NDA 20221

NDA 20-221

Ap Ltr	Pharm./Tox
AE Ltr	Exclusivity Summ.
Draft LBLG	Pediatric pts
MOR	Chem
Stat	Bio
Micro	Fonsi and E.A.

Ap Ltr

AE Ltr

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-221

U.S. Bioscience, Inc.
One Tower Bridge
100 Front Street
West Conshohocken, PA 19428

DEC 8 1995

Attention: Barbara Scheffler
Senior Vice President for Clinical Operations
and Regulatory Affairs

Dear Ms. Scheffler:

Please refer to your September 30, 1991 new drug application and your resubmission dated July 12, 1994 submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for ETHYOL (amifostine) for Injection 500 mg.

We acknowledge receipt of your amendment dated October 27, 1995 in response to our approvable letter of October 6, 1995.

This new drug application provides for the use of ETHYOL® for Injection to reduce the cumulative renal toxicity associated with repeated administration of cisplatin in patients with advanced ovarian cancer. In this setting, the effectiveness of the cyclophosphamide-cisplatin chemotherapy was not altered by Ethyol. There are at present only limited data on the effects of Ethyol on the efficacy of chemotherapy in other settings. Ethyol should not be administered to patients receiving chemotherapy for malignancies that are commonly curable (e.g. certain malignancies of germ cell origin), except in the context of a clinical study.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed revised draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-221. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Oncology Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and
Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods had not been validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact Linda McCollum, Consumer Safety Officer, at (301) 594-5771.

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

ENCLOSURE

NDA 20-221

U.S. Bioscience, Inc.
One Tower Bridge
100 Front Street
West Conshohocken, PA 19428

OCT - 6 1995

Attention: Barbara Scheffler
Senior Vice President Clinical Operations
and Regulatory Affairs

Dear Ms. Scheffler:

Please refer to your September 30, 1991 new drug application and your resubmission dated July 12, 1994 submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for ETHYOL (amifostine) 500 mg for Injection.

We acknowledge receipt of your amendments dated July 21, September 21, October 20 and 24, and November 28, 1994; and January 31, March 24, April 28, June 6 and 29, July 10 and 21, 1995.

We have completed the review of this application as submitted with draft labeling, and it is approvable with the attached draft labeling for the indication of reducing the cumulative nephrotoxicity of cisplatin in patients undergoing treatment with cisplatin-based chemotherapy for advanced ovarian cancer. Before the application may be approved, however, it will be necessary for you to submit revised draft labeling and to provide additional information (including plans for post-marketing studies, if appropriate) to address the following Agency concerns.

CLINICAL

1. Limited data are currently available regarding the preservation of anticancer efficacy when amifostine is administered prior to cisplatin chemotherapy in settings other than advanced ovarian cancer. Interference with the efficacy of cancer treatment would be of greatest concern in settings where chemotherapy can be curative. Therefore, without implying that amifostine has shown tumor protection, labeling should include a warning that amifostine should not at present be used in conjunction with cisplatin in settings where cisplatin-based chemotherapy is potentially curative (e.g., certain tumors of germ cell origin)

   These and other specific comments and recommendations regarding ETHYOL® (amifostine) labeling are incorporated in the attached copy of ETHYOL proposed labeling (revised from proposed labeling

submitted by U.S. Bioscience in a July 14, 1995 submission to NDA 20-221).

Although the approvable labeling provides for a limited indication (reduction of the cumulative nephrotoxicity of cisplatin in patients undergoing treatment with cisplatin-based chemotherapy for advanced ovarian cancer), we acknowledge your submissions of additional data regarding the use of ETHYOL in patients receiving cisplatin-based chemotherapy for other malignancies; and the potential value of ETHYOL in reducing the cumulative nephrotoxicity of cisplatin in patients with other malignancies. Appropriate labeling of chemotherapy protective agents is a complex issue that deserves expert and public discussion. We will be pleased to bring the additional data you have for prompt consideration at a meeting of the Oncologic Drugs Advisory Committee.

2. Under 21 CFR 314.50(d)(5)(vi)(b), we also request that you update your NDA by submitting all new safety information you now have regarding ETHYOL. Please provide updated information as listed below:

   a. Retabulate all safety data including results of trials that were still ongoing at the time of NDA submission. The tabulation can take the same form as was used in table 19 of the integrated summary of safety that was included in your July 12, 1994 submission.

   b. Retabulate deaths and adverse drop-outs with new deaths and adverse drop-outs identified. Discuss, if appropriate.

   c. Provide details of significant changes or findings, if any.

   d. Summarize worldwide experience on the safety of this drug.

   e. Submit case report forms not previously provided for each patient who died during a clinical study or who did not complete a study because of an adverse event that was deemed possibly, probably, or definitely related to Ethyol® amifostine.

   This update should cover all studies and uses of the drug, including:(1) those involving indications not being sought in the present submission, (2) all dosage forms, and (3) all dose levels.

PHARMACOKINETICS

1. There is some evidence for gender differences in the clearance of ETHYOL. This is based on data combined from two pharmacokinetic studies (ETH-PK 1 & 3). On average, there appeared to be a 30% lower clearance of amifostine in women than in men. These results can only be considered preliminary, however, in view of the small sample size, and the inclusion of data from subjects who received either the 740 or 910 mg/m^2 dose. As you hope to expand claims for amifostine beyond ovarian cancer, it will be important to examine the potential for differences in pharmacokinetics between men and women. You should design a study to address this issue.

2. The possibility that ETHYOL or its active metabolite might affect the pharmacokinetics of co-administered chemotherapeutic agents has not been adequately addressed. You should describe the effects of ETHYOL administration on the pharmacokinetics of representative chemotherapeutic agents that will be given while WR-1065 is still present.

3. Plasma protein binding of amifostine and its metabolites over the therapeutic range has not been characterized.

4. The clinical trial entitled "Phase I Study of Bone Marrow Protection by Ethyol (amifostine) in Patients with Solid Tumors Treated with Carboplatin" is apparently ongoing and will provide information on the pharmacokinetics of the active metabolite. We would like to know when the results of that study are anticipated. A report of the study should be provided to the agency as soon as possible. The report should include subject demography, including any medications taken during the study periods, individual and mean plasma concentration-time data for amifostine and WR-1065 after single versus multiple dosing, and assay validation for all species measured.

CHEMISTRY

The following comments concern the drug substance:

1. Specifications for the amifostine reference standard should include Assay by HPLC and Other Impurities.

2. Describe how mass balance was determined in the forced degradation studies. Were molecular weights used as for the drug product? See deficiency # 4 for the drug product.

3. The specification for methanol should be set at a much lower limit. All lots of drug substance, with the exception of one have methanol limits well below ppm. In fact all values are less than ppm. A specification of NMT ppm is reasonable based on the data.

The following comments concern the drug product:

1. The proposed release specifications for the drug product are unacceptably broad. An appropriate (lower than previously proposed NMT %) limit should be established for Total Related Substances. In addition the proposed % Assay limits are also too broad for release specifications.

2. The regulatory (shelf life) specifications for the drug product also need to be revised as follows:

   a. Establish a specification for Total Related Substances.

   b. The proposed % limit for thiol is unacceptably high. A % limit would be acceptable to the Agency.

   c. Examination of the stability data provided in Attachment 4 indicates a specification for reconstitution time of "NMT minutes" is unreasonable. While a NMT minute specification might have been proposed earlier in a meeting we now have more complete data to analyze. The Agency would find a reconstitution time of "NMT minutes" acceptable.

3. Please submit copies of the revised vial labels, carton labels and package insert for review.

4. Please clarify in your response to deficiency #26, Agency letter dated February 27, 1995, which tables are reporting mass balance data by weight percent and which tables are reporting mass balance based on millimoles. For example, it is unclear what units are used in the data for the mass balance in the forced degradation studies. It appears that in the data for forced degradation (heat treated) weight percent was used (since there is a column for other related substances and no molecular weight can be used to determine millimoles for unidentified

related substances). In this experiment good mass balance was obtained even though there was % thiol. However, if weight percent was used to determine mass balance (as appears to be the case) it is not clear why good mass balance was obtained in light of your claim that one needs to use molecular weights to obtain good mass balance.

5. The stability data generated at 5°C do not warrant the proposed 18 month expiration dating period for storage at 2-8°C. A 3.5% Thiol limit would support a 12 month expiration dating period.

Please submit revised draft labeling incorporating the attached revisions. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Oncology Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising
and Communications HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Within 10 days after the date of this letter, you are required to amend the application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.110. In the absence of such action FDA may take action to withdraw the application. The drug may not be legally marketed until you have been notified in writing that the application is approved.

Should you have any questions, please contact Linda McCollum, Consumer Safety Officer, at (301) 594-5771.

Sincerely yours,

Robert Temple 10/6/95

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

cc:
Original NDA 20-221
HFD-150/Div. Files(draft Labeling)
HFD-2/MLumpkin
HFD-80
HFD-100
HFD-150/LMcCollum
DISTRICT OFFICE
HFD-240/SSherman (with draft labeling)
HFD-638 (with draft labeling)

drafted: ljm/September 15, 1995/rev. 091895/091995/092295/092595

r/d Initials: SCSO-Pease/9-21-95
MO-DeLao/9-22-95
SMO-Justice/9-30-95
CMC-Dietze/9-22-95
SCMC-Tolgyesi/9-22-95
PT-Schmidt/
SPT-DeGeorge/9-22-95
BPH-Zannikos/9-22-95
SBPH-Mehta/9-22-95
STT-Gnecco/
SSTT-Wilson/

Final: DD-Justice/9-30-95 (Acting)
DD-DeLap/10-3-95 (Acting)

doc. id. N20221AE.sep

APPROVABLE (AE)

Draft LBLG

ETHYOL ®

(amifostine) for Injection

DESCRIPTION

ETHYOL (amifostine) is an organic thiophosphate cytoprotective agent known chemically as ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester) and has the following structural formula:

$$H_2N(CH_2)_3NH(CH_2)_2S\text{-}PO_3H_2$$

Amifostine is a white crystalline powder which is freely soluble in water. Its empirical formula is $C_5H_{15}N_2O_3PS$ and it has a molecular weight of 214.22.

ETHYOL is supplied as a sterile lyophilized powder mixture with mannitol requiring reconstitution for intravenous infusion. Each single-use 10 ml vial contains 500 mg of amifostine (anhydrous basis) and 500 mg of mannitol.

CLINICAL PHARMACOLOGY

ETHYOL (amifostine) is a prodrug that is dephosphorylated by alkaline phosphatase in tissues to a pharmacologically active free thiol metabolite that can reduce the toxic effects of cisplatin. The ability to differentially protect normal tissues is attributed to the higher capillary alkaline phosphatase activity, higher pH, and better vascularity of normal tissues relative to tumor tissue, which results in

a more rapid generation of the active thiol metabolite as well as a higher rate constant for uptake. The higher concentration of free thiol in normal tissues is available to bind to, and thereby detoxify, reactive metabolites of cisplatin; and also can act as a scavenger of free radicals that may be generated in tissues exposed to cisplatin. Several preclinical studies in mice and rats have demonstrated that pretreatment with ETHYOL results in protection from nephrotoxicity following administration of single and multiple doses of cisplatin.

Pharmacokinetics: Clinical pharmacokinetic studies show that ETHYOL is rapidly cleared from the plasma with a distribution half-life of < 1 minute and an elimination half-life of approximately 8 minutes. Less than 10% of ETHYOL remains in the plasma 6 minutes after drug administration. ETHYOL is rapidly metabolized to an active free thiol metabolite. A disulfide metabolite is produced subsequently and is less active than the free thiol. After a 10-second bolus dose of 150 mg/m^2 of ETHYOL, renal excretion of the parent drug and its two metabolites was low during the hour following drug administration, averaging 0. 69 %, 2.64 % and 2.22 % of the administered dose for the parent. thiol and disulfide, respectively. Measurable levels of the free thiol metabolite have been found in bone marrow cells 5-8 minutes after intravenous infusion of amifostine. Pretreatment with dexamethasone or metoclopramide has no effect on ETHYOL pharmacokinetics.

