (various organs)
Sternal Marrow Vascular Tumor, Hemangioma	Angiosarcoma (various organs)
Fibrosarcoma (various organs)	Fibroma (various organs)
Undifferentiated Sarcoma, Connective Tissue	Undifferentiated Sarcoma (various organs)
Sarcoma, Undetermined Type, Uterus	Meningeal Sarcoma, Nervous System
Mast Cell Tumor, Connective Tissue	Osteoma, Connective Tissue
Leiomyosarcoma, Muscle	Rhabdomyoma (various organs)
Rhabdomyosarcoma (various organs)	Leiomyoma, Muscle
Chondrosarcoma, Bone	Osteoma, Bone
Osteosarcoma, Bone	Odontogenic Sarcoma, Bone
Neurilemoma, Gastrointestinal Tract	Leiomyoma (various organs)
Leiomyosarcoma (various organs)	Neurilemoma, Uterus
Astrocytoma, Nervous System	Ependymoma, Nervous System
Neurofibroma, Peripheral Nerve Neurilemoma	Neurofibrosarcoma, Peripheral Nerve
Oligodendroglioma, Nervous System	Papilloma, Choroid Plexus, Nervous System
Undifferentiated Tumor	Glioma, Mixed, Nervous System
Chondrosarcoma, Heart	Fibroadenoma, Site to be specified
Medullary Neuroblastoma/ Ganglioneuroma, Adrenal	Medullary Pheochromocytoma, Adrenal