Clinical Studies: A randomized controlled trial compared six cycles of cyclophosphamide 1000 mg/m^2 and cisplatin 100 mg/m^2, with or without amifostine pretreatment at 910 mg/m^2, in two successive cohorts of 121 patients with advanced ovarian cancer. In both cohorts, after multiple cycles of chemotherapy, pretreatment with ETHYOL significantly reduced the cumulative renal toxicity associated with cisplatin as assessed by the proportion of patients who had a ≥40% decrease in creatinine clearance from pretreatment values, protracted elevations in serum creatinine (> 1.5 mg/dL), or severe hypomagnesemia. Subgroup analyses suggested that the effect of ETHYOL was present in patients who had received nephrotoxic antibiotics, or who had pre-existing

diabetes or hypertension (and thus may have been at increased risk for significant nephrotoxicity), as well as in patients who lacked these risks. Selected analyses of the effects of ETHYOL in reducing the cumulative renal toxicity of cisplatin in the randomized ovarian cancer study are provided in Tables 1 and 2, below.

TABLE 1

Proportion of Patients with ≥40% Reduction in Calculated Creatinine Clearance*

	Amifostine + CP	CP	p-value 2-sided
All Patients	16/122 (13%)	36/120 (30%)	0.001
First Cohort	10/63	20/58	0.018
Second Cohort	6/59	16/62	0.026

* Creatinine clearance values were calculated using the Cockcroft-Gault formula (give reference).

TABLE 2

NCI Toxicity Grades of Serum Magnesium Level for Each Patient's Last Cycle of Therapy

NCI-CTC Grade:	0	1	2	3	4	
(mEq/L)	>1.4	≤1.4->1.1	≤1.1->0.8	≤0.8->0.5	≤0.5	p-value*
All Patients						0.001
Amifostine + CP	92	13	3	0	0	
CP	73	18	7	5	1	
First Cohort						
Amifostine + CP	49	10	3	0	0	0.017
CP	35	8	6	3	1	
Second Cohort						
Amifostine + CP	43	3	0	0	0	0.012
CP	38	10	1	2	0	

In the randomized ovarian cancer study, ETHYOL had no detectable effect on the antitumor efficacy of cyclophosphamide-cisplatin chemotherapy. Objective response rates (including pathologically-confirmed complete remission rates), time to progression, and survival duration were all similar in the amifostine and control study groups. The table below summarizes the principal efficacy findings of the randomized ovarian cancer study.

TABLE 3

	Ethyol + CP		CP	
Complete pathologic tumor response rate	21.3% →		15.8% →	
Time to progression (months)				
Median (± 95% CI)	15.8	(13.2, 25.1)	18.1	(12.5, 20.4)
Mean (± Std error)	19.8	(±1.04)	19.1	(±1.58)
Hazard ratio (95% Confidence Interval)	.98 (.64, 1.4)			
Survival (months)				
Median (± 95% CI)	31.3	(28.3, 38.2)	31.8	(26.3, 39.8)
Mean (± Std error)	33.7	(± 2.03)	34.3	(± 2.04)
Hazard ratio (95% Confidence Interval)	.97 (.69, 1.32)			

center these numbers

INDICATIONS AND USAGE

ETHYOL is indicated to reduce the cumulative renal toxicity associated with repeated administration of cisplatin in patients with advanced ovarian cancer. In this setting, the effectiveness of cyclophosphamide-cisplatin chemotherapy was not altered by Ethyol. There are at present only limited data on the effects of ETHYOL on the efficacy of chemotherapy in other settings. ETHYOL should not be administered to patients receiving chemotherapy for malignancies that are commonly curable (e.g., certain malignancies of germ cell origin), except in the context of a clinical study.

CONTRAINDICATIONS

ETHYOL is contraindicated in patients with known sensitivity to aminothiol compounds or mannitol.

WARNINGS

1. Effectiveness of the cytotoxic regimen.

Limited data are currently available regarding the preservation of antitumor efficacy when amifostine is administered prior to cisplatin chemotherapy in settings other than advanced ovarian cancer. Although some animal data suggest interference is possible, in most tumor models the antitumor effects of chemotherapy are not altered by amifostine. The possibility of interference with the efficacy of cancer treatment would be of particular concern in those settings where chemotherapy is potentially curative. ETHYOL should therefore not be used in patients receiving chemotherapy for malignancies that are potentially curable (e.g., certain malignancies of germ cell origin), except in the context of a clinical study.

2. Hypotension.

Patients who are hypotensive or in a state of dehydration should not receive ETHYOL. Patients receiving antihypertensive therapy that cannot be stopped for 24 hours preceding ETHYOL treatment also should not receive ETHYOL. Patients should be adequately hydrated prior to ETHYOL infusion and kept in a supine position during the infusion. Blood pressure should be monitored every 5 minutes during the infusion. It is important that the duration of infusion be 15 minutes, as administration of ETHYOL as a longer infusion is associated with a higher incidence of side effects. If hypotension requiring interruption of therapy occurs, patients should be placed in the Trendelenburg position and be given an infusion of normal saline using a separate i.v. line. Guidelines for interrupting and re-starting ETHYOL infusion if a decrease in systolic blood pressure should occur are provided in the DOSAGE AND ADMINISTRATION section.

3. Nausea and vomiting.

Antiemetic medication should be administered prior to and in conjunction with ETHYOL (see DOSAGE and ADMINISTRATION). When ETHYOL is administered with highly emetogenic chemotherapy, the fluid balance of the patient should be carefully monitored.

4. Hypocalcemia.

Reports of clinically relevant hypocalcemia are rare, but serum calcium levels should be monitored in patients at risk of hypocalcemia, such as those with nephrotic syndrome. If necessary, calcium supplements can be administered.

PRECAUTIONS

General

Patients should be adequately hydrated prior to the infusion and blood pressure should be monitored during the infusion. ETHYOL should be administered as a 15-minute infusion. (See DOSAGE AND ADMINISTRATION)

The safety of ETHYOL administration has not been established in elderly patients, or patients with pre-existing cardiovascular or cerebrovascular conditions such as ischemic heart disease, arrhythmias, congestive heart failure, or history of stroke or transient ischemic attacks. ETHYOL should be used with particular care in these and other patients in whom the common ETHYOL adverse effects of nausea/vomiting and hypotension may be more likely to have serious consequences.

Drug Interactions

There are no known drug interactions with ETHYOL. However, special consideration should be given to the administration of ETHYOL in patients receiving anti-hypertensive medications or other drugs that could potentiate hypotension.

Carcinogenesis, Mutagenesis and Impairment of Fertility

No long term animal studies have been performed to evaluate the carcinogenic potential of ETHYOL. ETHYOL was negative in the Ames test and in the mouse micronucleus test. The free thiol metabolite, however, was positive in the Ames test with S9 microsomal fraction in the TA1535 *Salmonella typhimurium* strain and at the TK locus in the mouse L5178Y cell assay. The metabolite was negative in the mouse micronucleus test and negative for clastogenicity in human lymphocytes.

Pregnancy

Pregnancy Category C. ETHYOL has been shown to be embryotoxic in rabbits at doses of 50 mg/kg, *approximately sixty percent of the recommended dose in humans on a body surface area basis.* There are no adequate and well-controlled studies in pregnant women. ETHYOL should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

No information is available on the excretion of ETHYOL or its metabolites into human milk. Because many drugs are excreted in human milk and because of the potential for adverse reactions in nursing infants, it is recommended that breast feeding be discontinued if the mother is treated with ETHYOL.

ADVERSE REACTIONS

ETHYOL produced a transient reduction in blood pressure in 62% of patients treated. The mean time of onset was 14 minutes into the 15-minute period of ETHYOL infusion, and the mean duration was 6 minutes. In some cases, the infusion had to be prematurely terminated due to a more pronounced drop in systolic blood pressure. In general, the blood pressure returned to normal within 5-15 minutes. Fewer than 3% of patients discontinued ETHYOL due to blood pressure reductions. Short term, reversible loss of consciousness has been reported rarely. Blood pressure reductions during ETHYOL administration have not been reported to cause long-term CNS, cardiovascular, or renal sequelae, but clinical studies performed to date have not evaluated the safety of ETHYOL in elderly patients or patients with pre-existing cardiovascular or cerebrovascular conditions.

Hypotension that requires interruption of ETHYOL infusion should be treated with fluid infusion and postural management of the patient (supine or Trendelenburg position). If the blood pressure returns

to normal within 5 minutes and the patient is asymptomatic, the infusion may be restarted, so that the full dose of ETHYOL can be administered.

Nausea and/or vomiting occur frequently after amifostine infusion and may be severe. In the ovarian cancer randomized study, the incidence of severe nausea/vomiting on day I of cyclophosphamide-cisplatin chemotherapy was 10% in patients who did not receive ETHYOL, and 19% in patients who did receive ETHYOL. Other effects which have been described during or following ETHYOL infusion are flushing/feeling of warmth, chills/feeling of coldness, dizziness, somnolence, hiccups and sneezing. These effects have not generally precluded the completion of chemotherapy.

Decrease in serum calcium concentrations is a known pharmacological effect of ETHYOL. At the recommended doses, clinically significant hypocalcemia has occurred rarely (< 1%).

Allergic reactions, ranging from mild skin rashes to rigors, have occurred rarely (< 1%). There has been no reported occurrence of anaphylaxis with ETHYOL.

OVERDOSAGE

In clinical trials, the maximum single dose of ETHYOL was 1300 mg/m^2. No information is available on single doses higher than this in adults. In the setting of a clinical trial, children have received single ETHYOL doses of up to 2700 mg/m^2 with no unexpected effects. Multiple infusions (up to three) of 740-910 mg/m^2 doses of ETHYOL have been administered within a 24-hour period under study conditions without unexpected effects. Administration of ETHYOL at 2 and 4 hours after the initial dose has not led to increased or cumulative side effects, such as increased nausea and vomiting or hypotension. The most likely symptom of overdosage is hypotension, which should be managed by infusion of normal saline and other supportive measures, as clinically indicated.

198 DOSAGE AND ADMINISTRATION

199

200 In adults, the recommended starting dose of ETHYOL is 910 mg/m^2 administered once daily as a
201 15-minute i.v. infusion, starting within 30 minutes prior to chemotherapy.

202

203 The 15-minute infusion is better tolerated than more extended infusions. Further reductions in
204 infusion times have not been systematically investigated.

205

206 The infusion of ETHYOL should be interrupted if the systolic blood pressure decreases significantly
207 from the baseline value as listed in the guideline below:

208

209

210

Guideline for Interrupting ETHYOL Infusion Due to Decrease in Systolic Blood Pressure

	Baseline Systolic Blood Pressure (mm Hg)				
	<100	100-119	120-139	140-179	≥180
Decrease in systolic blood pressure during infusion of ETHYOL (mm Hg)	20	25	30	40	50

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218 If the blood pressure returns to normal within 5 minutes and the patient is asymptomatic, the infusion
219 may be restarted so that the full dose of ETHYOL may be administered. If the full dose of ETHYOL
220 cannot be administered, the dose of ETHYOL for subsequent cycles should be 740 mg/m^2.

221

222 Only limited experience is available for the usage of ETHYOL in children or elderly patients (more
223 than 70 years of age).

224

It is recommended that antiemetic medication, including dexamethasone 20 mg i.v. and a serotonin 5-HT_3 receptor antagonist, be administered prior to and in conjunction with ETHYOL. Additional antiemetics may be required based on the chemotherapy drugs administered.

Reconstitution

ETHYOL (amifostine) for Injection is supplied as a sterile lyophilized powder mixture requiring reconstitution for intravenous infusion. Each single use vial contains 500 mg of amifostine (anhydrous basis) and 500 mg of mannitol.

Prior to intravenous injection, ETHYOL for Injection is reconstituted with 9.5 ml of sterile Sodium Chloride Injection, USP 0.9%. The reconstituted solution (500 mg amifostine/10 ml) is chemically stable for up to 5 hours at room temperature (approximately 25°C) or up to 24 hours under refrigeration (2°C to 8°C).

ETHYOL prepared in polyvinylchloride (PVC) bags at concentrations ranging from 5 mg/ml to 40 mg/ml is chemically stable for up to 5 hours when stored at room temperature (25°C) or up to 24 hours when stored under refrigeration (2°C to 8°C).

CAUTION: Parenteral products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Do not use if cloudiness or precipitate is observed.

Incompatibilities

The compatibility of amifostine with solutions other than 0.9% Sodium Chloride for Injection, or Sodium Chloride solutions with other additives, has not been examined. The use of other solutions is not recommended.

HOW SUPPLIED

ETHYOL (amifostine) for Injection is supplied as a sterile lyophilized powder in 10 ml single-use vials (NDC58178-015-01). Each single-use vial contains 500 mg of amifostine (anhydrous basis) and 500 mg of mannitol. The vials are available packaged as 3 or 6 vials per carton as follows:

3 pack - 3 vials per carton (NDC58178-015-03)

6 pack - 6 vials per carton (NDC58178-015-06)

Store the lyophilized dosage form in a refrigerator (2°C to 8°C).

Manufactured by: Ben Venue Laboratories, Inc.
Bedford, OH 44146
For: U.S. Bioscience, Inc.
West Conshohocken, PA 19428

US Patent No. _____

CAUTION: Federal law prohibits dispensing without prescription.

Exclusivity Summary Ped. Pts

EXCLUSIVITY SUMMARY FOR NDA # 20-221 SUPPL # N/A

Trade Name ETHYOL for Injection Generic Name Amifostine

Applicant Name US Bioscience, Inc. HFD # 150, DODP

Approval Date If Known Pending

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

   (a) Is it an original NDA?

   YES /_X_/ NO /__/

   (b) Is it an effectiveness supplement?

   YES /__/ NO /_X_/

   If yes, what type? (SE1, SE2, etc.) _____

   (c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

   YES /_X_/ NO /__/

   If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

   _____

   _____

   If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

   _____

   _____

Form OGD-011347 Revised 8/7/95
cc: Original NDA # Division File HFD-85 Mary Ann Holovac HFD-150 CSO

(d) Did the applicant request exclusivity?

YES /_X_/ NO /__/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

Seven years after Approval, re: Orphan Drug Status application # 89-83-427-6

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES /__/ NO /_X_/

If yes, NDA # _____. Drug Name _____.

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /__/ NO /X/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /__/ NO /_X_/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

2. Combination product.

If the product contains more than one active moiety(as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /__/ NO /_ _/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA number(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /_ _/ NO /__/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /_ _/ NO /__/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

_____

_____

_____

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /__/ NO /__/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /__/ NO /__/

If yes, explain:_____

_____

_____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /__/ NO /__/

If yes, explain: _____

_____

_____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

_____

_____

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation' to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

(a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1 YES /__/ NO /_ _/

Investigation #2 YES /__/ NO /__/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

NDA # _____ _____

NDA # _____ _____

(b) For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /__/ NO /_ _/

Investigation #2 YES /__/ NO /__/

If you have answered "yes" for one or more investigation, identify the NDA in which a similar investigation was relied on:

NDA # _____ _____

NDA # _____ _____

(c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

NDA # _____ _____

NDA # _____ _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, *before or during the conduct of the investigation*, 1) *the applicant was the sponsor* of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

   (a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1 IND # __ ____

YES /_ _/ Explain: _____

NO /__/ Explain: _____

Investigation #2 IND # _____

YES /__/ Explain: _____

NO /__/ Explain: _____

   (b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1 IND # _____

YES /__/ Explain: _____

NO /__/ Explain: _____

Investigation #2 IND # _____

YES /__/ Explain: _____

NO /__/ Explain: _____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /__/ NO /__/

If yes, explain: _____

Signature _____ Date 9.15.95

Title CSO

_____ 12/7/95

Division or Office Director Signature Date

Title

Form OGD-011347 Revised 8/7/95 Division File HFD-85 Mary Ann Holovac HFD-150 CSO
cc: Original NDA #

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-221 Trade (generic) names Ethyol (Amifostine) for Injection

Check any of the following that apply and explain, as necessary, on the next page:

____ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.

____ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. -The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.

____ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.

____ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)

____ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).

____ a. The applicant has committed to doing such studies as will be required.

____ (1) Studies are ongoing.
____ (2) Protocols have been submitted and approved.
____ (3) Protocols have been submitted and are under review.
____ (4) If no protocol has been submitted, on the next page explain the status of discussions.

____ b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

____ 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

√ 5. If none of the above apply, explain.

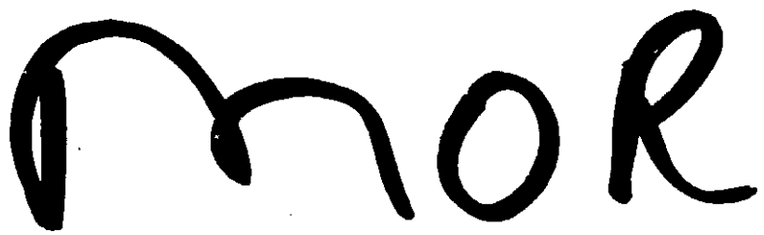
Explain, as necessary, the foregoing items:

NDA does not address use in
children but if approved
will have appropriate [illegible]
& labeling changes.
Pediatric studies are underway
as are recommended.

[illegible] ______________________ Signature of Preparer

3/4/92 ______________________ Date

cc: Orig NDA 20-221
HFD-150/Div File
NDA Action Package



Medical Officer Review - NDA 20-221
Amendment Submitted October 27, 1997
Response to Approvable Letter, Including Safety Update

Reviewing Medical Officer: Robert J. DeLap, MD, PhD
Date of completion of this review: December 7, 1995

This submission represents the responses of the Applicant (US Bioscience) to clinical, pharmacology, biopharmaceutics, and chemistry issues, that were identified in the October 6, 1995 Agency "Approvable" letter as requiring resolution prior to final approval of this New Drug Application (NDA 20-221, ETHYOL® amifostine). In proposed labeling for ETHYOL, US Bioscience has agreed to accept the INDICATIONS section as specified in the prior Approvable letter; and has proposed several additions and changes to the CLINICAL PHARMACOLOGY, WARNINGS, PRECAUTIONS, ADVERSE REACTIONS, and DOSAGE AND ADMINISTRATION sections. A safety update is also included in this submission.

The biopharmaceutics and chemistry/manufacturing issues have been addressed sufficiently to allow approval of this New Drug Application at this time (refer to those reviews). Labeling is acceptable with additional final changes as per the attached copy. The safety update provided with this submission is adequate and does not reveal any apparent substantive new concerns regarding the safety of ETHYOL, or significant changes in frequency of adverse effects.

Summary: NDA 20-221 should be approved with the attached labeling.

Robert J. DeLap, MD, PhD
Medical Officer and Acting Division Director

CC: NDA 20-221
HFD-150 / R. Justice
HFD-150 / L. McCollum

1. Regulatory History

ETHYOL® amifostine (also known as WR-2721; chemically characterized as 2-[(3-aminopropyl)amino]-ethanethiol, dihydrogen phosphate ester) has been studied clinically for many years, as an agent that may selectively protect normal tissues from the toxic effects of cancer chemotherapy or radiation therapy, without substantially reducing the efficacy of treatment. A New Drug Application for ETHYOL® amifostine was filed by U.S. Bioscience in 1991. This application has subsequently been extensively amended to include additional data and analyses. The regulatory history of this application may be briefly summarized as follows.

A. September 30, 1991 New Drug Application (NDA 20-221)

The original New Drug Application for ETHYOL® amifostine was submitted by US Bioscience on September 30, 1991. The original application was based primarily on the results of a single randomized trial in patients with advanced ovarian cancer. Following debulking surgery, patients were treated with cyclophosphamide and cisplatin (control arm), or amifostine followed by cyclophosphamide and cisplatin. Patients were to receive 6 cycles of treatment, with treatment repeated at 3 week intervals. After treatment of the first 121 of 200 planned patients, an interim analysis revealed that there had been fewer episodes of neutropenia with fever ("febrile neutropenia") among the amifostine-treated patients. Response rates were similar on the two study arms, suggesting that amifostine had not reduced the anticancer efficacy of this treatment. Accrual to the study was interrupted, and these data were submitted to the FDA in the original New Drug Application.

After FDA and Oncologic Drugs Advisory Committee review, the 1991 application was determined to be non-approvable. There were several concerns regarding the clinical data and analyses included in the application. The protocol for the ovarian cancer randomized trial had specified several study endpoints, and did not provide for the necessary statistical adjustments for multiple analyses. The protocol did not precisely define a "febrile neutropenia" event, and did not prospectively specify that such events would be the primary study endpoint. Also, collection of the data needed to determine whether a patient had experienced a "febrile neutropenia" event was inconsistent and frequently inadequate. For example, data on patient temperatures during periods of grade 4 neutropenia were often absent. There was also concern that the size of the study (63 amifostine patients and 58 control patients) was inadequate to exclude the possibility of a significant reduction in treatment efficacy with amifostine treatment. Finally, significant deficiencies were also identified in the chemistry and biopharmaceutical parts of the application.

For the above reasons, the clinical data provided in the 1991 application were considered to be positive, but non-definitive. A second adequate and well controlled study was needed, to prospectively verify whether amifostine has significant efficacy in reducing the frequency of neutropenia-associated clinical events in patients receiving cyclophosphamide-cisplatin chemotherapy for advanced ovarian cancer. More data were also required to better evaluate whether amifostine has significant tumor-protective effects. Subsequently, US Bioscience

and the Agency (with the advice of the Oncologic Drugs Advisory Committee) agreed that the advanced ovarian cancer study would be completed, with accrual of 200 patients as originally planned. The efficacy of amifostine in significantly reducing the incidence of "grade 4 neutropenia with fever (with or without sepsis)", as observed in the applicant's analyses of results in the first 121 patients treated in the study, was identified as the primary study endpoint to be replicated in patients enrolled in the second half of this study. The anticancer efficacy of this treatment (particularly response rate, but also time to disease progression and survival duration) would also be examined in updated analyses of all 200 patients, to better assess whether amifostine has significant adverse effects on treatment efficacy.

B. July 12, 1994 amendment 017 to NDA 20-221

A major amendment to this New Drug Application was submitted on July 12, 1994 (NDA 20-221, amendment 017). The amended application now included data from 242 patients treated in the applicant's randomized ovarian cancer study. Separate analyses were provided for the original 121 patient cohort, for the new 121 patient cohort, and for all 242 patients combined. As noted above, the primary efficacy hypothesis to be tested was whether the incidence of "grade 4 neutropenia with fever, with or without sepsis" (modified to "neutropenia-associated clinical events" in the amended submission) would be significantly reduced in the amifostine study arm in the new patient study cohort, to confirm the observation made in the original 121 patient cohort. Unfortunately, on FDA analyses, the incidence of neutropenia-associated clinical events was not significantly reduced in the amifostine treatment arm of the new 121 patient study cohort (although patients in the amifostine treatment arm did have a slightly smaller total number of these events). Thus, the primary efficacy hypothesis was not confirmed in the new 121 patient cohort. In other analyses of this study performed by U.S. Bioscience, amifostine pretreatment was associated with a lesser frequency of grade 4 neutropenia in later treatment cycles: and there was a trend indicating more rapid recovery from grade 4 neutropenia for patients in the amifostine arm, in later treatment cycles.

While the renal toxicity of chemotherapy was relatively mild in the randomized ovarian cancer study, several analyses indicated that the cumulative renal toxicity of chemotherapy was reduced in the amifostine-treated patient group. After repeated treatment with cisplatin-cyclophosphamide, the incidence of prolonged elevation of serum creatinine levels (defined as a serum creatinine level of 1.5 mg/dl or higher, that was still present 21 days or longer after a chemotherapy treatment) was greater in control group patients. Similarly, at the time of initiation of the 5th and 6th cycles of chemotherapy, patients in the control arm were noted to have serum creatinine levels that had increased by a median of 25-31% (compared to pre-study baseline), while serum creatinine levels in patients in the amifostine pretreatment group had increased by only 11-12%. However, in Agency analyses of the time to first occurrence of a serum creatinine > 1.5 mg/dl, no advantage was seen for patients in the amifostine group (in fact, in the new patient study cohort the amifostine arm was statistically inferior to the control arm, possibly because of increased nausea / vomiting / dehydration in the amifostine group, causing more prerenal azotemia). Finally, over the course of the study, the applicant reported

that 6 control arm patients were taken off study because of renal toxicity, compared to none in the amifostine arm (although medical officer review suggested a lesser difference between the two study arms in the incidence of treatment termination related to renal toxicity).

Analyses of the anticancer efficacy of treatment in the 242 patients in the randomized ovarian cancer study population revealed no differences between the amifostine and control groups. These analyses included comparisons of pathologically-confirmed complete response rate (as documented by a negative "second look" laparotomy, revealing no evidence of residual cancer after completion of chemotherapy), as well as analyses of time to disease progression and analyses of survival duration in the amifostine and control groups. However, this study had limited statistical power to exclude the possibility of a significantly inferior time to progression (and/or survival duration) for patients in the amifostine group.