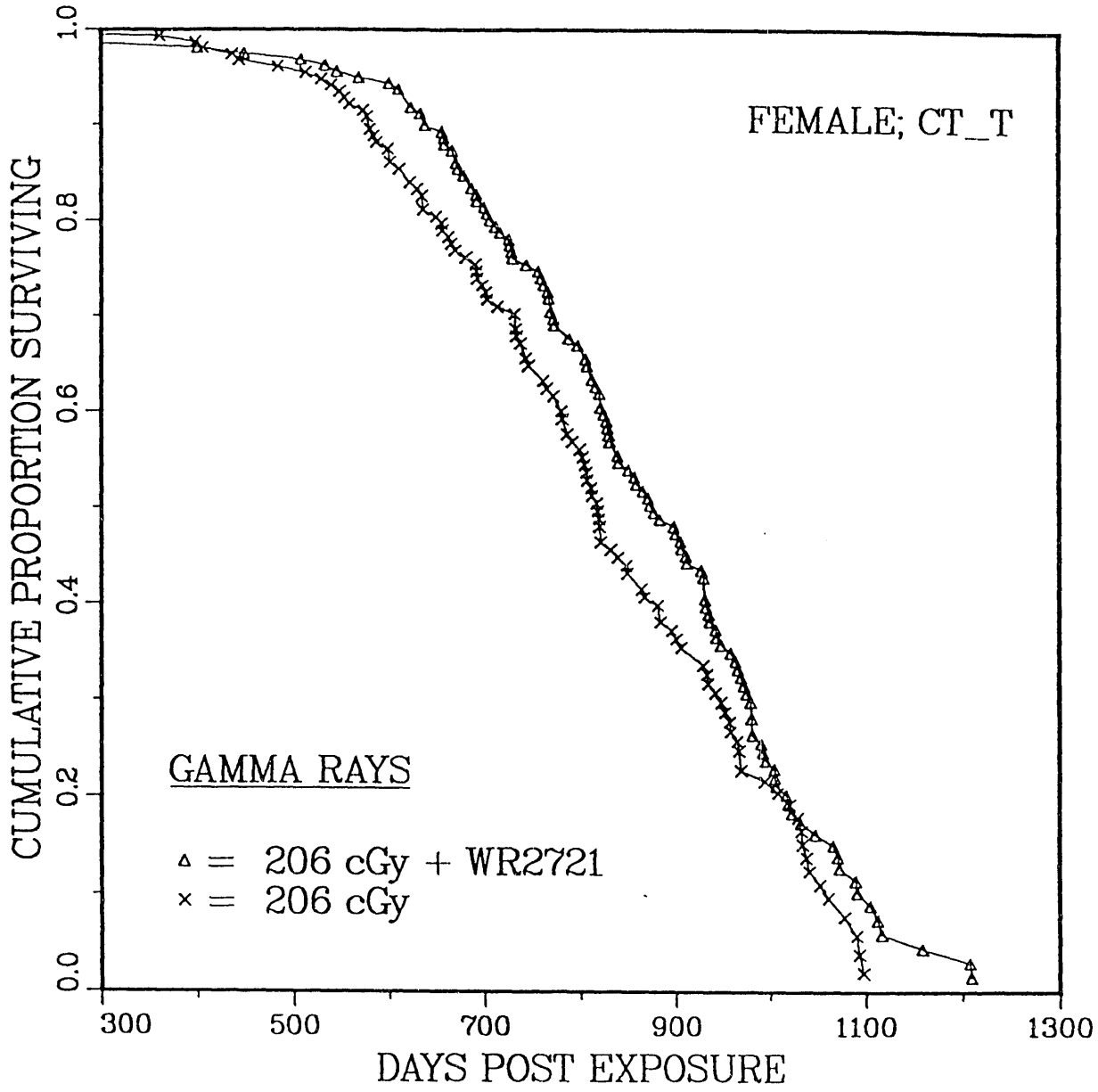
TABLE 2

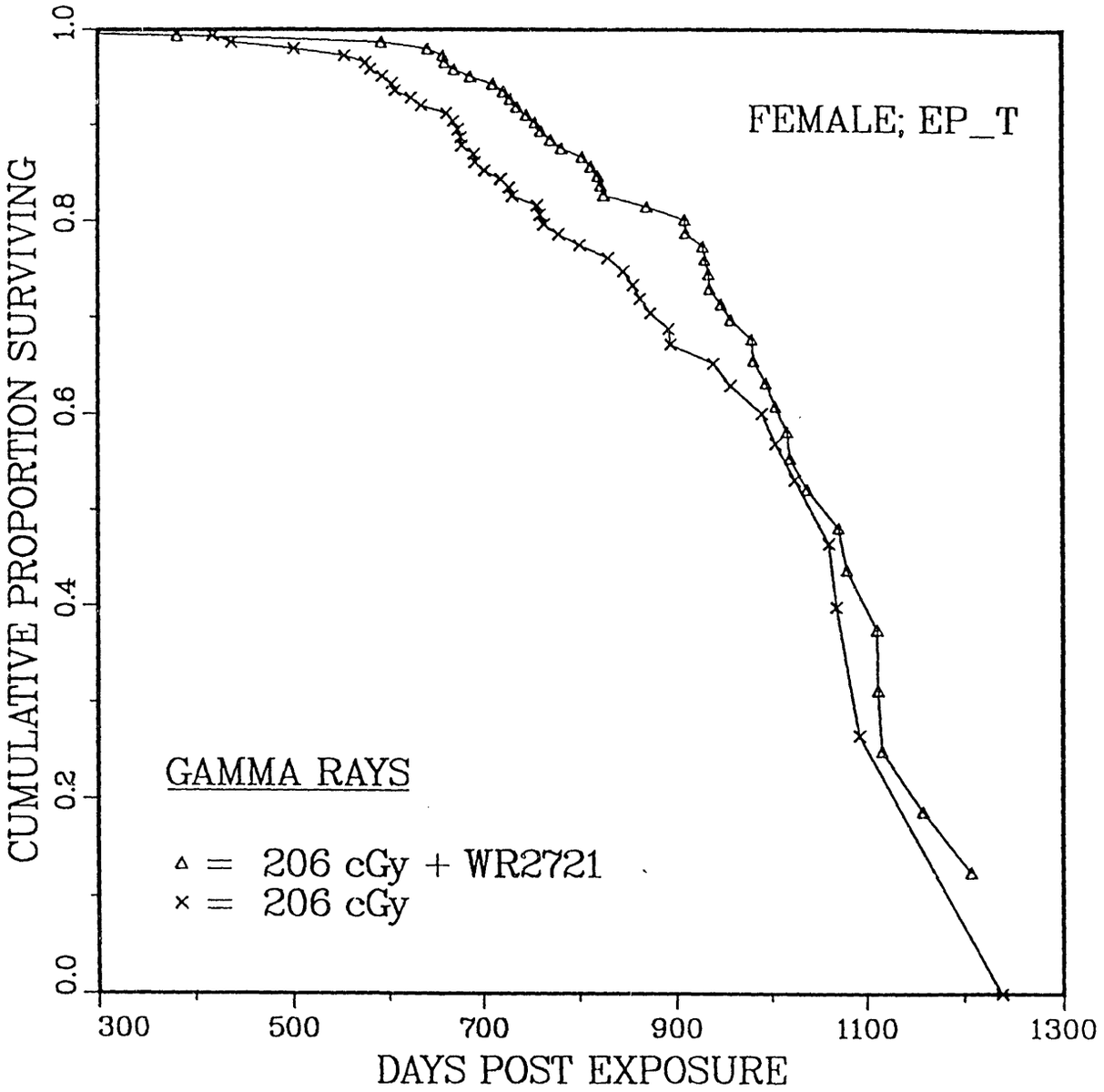
Primary Epithelial Tumors <EP_T>	
Alveologenic Tumor, benign	Alveologenic Tumor, malignant
Cystadenoma	Adenocarcinoma (various organs)
Adenoacanthoma	Tumor, undetermined cell type
Acidophilic Adenoma	Carcinoma (various organs)
Adenoma (various organs)	Renal Pelvic Transitional-Cell Carcinoma
Squamous Cell Carcinoma	Transitional Cell Carcinoma
Hepatocarcinoma	Hyperplastic Nodule
Cholangiocarcinoma	Cholangioma (Cholangiomatosis)
Plaque, Pyloric Region Polyp	Polyps
Undifferentiated Carcinoma	Seminoma
Interstitial Cell Tumor (Leydig)	Sertoli Cell Tumor
Embryonal Carcinoma	Basal Cell Carcinoma (Hair Follicle Tumor)
Sebaceous Gland Adenoma	

LEGENDS

Figure 1. Survival curves resulting from neoplasms, as determined by histopathological analysis of tissues taken from deceased animals that had been irradiated (206 cGy) either with or without WR-2721. Using the Wilcoxon test, the curves were found to differ at the $p = 0.022$ level. CT_T represents tumors derived from connective tissues (see Table 1).

Figure 2. Survival curves resulting from neoplasms, as determined by histopathological analysis of tissues taken from deceased animals that had been irradiated (206 cGy) either with or without WR-2721. Using the Wilcoxon test, the curves were found to differ at the $p = 0.025$ level. EP_T represents tumors derived from epithelial tissues (see Table 2).


FEMALE; CT_T
CUMULATIVE PROPORTION SURVIVING
1.0
0.8
0.6
0.4
0.2
0.0
GAMMA RAYS
Δ = 206 cGy + WR2721
× = 206 cGy
300
500
700
900
1100
1300
DAYS POST EXPOSURE


FEMALE; EP_T
CUMULATIVE PROPORTION SURVIVING
1.0
0.8
0.6
0.4
0.2
0.0
GAMMA RAYS
△ = 206 cGy + WR2721
× = 206 cGy
300
500
700
900
1100
1300
DAYS POST EXPOSURE

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