The amended application also included supportive data from several other smaller studies of amifostine. The FDA Medical Officer review of NDA 20-221, amendment 017, provides a detailed review of this submission.

Following review of this amended application at the December 12, 1994 meeting of the Oncologic Drugs Advisory Committee, the Committee voted 9-0 that the application should not be approved. The Committee was not convinced that clinical efficacy of amifostine had been adequately demonstrated in the applicant's studies. The issue of possible tumor protection was deferred by the Committee, as it was deemed unnecessary to discuss this issue absent a finding of amifostine efficacy in protection of bone marrow or other normal tissues.

C. January 31, 1995 amendment 021 to NDA 20-221

The application was subsequently amended on January 31, 1995, with additional data and analyses to further examine the potential efficacy of amifostine in reducing the cumulative toxicities of cisplatin-cyclophosphamide chemotherapy (particularly, cumulative renal injury). The considerations and events that resulted in FDA acceptance of this current submission for review were as follows.

As discussed above, the possible efficacy of amifostine in reducing the incidence of "grade 4 neutropenia with fever, with or without sepsis" was identified as the primary efficacy endpoint to be replicated in patients enrolled in the second half of the randomized ovarian cancer study, because of the positive results observed in the original 121 patient cohort. In retrospect, considering all of the data now available regarding the clinical effects of amifostine, this may not have been an optimal test of amifostine efficacy. In this ovarian cancer study chemotherapy was initiated early after major abdominal surgery. Many of the study patients were probably at high risk for infection, regardless of any effects amifostine may have had on the duration or severity of chemotherapy-induced neutropenia, for reasons such as their (a) multiple recent invasive procedures (surgery, central lines, catheters); (b) incomplete wound healing; (c) poor nutritional status; and (d)

disturbed bacterial flora, due to antibiotics administered around the time of surgery, with possible colonization by pathogenic bacteria during the hospital stay for surgery. In fact, most of the neutropenia-associated events observed in this study occurred in the first treatment cycle. The marked apparent difference in the incidence of these events (favoring the amifostine treatment group) that was observed in the initial 121 patient cohort in the randomized ovarian cancer study could have been largely a chance occurrence. This possibility is supported by the absence of data indicating significant differences in the incidence or duration of grade 4 neutropenia in the two treatment groups, in the first treatment cycle.

Based on current understanding of the mechanism of action of amifostine, this agent might be expected to act primarily to reduce the cumulative toxic effects of treatment with certain chemotherapy drugs (notably, cisplatin and certain alkylating agents). Clinically, this could be manifested by reduced cumulative injury to the bone marrow and potentially other organ systems. As noted above, consistent with this concept, amifostine pretreatment was associated with a lesser frequency of grade 4 neutropenia in later treatment cycles in the ovarian cancer randomized study; and there was a trend indicating more rapid recovery from grade 4 neutropenia for patients in the amifostine arm, in later treatment cycles. Similarly, in exploratory medical officer analyses of the occurrence of neutropenia-associated clinical events in the 2nd through 6th treatment cycles, the amifostine-pretreated patient groups experienced fewer such events in both halves of this study (although the total number of neutropenia-associated clinical events that occurred after the first treatment cycle was quite small). Similarly, as noted above, there was evidence of decreased cumulative renal toxicity in amifostine-pretreated patients (manifested by changes in serum creatinine levels), a finding that was reproduced in both halves of the ovarian cancer randomized study.

After the December 12, 1994 Advisory Committee action, a meeting was held between representatives of US Bioscience and the FDA on December 21, 1994. Based on the above considerations, it was agreed that the FDA would consider a resubmission with additional analyses of the applicant's clinical data. This resubmission would focus on the effects of amifostine in reducing the cumulative toxic effects of cisplatin-cyclophosphamide chemotherapy (particularly, the cumulative renal toxicity of cisplatin); would include a rationale for possible early amifostine approval under the accelerated approval mechanism; and would describe follow-up studies that the applicant could perform, to confirm the clinical efficacy of amifostine. Subsequent U.S. Bioscience submissions have included amendment 021 to NDA 20-221, and additional submissions of data and analyses from the randomized ovarian cancer study and other smaller studies, examining the effects of amifostine on the cumulative toxicities of cisplatin-based chemotherapy. Amendment 021 and several subsequent submissions to NDA 20-221 are the subject of this medical officer review.

2. Scope of Review

Materials reviewed included:

(a) Amendment 021 (submitted January 31, 1995) to NDA 20-221

(b) Correspondence and minor medical amendment submissions from U.S. Bioscience, dated April 20, 1995; April 28, 1995; May 22, 1995; June 30, 1995; July 14, 1995; and July 21, 1995.

(c) The U.S. Bioscience submission overview, provided for the June 9, 1995 meeting of the Oncologic Drugs Advisory Committee regarding this amended submission

NOTE: The above submissions supplement the extensive medical and statistical data and analyses that were previously submitted to NDA 20-221 in amendment 017, and provide additional and revised data and analyses that (a) focus on the possible efficacy of amifostine in reducing the cumulative toxic effects of cisplatin-cyclophosphamide chemotherapy (particularly, the cumulative renal toxicity of cisplatin) and (b) provide some further evidence that amifostine does not significantly reduce the anticancer efficacy of cisplatin-based chemotherapy (included are data from phase II clinical studies in patients with non-small cell lung cancer, and data from an ongoing randomized trial in patients with head and neck cancers).

This review specifically addresses the additional and revised data and analyses provided in Amendment 021 and subsequent submissions, and should be considered as a supplement or addendum to the more extensive medical officer review of NDA 20-221, amendment 017. Parts of the medical officer review of amendment 017 are duplicated in this review, as needed for purposes of clarity.

(also refer to the Statistical review of NDA 20-221, amendment 021, completed by Dr. Clare Gnecco).

3. Amifostine effects on cisplatin-associated cumulative renal toxicity

One of the study endpoints listed in the original protocol for the ovarian cancer randomized study was "incidence of nephrotoxicity defined by the need to delay or reduce the dose of cisplatin". Several analyses have been performed to evaluate the effects of amifostine on the occurrence and severity of cisplatin-associated renal toxicity in this study. In general, study patients did not experience severe cumulative renal toxicity, probably because the study protocol specified that cisplatin treatment would be interrupted if a patient's serum creatinine remained above 1.5 mg/dl at the time of scheduled re-treatment; and protocol treatment was to be terminated if a patient's serum creatinine remained > 1.5 mg/dl for more than 35 days following a chemotherapy treatment. However, evidence of cumulative renal toxicity was observed in study patients, and cumulative renal toxicity was reduced in patients who received amifostine in this study. Selected Amendment 021 analyses, that examined the effects

of amifostine on cisplatin-associated cumulative renal toxicity in the ovarian cancer randomized study, are discussed below.

A. Proportion of patients who required a delay or discontinuation of cisplatin therapy due to nephrotoxicity

The US Bioscience analysis of the proportion of ovarian cancer study patients whose cisplatin treatment was delayed or discontinued due to nephrotoxicity is summarized in Table 1 of amendment 021 to NDA 20-221 (duplicated as Table 2 in the US Bioscience ODAC meeting presentation package):

TABLE 2

Proportion of Patients With Elevations in Serum Creatinine ≥1.5 mg/dL Requiring a Delay or Discontinuation of Cisplatin[a]

	Amifostine + CP	CP	p-value[b]
Original Cohort			
Cycle 4	2/36	5/33	0.190
Cycle 5	2/32	9/35	0.033
Cycle 6	4/27	11/23	0.012
Last Cycle[c]	4/61	11/53	0.026
Last Cycle[d]	3/48	9/40	0.028
New Cohort			
Cycle 4	0/33	1/45	0.392
Cycle 5	0/29	5/40	0.050
Cycle 6	0/15	5/21	0.045
Last Cycle[c]	2/55	6/61	0.190
Last Cycle[d]	0/39	6/50	0.026
Entire Study			
Cycle 4	2/69	6/78	0.203
Cycle 5	2/61	14/75	0.006
Cycle 6	4/42	16/44	0.003
Last Cycle[c]	6/116 (5%)	17/114 (15%)	0.014
Last Cycle[d]	3/87 (3%)	15/90 (17%)	0.004

[a] Cisplatin was to be delayed if serum creatinine >1.5 mg/dL at Day 22; cisplatin was to be discontinued if serum creatinine >1.5 mg/dL at Day 35.
[b] Based on Pearson's Chi Square Test (2-sided).
[c] All patients
[d] Patients who had at least four cycles of therapy.

The above analysis was reportedly performed using a last observation carried forward approach, in which patients withdrawn from therapy in earlier cycles due to renal toxicity or due to nausea/vomiting associated with protracted elevations in serum creatinine at day 35 were included, with their last serum creatinine value carried forward. As noted in the statistical review of this submission (performed by Dr. Clare Gnecco), such analyses may be problematic depending on the pattern of missing data. Another way to evaluate this issue is simply to determine the number of patients who had a serum creatinine of > 1.5 mg/dl documented 22 days after treatment (when the next treatment cycle would be due). The following reviewer table was generated by extracting, from the full database of patient serum creatinine levels, those levels that were obtained closest to day 22 after each treatment (within a window of day 22 ± 7 days; selecting the later level if two "closest" levels were obtained an equal number of days prior to and after day 22; and selecting the highest level if multiple levels were obtained on the same day).

Reviewer Table 1

Patients with a serum creatinine > 1.5 on or about day 22 after treatment

	Amifostine + CP	CP
Original cohort		
Cycle 4	2/43	3/38
Cycle 5	2/39	6/35
Cycle 6	3/30	5/24
Last cycle*	4/47	9/39
New cohort		
Cycle 4	0/38	1/49
Cycle 5	1/33	4/42
Cycle 6	0/20	2/21
Last cycle*	0/39	6/50
All patients		
Cycle 4	2/81	4/87
Cycle 5	3/72	10/77
Cycle 6	3/50	7/45
Last cycle*	4/86	15/89

*Last cycle for patients who had at least 4 cycles of therapy.

This reviewer analysis supports the US Bioscience analysis, in that it shows that fewer patients on the amifostine treatment arm had a serum creatinine of > 1.5 mg/dl at 22 days post-treatment in the last 3 treatment cycles (thus, fewer delays due to this sign of renal toxicity would have been indicated). The numbers in reviewer table 1 are smaller than in the US Bioscience table, predictably due to differences in the analyses performed.

B. Cumulative incidence of treatment-limiting cisplatin-related nephrotoxicity

US Bioscience reported an analysis of the cumulative incidence of treatment-limiting cisplatin nephrotoxicity in Table 4 of amendment 021 to NDA 20-221 (table 3A in the US Bioscience ODAC meeting presentation package):

TABLE 3A

Cumulative Incidence of Treatment-Limiting Cisplatin-Related Nephrotoxicity[a]: (All Patients)

	Amifostine + CP	CP	p-value (2-sided)
Original Cohort[b]	2/63	11/58	0.005
New Cohort	0/59	4/62	0.047
Entire Study[b]	2/122	15/120	0.001

[a] Protracted elevations in serum creatinine at day 35 and/or complications from hypomagnesemia.

[b] Three of the patients in the original cohort (2 amifostine + CP: 301 and 320 and 1 CP: 305) who had protracted elevations in serum creatinine also had severe nausea and vomiting and clinical dehydration which resulted in withdrawal from the study. These three patients were treated prior to the allowance of high dose dexamethasone as an antiemetic. These patients were included in the analyses. Excluding them would reduce the p-values for the original cohort and the entire study to <0.001

While 15 patients reportedly experienced treatment-limiting cisplatin-related nephrotoxicity in the control arm of this study, versus 2 patients in the amifostine arm, it should be noted that far fewer patients actually had their treatment discontinued due to the nephrotoxicity of treatment. Several of these "treatment-limiting" nephrotoxicity events occurred after patients had already completed their planned course of 6 cycles of treatment. For example, persistence of a serum creatinine of > 1.5 mg/dl for more than 35 days after treatment cycle 6 would count as "treatment-limiting nephrotoxicity", but could not result in premature treatment termination.

The current submissions do not include details regarding actual premature treatment terminations due to nephrotoxicity, but such details were provided by US Bioscience in the prior amendment 017 to NDA 20-221, and were considered in the FDA medical officer review of that amendment. To reiterate pertinent sections from the medical officer review of amendment 017:

US Bioscience reported that a total of 6 control arm patients were taken off study due to renal toxicity over the course of the ovarian cancer randomized study, compared to none in the amifostine arm. On Agency review, 4 of these 6 patients (numbers , had serum creatinine elevations that had not resolved to a level of ≤ 1.5 mg/dl by day 35 after a chemotherapy treatment (a protocol criterion for treatment termination); one patient (number had seizures and was found to be hypomagnesemic after 1 cycle of cyclophosphamide-cisplatin treatment (serum creatinine level was unchanged); and the last patient (number had a reduced creatinine clearance of 42 ml/min after her second protocol chemotherapy treatment (although her serum creatinine level of 1.3 mg/dl, documented on days 9, 23, and 28 of cycle 2, was actually lower than her baseline day 1, cycle 1 serum creatinine level of 1.5 mg/dl).

Among patients in the amifostine study groups, patients went off study due to nausea and vomiting with renal dysfunction (and may have had serum creatinine levels that were above 1.5 mg/dl as of day 35 of their last cycle, but day 35 values were not obtained). Amifostine patient received a second cycle of chemotherapy (cyclophosphamide alone, no cisplatin or amifostine) on day 28 after cycle 1 treatment, with a serum creatinine level of 2.6 mg/dl on that day. Her serum creatinine level was still elevated (1.7 mg/dl) on day 14 of cycle 2 (42 days after cycle 1 treatment), and finally fell to 1.2 mg/dl by day 23 of treatment cycle 2. Amifostine patient went off study after treatment cycle 1 with an elevated serum creatinine that probably persisted beyond day 35, but this patient's day 35 renal dysfunction appeared to be due to G-CSF-induced leukocytosis and hyperuricemia (rather than chemotherapy). Finally, two patients experienced acute clinical deterioration in treatment cycle 1 (including deterioration in renal function), and died (amifostine patient and control patient , Although the immediate cause of death of these patients was not clearly determinable from the case records, they should probably be considered for inclusion in analyses of patients who went off study for reasons related to renal toxicity. In summary, one of the six patients stated by the applicant to have discontinued treatment due to renal toxicity probably had minimal or no renal toxicity; and 3 or 4 amifostine patients (and possibly one additional control patient) should probably have been included as having discontinued treatment with renal toxicity.

It should be noted that amifostine patients are now included as -patients who experienced treatment-limiting cisplatin-related nephrotoxicity, in the above Amendment 021 US Bioscience analysis. To reiterate, while this analysis demonstrates a difference between the two treatment groups in the occurrence of treatment-limiting cisplatin-related nephrotoxicity, only a small number of patients actually had their treatment terminated prematurely due to cisplatin nephrotoxicity.

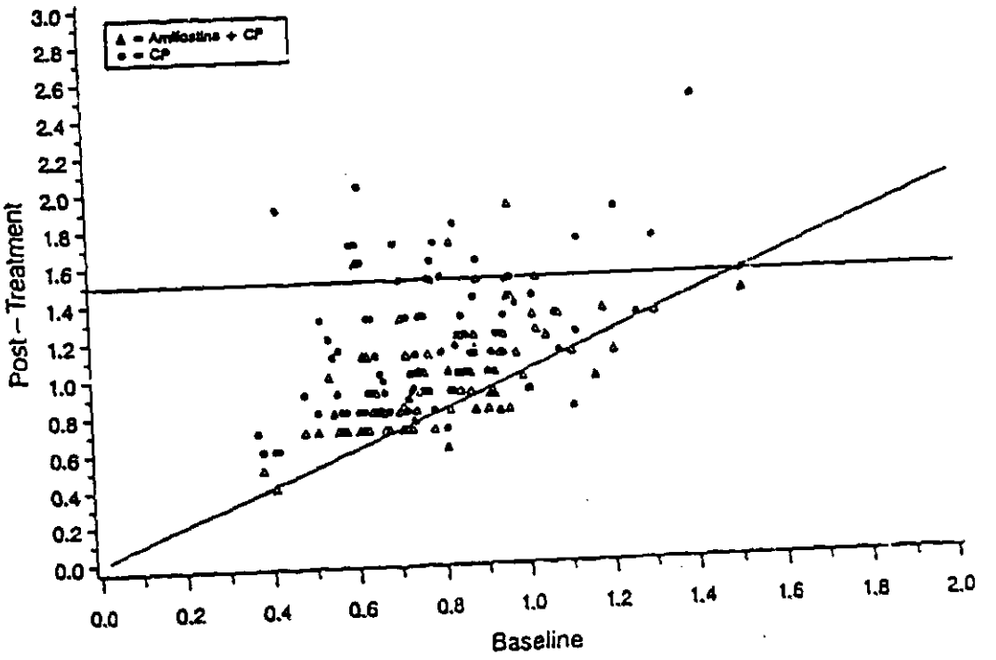
C. Change in serum creatinine levels over the course of treatment

Analyses of changes in serum creatinine levels over the course of treatment, in patients who participated in the randomized ovarian cancer study, currently provide the clearest and most consistent evidence supportive of amifostine efficacy in reducing cisplatin-associated cumulative renal injury. Figure 3C in the US

Bioscience ODAC meeting presentation package is a scatter plot of post-treatment versus baseline serum creatinine levels (for patients who received at least 4 cycles of treatment), and is reproduced below:

FIGURE 3C

Serum Creatinine: > = 4 Cycles Only
Entire Study



Most of the points on this scatter plot fall above the line of equivalence, indicating an increase in serum creatinine (deterioration in renal function). It can also be appreciated that the data points that show the largest deterioration in renal function are generally from patients on the study control arm.

The reviewer table on the following page was generated by extracting, from the full database of patient serum creatinine levels, (i) baseline (pretreatment) serum creatinine levels and (ii) those levels that were obtained closest to day 22 after treatment cycles 4, 5, and 6 (within a window of day 22 ± 7 days; selecting the later level if the two "closest" levels were obtained an equal number of days prior to and after day 22; and selecting the highest level if multiple levels were obtained on a single day). All values from patients who had both baseline and post-treatment cycle 4 values were averaged to obtain mean baseline and post-treatment cycle 4 serum creatinine levels. Baseline versus post-treatment mean serum creatinine values were similarly determined for treatment cycles 5 and 6, and for patients' last treatment cycles (for patients who received at least 4 cycles of treatment).

Reviewer Table 2

Pre-and post-treatment mean serum creatinine levels

"TOTAL" 242 Patient Cohort

	Cyclophosphamide + cisplatin			Amifostine, cyclophosphamide, cisplatin		
	n	Mean baseline value	Mean follow-up value	n	Mean baseline value	Mean follow-up value
Patients enrolled	120	N/A	N/A	122	N/A	N/A
With baseline creatinine	116	.79	N/A	119	.81	N/A
With baseline and cycle 4 creatinine values	85	.80	.99	79	.81	.93
With baseline and cycle 5 creatinine values	76	.79	1.09	69	.81	.98
With baseline and cycle 6 creatinine values	45	.79	1.16	49	.81	.99
With baseline creatinine and a creatinine value after cycle 4, 5, and/or 6	87	.79	1.15*	84	.81	.99*

*Mean follow-up value uses the last follow-up value (cycle 4, 5, or 6) available for each patient.

The above data demonstrate a progressive rise in mean serum creatinine with continued cisplatin chemotherapy; and indicate that amifostine can reduce this progressive loss of renal function.

D. Change in patients' calculated creatinine clearance over the course of treatment

While cumulative renal toxicity (as manifested by rising serum creatinine levels) was reduced in the amifostine treatment group in this study, the overall level of renal toxicity did not appear to be very striking, even in patients who did not receive amifostine. Also, there were concerns that some of the effects observed on renal function could have been due to confounding factors (e.g., differences in the use of nephrotoxic antibiotics in the two study groups). Of course, the study protocol included appropriate safeguards to minimize the possibility of severe renal injury, including provisions to delay or discontinue cisplatin for persistent elevation of serum creatinine to > 1.5 mg/dl. US Bioscience has now provided comparisons of calculated creatinine clearances in the two study groups over the course of treatment (using the formula to derive calculated creatinine clearances from serum creatinine values), US Bioscience has also provided analyses of changes in calculated creatinine clearance in the two study arms that are stratified for baseline creatinine clearance, exposure to nephrotoxic antibiotics, and preexisting hypertension or diabetes.

In brief, these analyses show that patients in the control arm of the ovarian cancer randomized study were significantly more likely to experience a 40% or greater decline in calculated creatinine clearance following cisplatin-cyclophosphamide chemotherapy (Table 7 of amendment 021 to NDA 20-221, appearing with slight corrections as table 4B in the US Bioscience ODAC meeting presentation package). Treatment arm was the only statistically significant factor predictive of a 40% or greater decrease in creatinine clearance in treated patients (ANOVA, including nephrotoxic antibiotic exposure, hypertension, diabetes, and treatment arm in the analytic model). Reproduced below are selected, pertinent tables and figures from the US Bioscience ODAC meeting presentation package.

TABLE 4B

Proportion of Patients With ≥40% Reduction in Creatinine Clearance

	Amifostine + CP	CP	χ^2(2-sided)
Original Cohort			
Cycle 4	4/48	10/40	0.034
Cycle 5	4/42	8/35	0.111
Cycle 6	5/35	11/29	0.031
Last Cycle[a]	10/63	20/58	0.018
Last Cycle[b]	6/48	17/41	0.002
New Cohort			
Cycle 4	0/40	7/49	0.013
Cycle 5	2/34	7/44	0.172
Cycle 6	2/25	6/28	0.177
Last Cycle[a]	6/59	16/62	0.026
Last Cycle[b]	3/40	13/50	0.023
Entire study			
Cycle 4	4/88	17/89	0.003
Cycle 5	6/76	15/79	0.044
Cycle 6	7/60	17/57	0.015
Last Cycle[a]	16/122 (13%)	36/120 (30%)	0.001
Last Cycle[b]	9/88 (10%)	30/91 (33%)	<0.001

[a] All patients

[b] Patients who had at least four cycles of therapy

TABLE 5

Proportion of Patients With Creatinine Clearance <60 cc/min Following the Last Cycle of Chemotherapy Stratified by Pretreatment Creatinine Clearance (All Patients)

Pretreatment Creatinine Clearance	Amifostine + CP		CP	χ^2 CMH	p-value (2-sided)
Original Cohort					
<60	8/13		6/6		
60-79	10/18		11/16		
80-99	3/15		6/14		
≥100	3/17		4/20	3.504	0.061
New Cohort					
<60	11/11		14/15		
60-79	9/22		10/14		
80-99	2/13		6/13		
≥100	2/11		4/19	3.828	0.050
Entire Study					
<60	19/24		20/21		
60-79	19/40	p=0.062*	21/30		
80-99	5/28	p=0.035*	12/27		
≥100	5/28		8/39	8.509	0.004

* 2-sided p-values based on Pearson's Chi Square statistic

TABLE 6

Proportion of Patients With Creatinine Clearance <60 cc/min Following the Last Cycle of Chemotherapy Stratified on Pretreatment Creatinine Clearance (Patients Who Received at Least 4 Cycles of Chemotherapy)

Pretreatment Creatinine Clearance	Amifostine		CP	χ^2 CMH	p-value (2-sided)
Original Cohort					
<60	6/9		5/5		
60-79	8/11		7/8		
80-99	3/14		6/11		
≥100	2/14		4/17	4.936	0.026
New Cohort					
<60	8/8		12/13		
60-79	7/15		9/10		
80-99	0/10		4/8		
≥100	1/7		4/19	6.913	0.009
Entire Study					
<60	14/17		17/18		
60-79	15/26	p=0.027*	16/18		
80-99	3/24	p=0.005*	10/19		
≥100	3/21		8/36	12.389	<0.001

* 2-sided p-values based on Pearson Chi Square statistic

FIGURE 5C

Creatinine Clearance: > = 4 Cycles Only
Entire Study

(scatterplot - all patients with pre-and post-treatment values)

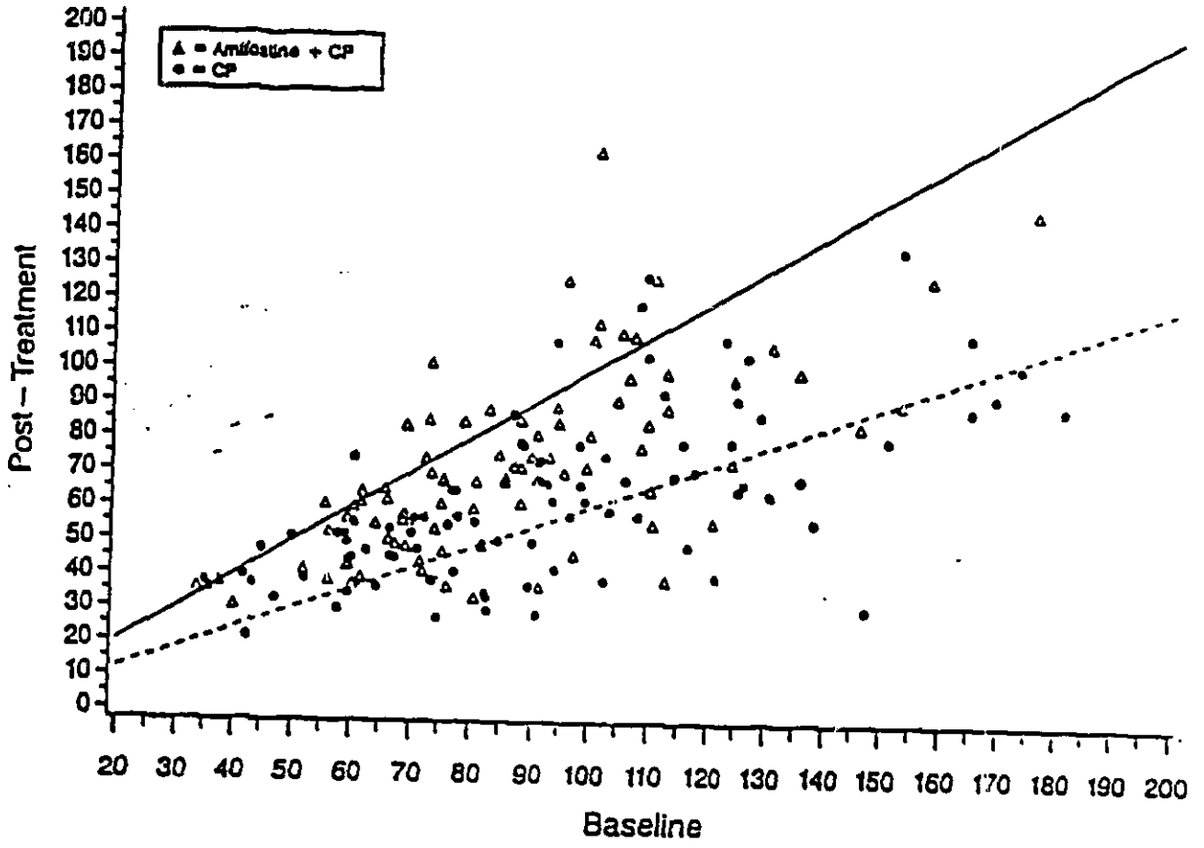


TABLE 10A

Number of Patients Who Received Nephrotoxic Antibiotics
(All Patients)

	Amifostine + CP	CP	p-Value
Original Cohort	7/63	12/58	0.150
New Cohort	8/59	8/62	0.916
Entire Study	15/122	20/120	0.335

TABLE 11A

Incidence of ≥40% Reduction in Creatinine Clearance Following the
Last Cycle of Chemotherapy Stratified in Use of Nephrotoxic Antibiotics
(All Patients)

	Amifostine + CP		CP	χ^2_{corr}	p-value (2-sided[a])
Original Cohort					
Nephrotoxic antibiotics	1/7		5/12		
No nephrotoxic antibiotics	9/56		15/46	5.184	0.023
New Cohort					
Nephrotoxic antibiotics	1/8		5/8		
No nephrotoxic antibiotics	5/51		11/54	5.150	0.023
Entire Study					
Nephrotoxic antibiotics	2/15 (13%)	p=0.026[a]	10/20 (50%)		
No nephrotoxic antibiotics	14/107 (13%)	p=0.019[a]	26/100 (26%)	9.560	0.002

[a] 2-sided p-values based on Pearson's Chi Square statistic

TABLE 12A

Incidence of ≥40% Reduction in Creatinine Clearance Following the Last Cycle of Chemotherapy Stratified on Whether or Not the Patient Had Diabetes (All Patients)

	Amifostine + CP		CP	χ^2_{CMH}	p-value (2-sided)
Original Cohort					
Diabetes	0/2		3/4		
No Diabetes	10/61		17/54	5.065	0.024
New Cohort					
Diabetes	0/3		2/4		
No Diabetes	6/56		14/58	4.800	0.028
Entire Study					
Diabetes	0/5	p=0.030*	5/8		
No Diabetes	16/117	p=0.009*	31/112	9.714	0.002

* 2-sided p-value based on Pearson's Chi Square statistic

TABLE 13A

Proportion of Patients With ≥40% Reduction in Creatinine Clearance Following the Last Cycle of Chemotherapy - Stratified by Hypertension (All Patients)

	Amifostine + CP		CP	χ^2_{CMH}	p-value (2-sided)
Original Cohort					
Hypertension	1/3		1/5		
No Hypertension	9/60		19/53	5.542	0.019
New Cohort					
Hypertension	1/6		3/7		
No Hypertension	5/53		13/55	4.835	0.028
Entire Study					
Hypertension	2/9 (22%)	p=0.586*	4/12 (33%)		
No Hypertension	14/113 (12%)	p=0.002*	32/108 (30%)	9.941	0.002

* 2-sided p-value based on Pearson's Chi Square statistic

TABLE 14

Multi-Factoral Analysis of ≥40% Reduction in Creatinine Clearance

	p-Values (2-sided Chi Square)		
Factor	Original Cohort	New Cohort	Entire Study
Nephrotoxic antibiotics	0.656	0.067	0.099
Hypertension	0.906	0.484	0.737
Diabetes	0.234	0.474	0.172
Treatment arm	**0.031**	**0.031**	**0.003**

Examination of U.S. Bioscience figure 5C (reproduced above on page 16) shows that some patients had post-treatment calculated creatinine clearances in the range of ml/min, with control arm patients comprising the majority of patients with those lower post-treatment creatinine clearances. Conversely, amifostine arm patients predominate at and above the solid line of identity in this figure, indicating that patients who had little or no deterioration in calculated creatinine clearance tended to be members of the amifostine treatment group.

Interestingly, U.S. Bioscience tables 11A, 12A, 13A, and 14 (also reproduced above) also show that study treatment was an important determinant of whether a patient would experience cisplatin-associated cumulative nephrotoxicity, whereas nephrotoxic antibiotic exposure, preexisting diabetes, or preexisting hypertension were not significant predictors of nephrotoxicity in study patients, in a multi-factorial analysis of variance. However, control group patients with nephrotoxic drug exposures or preexisting diabetes appeared to be particularly likely to experience cumulative cisplatin-associated nephrotoxicity, whereas amifostine group patients with similar histories did not appear to have an increased risk of nephrotoxicity (tables 11A and 12A).

It should be noted that the above analyses of calculated creatinine clearance were not prospectively specified in the original study protocol. However, performance of analyses of calculated creatinine clearance were suggested to U.S. Bioscience by this reviewer as a way to further evaluate the possible renal-protective effects of amifostine. Similarly, analyses of treatment toxicities that focused on patients who had completed at least 4 cycles of chemotherapy were not prospectively specified in the study protocol, but such analyses were suggested by this reviewer as a way to better evaluate whether amifostine protects against cumulative treatment toxicities.

U.S. Bioscience staff independently decided to specify a deterioration in calculated creatinine clearance of ≥ 40% (or in some analyses, a deterioration of calculated creatinine clearance to below 60 ml/min) as the cutoffs for defining serious renal injury in the above analyses. These cutoffs were reportedly determined prior to examination of the study data; and they are considered by this reviewer to be reasonable cutoffs. It is clear that if different cutoffs had been selected, the quantitative results of these analyses would have been different. For example, specification of a 50% or 60% deterioration in creatinine clearance as the value that would define serious renal injury (or a requirement that calculated creatinine

clearance had to fall below 50 or 40 ml/min to be considered serious) would have resulted in identification of a much smaller number of cases of serious deterioration in creatinine clearance in both treatment groups. However, examination of the raw study data indicates that the primary qualitative conclusion of these analyses (i.e., lesser nephrotoxicity in the amifostine treatment groups) would not be altered by selection of different cutoff values to identify significant nephrotoxicity.

E. Change in serum magnesium levels over the course of treatment

Hypomagnesemia is a recognized complication of cisplatin chemotherapy. Cisplatin appears to produce a renal tubular defect that results in magnesium wasting in urine, which can worsen with continued cisplatin treatment and may persist for months to years after cisplatin treatment is terminated. Renal magnesium wasting can also be caused by aggressive hydration and diuresis. Patients receiving cisplatin chemotherapy are commonly treated with aggressive hydration and diuretics, to protect the kidneys from cisplatin-associated nephrotoxicity. Thus, hypomagnesemia may be multifactorial in patients undergoing cisplatin chemotherapy.

The current submission includes analyses indicating that amifostine may reduce the severity of hypomagnesemia following cisplatin treatment, summarized in tables 8A and 17 of the U.S. Bioscience ODAC meeting presentation package:

TABLE 8A NCI Toxicity Grades of Serum Magnesium Levels for Each Patient's Last Cycle of Therapy

NCI-CTC Grade: (mEq/L)	0 >1.4	1 ≤1.4->1.1	2 ≤1.1->0.8	3 ≤0.8->0.5	4 ≤0.5	p-value*
Original Cohort						
Amifostine+ CP	49	10	3	0	0	0.017
CP	35	8	6	3	1	
New Cohort						
Amifostine + CP	43	3	0	0	0	0.012
CP	38	10	1	2	0	
Entire Study						
Amifostine + CP	92	13	3	0	0	0.001
CP	73	18	7	5	1	

* Based on 2-sided Mantel-Haenszel Chi-Square statistic.

TABLE 17 EORTC Trial:

NCI Toxicity Grades of Serum Magnesium Levels in Patients With Head and Neck Cancer Treated With Cisplatin, 70 mg/m² ± Amifostine Weekly x 6

	0 >1.4	1 ≤1.4 - >1.1	2 ≤1.1 - >0.8	3 ≤0.8 - >0.5	4 ≤0.5	p-value*
Amifostine + Cisplatin	6	2	11	9	0	0.011
Cisplatin	0	0	6	12	3	

The above analyses indicate that patients treated with amifostine in the U.S. Bioscience ovarian cancer randomized study, and also patients who received amifostine in an EORTC randomized study of weekly cisplatin ± amifostine in head and neck cancer, had a reduced incidence of severe hypomagnesemia following treatment. However, it should be noted that magnesium levels were determined irregularly in the ovarian cancer randomized study. A pre-treatment baseline magnesium level was not reported for one-third of the patients enrolled in this study. Fewer than one-half of the study patients had both a baseline magnesium value and at least one follow-up value in treatment cycle 4, 5, or 6. It is also of concern that on reviewer evaluation of the serum magnesium test results, there seemed to be minimal evidence of cumulative hypomagnesemia in study subjects, or of a beneficial effect of amifostine on mean serum magnesium levels. This evaluation is summarized in reviewer table 3 on the following page. This table was generated by extracting, from the full study database of patient serum magnesium values, (i) baseline serum magnesium levels and (ii) follow-up levels that were obtained in treatment cycles 4, 5, and 6 (selecting the level closest to day 22 of the treatment cycle, if multiple values were obtained). All values from patients who had both baseline and post-treatment cycle 4 values were averaged to obtain mean baseline and post-treatment cycle 4 serum magnesium levels. Mean baseline and post-treatment serum magnesium values were similarly determined for treatment cycles 5 and 6, and for patients' last treatment cycles (for patients who received at least 4 cycles of treatment).

Pre-and post-treatment mean serum magnesium levels
"TOTAL" 242 Patient Cohort

	Cyclophosphamide + cisplatin			Amifostine, cyclophosphamide, cisplatin		
	n	Mean baseline value	Mean follow-up value	n	Mean baseline value	Mean follow-up value
Patients enrolled	120	N/A	N/A	122	N/A	N/A
With baseline magnesium	80	1.93	N/A	82	1.91	N/A
With baseline and cycle 4 magnesium values	55	1.92	1.75	49	1.87	1.80
With baseline and cycle 5 magnesium values	46	1.94	1.76	41	1.88	1.87
With baseline and cycle 6 magnesium values	19	1.86	1.78	25	1.84	1.78
With baseline magnesium and a magnesium value after cycle 4, 5, and/or 6	58	1.93	1.77*	56	1.88	1.83*

*Mean follow-up value uses the last follow-up value (cycle 4, 5, or 6) available for each patient.

In summary, the effects of amifostine on cisplatin-associated renal magnesium wasting and hypomagnesemia lend additional support for the proposed renal-protective effects of amifostine in patients undergoing cisplatin chemotherapy. However, additional studies are needed to confirm and further evaluate the significance of effects of amifostine on cisplatin-associated renal magnesium wasting and hypomagnesemia.

4. Amifostine effects on other cumulative non-hematologic toxicities of cisplatin

Analyses of the cumulative incidence of neurotoxicity (defined as a decrease in neurological function on exam, or neurologic toxicities reported as an adverse event) were included in the applicant's study report. These analyses, summarized in table 10 in amendment 021 to NDA 20-221 (reproduced below), indicate a trend to lesser neurotoxicity in the amifostine group by the end of treatment, in both the original and new patient cohorts. This becomes a statistically significant result in the combined 242 patient "all patients" cohort.

TABLE 10

Cumulative Incidence of Neurologic Toxicity

	Amifostine + CP	CP	p-value (2-sided)
Original cohort	37/63	40/58	0.244
New cohort	30/59	41*/62	0.089
All patients	67/122	81/120	0.045

* 2 patients in the CP arm discontinued due to neurologic toxicity

U.S. Bioscience also analyzed the cumulative incidence of ototoxicity in the ovarian cancer randomized study, as indicated by a need for dose reduction of cisplatin due to ototoxicity. These analyses are summarized in Table 11 in amendment 021 to NDA 20-221 (reproduced below). There was a trend to less ototoxicity by this measure, which neared statistical significance in the all 242 patients analysis.

TABLE 11

Cumulative Incidence of Patients who had Cisplatin Dose Reduced or Discontinued Due to Ototoxicity

	Amifostine + CP	CP	p-value (2-sided)
Original cohort	5/63	10/58	0.122
New cohort	6/59	9/62	0.470
All patients	11/122	19/120	0.108

TABLE 13C

Incidence of Grade 4 Neutropenia
- All Patients (N=242) -

Cycle	Amifostine + CP	CP	χ^2	p-value[a]
1	74/121	73/120	0.003	0.959
2	56/113	59/113	0.159	0.690
3	38/103	40/98	0.324	0.569
4	35/90	35/94	0.053	0.818
5	17/78	42/85	13.350	<0.001
6	16/65	24/70	0.500	0.221
Last[b]	27/122 (22.1%)	51/120 (42.5%)	11.443	0.001
Last[c]	15/89 (16.9%)	38/90 (42.2%)	13.740	<0.001

[a] Based on Pearson Chi-Square test (2-sided).
[b] Following last cycle of chemotherapy received (through six cycles for original cohort and through five cycles for new cohort).
[c] Last cycle of chemotherapy for patients who had received at least four cycles of therapy.

TABLE 14

Proportion of Patients Who Experienced Grade 4 Neutropenia Whose Neutrophil Counts Failed to Recover to 1500/mm^3 by Day 22 (± 3 Days)

All Patients

Cycle	Amifostine + CP	CP	χ^2	p-value[a]
1	7/71	13/72	1.982	0.159
2	18/56	25/59	1.273	0.259
3	20/38	20/40	0.053	0.817
4	17/34	18/35	0.014	0.906
5	9/16	29/42	0.825	0.364
6	6/15	16/23	3.170	0.075
Last[b]	10/26	32/50	4.453	0.035
Overall	43/98 (43.9%)	64/99 (64.6%)	8.518	0.004

[a] Based on Pearson Chi-Square test (2-sided).
[b] Last cycle of chemotherapy received prior to study discontinuation.

6. Amifostine effects on the efficacy of cisplatin plus cyclophosphamide in ovarian cancer treatment

The data regarding amifostine effects on the efficacy of cyclophosphamide plus cisplatin in ovarian cancer treatment were described and discussed in detail in amendment 017 to NDA 20-221 and in the medical officer review of that amendment, and will not be described in detail in this review. In brief, it is clearly not possible to statistically prove that the efficacy of cyclophosphamide and cisplatin in ovarian cancer treatment is unaffected by amifostine (i.e., that amifostine selectively protects normal tissues but does not protect tumor tissues from the cytotoxic effects of this chemotherapy). However, the findings of the ovarian cancer randomized study did not reveal any evidence of significant tumor protection by amifostine. Pathologically-confirmed complete remission rates were similar in the amifostine and control groups in this study (the intent-to-treat analysis is reproduced here):

Study arm	Number of patients	Number who had "second look" surgery	Number with pathologic CR at second look	CRs observed / no. of patients (%)
amifostine	122	60	26	21%*
control	120	52	19	16%

*The difference in pathologic CR rate between the amifostine and control groups was +5.5% (95% confidence interval for the difference, -4.3% to +15.2%).

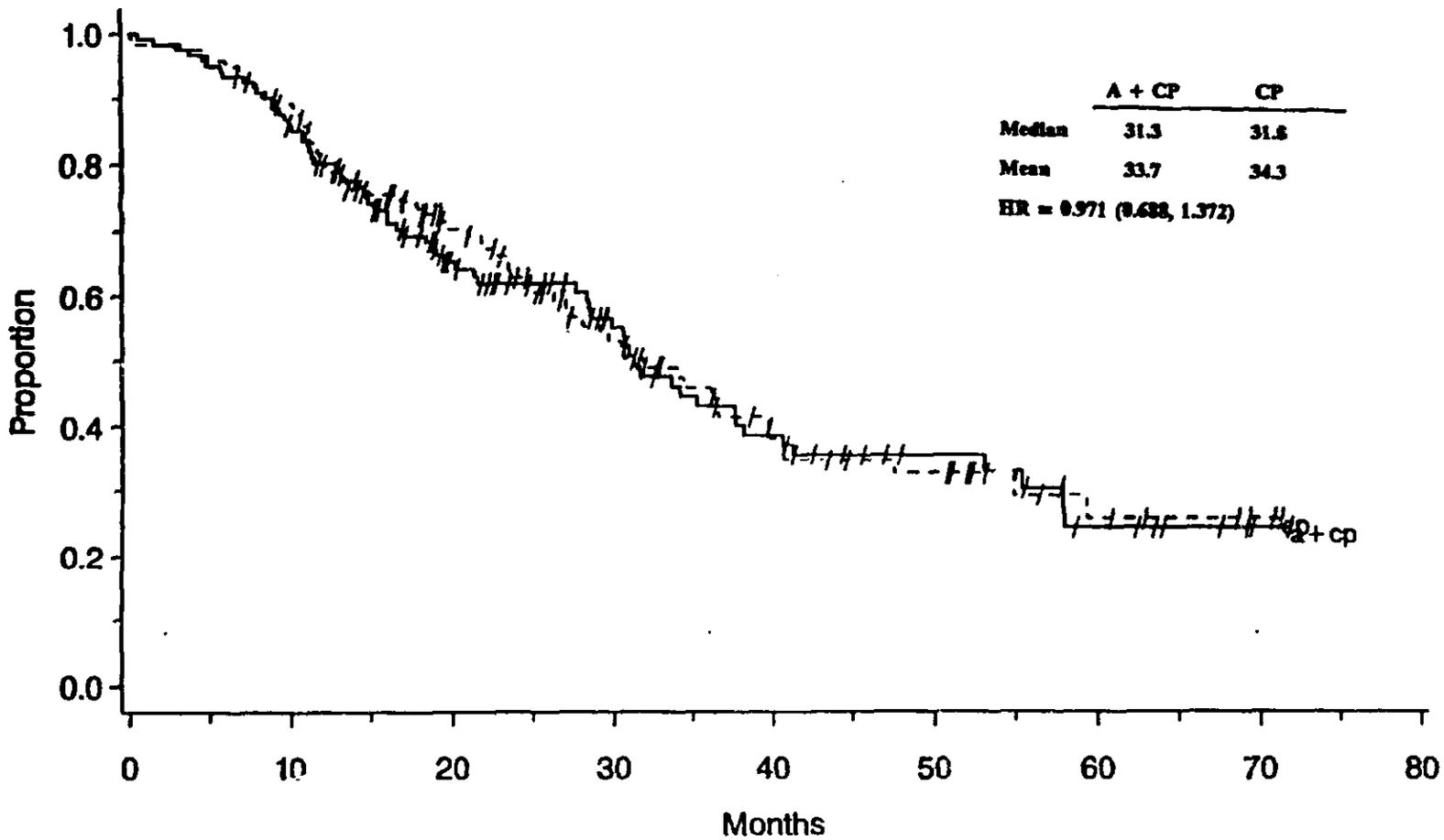
Similarly, overall patient survival was the same in the amifostine and control arms of this study; and "bootstrap" statistical simulations indicated that if the study had been expanded to the very large size that would have been needed to prove "equivalence" in survival (defined as a lower confidence bound for the hazard ratio for survival of 0.80), this criterion would likely have been met. The survival curves for ovarian cancer randomized study patients, updated to July 1994, are reproduced on the following page.

Product Limit Estimate

Survival Time

Cohort: All 242

Amifostine + Cisplatin (n=122) VS Cisplatin (n=120)



Log-Rank Chi-Square: 0.0270 Pr>Chi-Square: 0.8695

Wilcoxon Chi-Square: 0.0952 Pr>Chi-Square: 0.7577

Data Last Modified on 08NOV94

SAS Program: STAT:[CONLON]SURV_GRAPH.SAS;47

Macro: PWSURV

7. Summary of the above data

Data from the U.S. Bioscience ovarian cancer randomized study provide support for the efficacy of amifostine, in reducing cumulative toxicities of cisplatin-cyclophosphamide chemotherapy in patients with advanced ovarian cancer. The data are clearest with respect to protection from cisplatin-associated nephrotoxicity (deterioration in creatinine clearance). While patients in this clinical study generally experienced only a modest degree of cisplatin-associated renal injury, some patients had a more significant deterioration in renal function (defined as a ≥ 40% fall in calculated creatinine clearance, in the U.S. Bioscience study analyses). This degree of deterioration in renal function was observed less frequently in patients in the amifostine treatment groups.

The study data indicate that there was no significant reduction in pathological complete response rate in the amifostine study group (point estimate, 5.5% higher pathologic CR rate in the amifostine group; 95% confidence interval for the difference in pathologic CR rate, -4.3 to +15.2%). Similarly, overall patient survival was the same in the amifostine and control arms of this study. These study data do not demonstrate (but cannot statistically rule out) tumor protection by amifostine in this study population. However, based on the results of prior studies of cisplatin-based chemotherapy regimens in the treatment of advanced ovarian cancer, these chemotherapy regimens probably have a relatively modest effect on the survival duration of patients with advanced ovarian cancer; and long-term relapse-free survival appears to be a relatively rare event. Therefore, in this setting, concern over the possibility that amifostine might reduce the curative survival benefit of chemotherapy is tempered by the fact that currently-available chemotherapy regimens are probably not curative for the great majority of patients.

An important question relates to the long-term natural history of the cumulative toxicities of cisplatin-cyclophosphamide chemotherapy, and the long-term effects that amifostine may have on these toxicities. While chemotherapy with cisplatin is known to cause cumulative treatment toxicities, there may be some recovery from these treatment toxicities over time. Specifically, cisplatin-associated nephrotoxicity (as manifested by deterioration in creatinine clearance, and renal magnesium wasting with hypomagnesemia) may include both an acute or subacute (reversible) component, and a chronic (permanent) component. A fall of 40% in creatinine clearance would be of lesser concern if creatinine clearance subsequently improved over time to within 15-20% of the baseline value; and would be of greater concern if there was further deterioration over time. Also, if amifostine is shown to reduce long-term renal magnesium wasting in patients who have been treated with cisplatin, it must be noted that the long-term consequences of hypomagnesemia are not well characterized (although magnesium clearly is important in many physiologic processes); and measurements of serum magnesium may not accurately reflect total body magnesium (as most of this cation is intracellular).

In summary, results of the above U.S. Bioscience clinical trials include several statistically-significant findings indicative of amifostine efficacy in protecting normal tissues from the cumulative toxicities of treatment with cyclophosphamide plus cisplatin.

The clearest data relate to protection from cisplatin-associated nephrotoxicity. The clinical significance of this renal protection is not entirely clear, although a reduction in the number of patients who experience a ≥ 40% decline in creatinine clearance can be considered to be a tangible clinical benefit. Finally, the possibility that amifostine could protect tumors and reduce the efficacy of cancer treatment in at least some clinical settings cannot yet be dismissed.

The available data might not be considered sufficient to support conventional approval of this New Drug Application, under 21 CFR 314.105 (since there are some uncertainties regarding the durability and clinical significance of the observed protection from cisplatin nephrotoxicity, and other cumulative treatment toxicities, in patients treated with cisplatin and cyclophosphamide for advanced ovarian cancer). Accelerated approval under 21 CFR 314.510 could be an alternative, considering the observed effects of amifostine on serum creatinine levels and calculated creatinine clearance in patients undergoing cisplatin chemotherapy as a surrogate for clinical benefit. Approval under 21 CFR 314.510 would include the requirement that U.S. Bioscience conduct further studies of amifostine, to verify and further describe its clinical benefit. Approval under 21 CFR 314.510 could be withdrawn if due diligence was not shown in pursuing the required additional research; or in the event that the additional research failed to confirm the validity of the current study data as predictive of clinical efficacy. A regular or accelerated approval action would also depend on the prior resolution of any other remaining significant FDA concerns (e.g. any remaining chemistry/manufacturing and biopharmaceutical issues), to the Agency's satisfaction.

8. ODAC discussion of June 9, 1995

The above data (with a draft version of this review) were reviewed by the Oncologic Drugs Advisory Committee at its June 9, 1995 public meeting. In brief, the committee:

- voted 8-0 that amifostine was effective in reducing cumulative renal injury (as manifested by decreases in creatinine clearance) in cancer patients treated with 100 mg/m^2 cisplatin for a proposed 6 courses.

- voted 8-0 that current efficacy and safety data were sufficient to support at least accelerated approval of amifostine, for use to reduce the cumulative renal injury associated with cisplatin chemotherapy at 100 mg/m^2 with a planned program of 6 courses, with other agents unspecified, for previously untreated patients with advanced ovarian cancer.

- Deadlocked 4-4 on whether current efficacy and safety data were sufficient to support (at least) accelerated approval of amifostine, for the above indication.

- Discussed several follow-up studies that would be of interest to further elucidate the clinical utility of amifostine (but did not specifically identify a study that should be required to confirm the clinical efficacy of amifostine, as a part of an accelerated approval action).

9. Subsequent US Bioscience correspondence submissions to NDA 20-221

In 3 recent correspondence submissions to NDA 20-221, US Bioscience has submitted additional efficacy and safety data obtained in 2 currently-ongoing clinical trials, to further support the proposition that amifostine does not significantly interfere with the anticancer efficacy of cisplatin-based chemotherapy regimens. The current findings of these trials, as briefly summarized in these 3 submissions, are as follows:

A. Phase 2 study in stage IV non-small cell lung cancer (correspondence submission dated June 30, 1995)

This submission reports current data from a phase 2 study in patients with stage IV (metastatic) non-small cell lung cancer, in which 25 patients have been treated under the supervision of Dr. Joan Schiller at the University of Wisconsin. Patients received amifostine (initially 910 mg/m^2, now 740 mg/m^2) and cisplatin (120 mg/m^2) on day 1, followed by vinblastine injections (5 mg/m^2) on days 8, 15, and 22. Treatment was to be repeated at 4 week intervals, for a maximum of 6 cycles. The actual number of treatments administered ranged from 1-6 (median 4, only 3 patients received 6 cycles). According to the cover letter for this submission, objective partial responses of > 1 month duration were documented in 16 patients, for a partial response rate of 64%. Reported response durations ranged from 2-20$^+$ months (median 5 months; 4 patients had responses lasting 12 months or longer). Median survival was estimated at 17 months, with 65% of patients alive 1 year after entry. This study followed an earlier study of a similar regimen (administered prior to radiation therapy) in patients with stage III non-small cell lung cancer (also conducted by Dr. Schiller). In that study, response to chemotherapy was assessed prior to initiation of radiation therapy, and 23 objective responses were observed among 31 patients treated (response rate, 74%).

These data are submitted by the applicant as evidence that amifostine does not have tumor-protective effects in patients with non-small cell lung cancer, treated with this cisplatin-vinblastine regimen. The reported objective response rate is substantially higher, and survival is longer, that the results usually reported in phase 2 studies of chemotherapy regimens for this disease. The applicant suggests that amifostine might actually enhance the efficacy of chemotherapy in this setting. A phase 3 study is being initiated.

Full documentation / case records were not submitted for the 25 patients who participated in this study, but pre- and post-treatment chest CT scan photos are provided for 15 patients (14 who are described in appendix 3 of this submission as partial responders, and 1 described as stable disease). These photos appear to be supportive of the investigator's assessments of partial response. It is noted that the cover letter and the study report included in this submission both state that there were a total of 16 objective partial responses, but appendix 3 lists 18 patients as having attained a partial response. The brief study report provided includes relatively little information about treatment toxicity. The principal toxicity was stated to be hematotoxicity (associated with the weekly vinblastine injections, which were

not preceded by amifostine treatment). Calculated creatinine clearance , reportedly remained stable over the course of treatment, despite the relatively high dose of cisplatin administered. It is further stated that "none of the 11 patients who completed 4 or more cycles of treatment had a 40% or greater decline in calculated creatinine clearance after their last treatment" (but appendix 3 to this report indicates that 13 patients completed 4 or more cycles of treatment). However, it should be noted that Dr. Schiller published a research abstract on this study in 1995, in the Proceedings of the American Society of Clinical Oncology (copy included in this submission to NDA 20-221). This abstract provided some additional information regarding treatment toxicity, as follows:

Toxicities have included grade 3 or 4 renal dysfunction in 14% and 0% of patients, respectively; (and grade 3 or 4) neutropenia in 10% and 90%. Four patients were hospitalized for neutropenic fevers; 2 patients went off study for renal toxicity. Eighteen patients required interruption of amifostine for asymptomatic hypotension; all except 3 were able to continue. Other toxicities included grade 3 neuropathy (5 patients) and ototoxicity (3 patients) at cumulative cisplatin doses of 660, 600, 540, 480, 450, 420, 324, and 390 mg/m^2, respectively.

To summarize, this study provides evidence to support the contention that amifostine does not have significant tumor-protective effects in patients with non-small cell lung cancer, treated with this cisplatin-vinblastine regimen. The reported objective response rate is high, and patient survival is long, compared to the results commonly reported in phase 2 studies of chemotherapy for stage IV non-small cell lung cancer. Dr. Schiller's prior report of a 74% objective response rate in 31 patients with stage III non-small cell lung cancer is also an promising finding, although objective response rates are often higher in stage III than in stage IV disease. However, it must be noted that this treatment regimen had substantial toxicity. The high rate of grade 4 neutropenia, with 4 hospitalizations for neutropenia-fever, can be blamed largely on the weekly vinblastine injections (which were not preceded by amifostine treatment); but the neuropathy, ototoxicity, and renal toxicity were probably more related to the cisplatin therapy, and it appears that amifostine did not prevent these problems from developing in several patients. The applicant's suggestion that amifostine administration might be enhancing anticancer efficacy in this setting (via as-yet unknown mechanisms), if accepted, raises the issue of whether chemotherapy toxicities could also be enhanced (rather than reduced) in some settings. Based on current understandings of the mechanisms of amifostine action, it does not seem plausible that amifostine could protect normal tissues from the toxicity of chemotherapy, and simultaneously enhance the anticancer efficacy of this chemotherapy.

B. Randomized phase 2 study in patients with advanced head and neck cancers (Correspondence submissions dated July 14 and 21, 1995)

These submissions report data available from 59 currently-evaluable patients, who have been enrolled in an EORTC randomized trial of weekly cisplatin ± amifostine as initial chemotherapy for treatment of advanced head and neck cancers (it appears that the final enrollment in this study will be approximately 80 patients).

All patients receive cisplatin 70 mg/m^2/week for a planned 6 week course of treatment; half are randomized to receive amifostine (740 mg/m^2) immediately prior to each weekly cisplatin treatment. Most of the patients enrolled to date have been males with locally advanced disease (no distant metastases), and performance status 1 (eligibility criteria allow PS 0-2). Prior radiation therapy is permitted, but few of the patients enrolled to date have received prior treatment with radiation.

The preliminary toxicity findings of this study include grade 3/4 thrombocytopenia in 6 control arm patients versus 1 amifostine patient (but, on examination of listings of patient hematology studies that were included with this submission, the amifostine patient had a platelet nadir of 2,000 per cmm, which likely required platelet transfusions; while the lowest nadirs reported on the control arm were 12,000 and 19,000 per cmm). Also, the provided listings of hematology data listed only 5 control arm patients as having grade 3/4 thrombocytopenia. Grade 3/4 neutropenia reportedly occurred in 10 patients on the control arm, versus 8 patients on the amifostine arm (examination of the patient listings revealed 1 control arm patient who experienced grade 4 neutropenia, and 2 amifostine arm patients with grade 4 neutropenia). The study report states that treatment delays due to hematologic toxicities were more common in the control arm, resulting in a lower treatment dose intensity (i.e. the treatment was spread out over a longer period of time). However, on examination of the dosing listings provided for 29 patients in each study group, it appears that the total number of cisplatin treatments ultimately administered was similar in the two study groups:

Reviewer Table 4
Cisplatin treatments administered
EORTC study of weekly cisplatin in patients with head and neck cancers

	Number of Cisplatin treatments administered					
	1	2	3	4	5	6
Amifostine study arm (n = 29)	0	0	3	3	6	17
Control study arm (n = 29)	0	1	1	3	3	21

Patients who received amifostine appeared to experience less nephrotoxicity (as manifested by serum magnesium levels and calculated creatinine clearances pre- and post-therapy). Also, grade 2/3 ototoxicity (determined by repeat audiograms) and neurotoxicity (determined by measurement of vibratory sensation, using an instrument called a vibrometer) were reportedly observed less frequently among patients on the amifostine study arm.

Objective response rates were reportedly similar in the two study arms (63% in the amifostine arm, versus 58% for the control arm; there were 3 complete remissions on the amifostine arm, versus none on the control arm). No analyses of response duration, time to progression, or survival of study patients were provided in this update of the results of this ongoing study. Copies of EORTC case records of tumor measurements were provided as an appendix to this report.

US Bioscience has recently reported a patient death during treatment on this study (reported to IND , submission serial no. 226, dated July 10, 1995). The patient was a 47 year old man with a large tonsillar mass that periodically stimulated paroxysms of coughing. Following amifostine administration in his second week of study treatment, he appeared to have a choking episode with respiratory distress and hypotension, then convulsions and death. Further information is being sought.

To summarize, this study provides evidence that amifostine may not have significant tumor-protective effects in patients with head and neck cancers, treated with this weekly cisplatin regimen. The reported objective response rates for the two study arms are consistent with those reported in other studies of aggressive frontline chemotherapy treatments for these malignancies. The number of weekly cisplatin treatments was essentially the same in the two study arms, but patients in the amifostine arm reportedly required fewer treatment delays and thus were treated at a higher dose intensity. Clinical treatment toxicities (nephrotoxicity, neurotoxicity, ototoxicity) were reportedly less in the amifostine study arm (although the recently-reported patient death is a concern). A comprehensive analysis of the results of this study is not currently possible, given the preliminary nature of the study results that were submitted.

10. Other clinical studies addressing amifostine effects on chemotherapy efficacy

Two other clinical studies that have previously been cited by the applicant, in support of the claim that amifostine does not significantly interfere with the efficacy of cancer treatment. These were studies of relatively high doses of cisplatin, in patients with metastatic melanoma. Data from these studies were reported in volumes 30 and 31 of amendment 017 to NDA 20-221 (submitted July 12, 1994).

US Bioscience protocol WR-2721-B001 (study number 201D): Dr. D. Glover and Dr. J. Glick at the conducted this study of amifostine (740 mg/m^2) followed by cisplatin (60-150 mg/m^2) in metastatic melanoma (treatment to be repeated at 3-4 week intervals). Fifty-three patients were enrolled; 48 received initial treatment with cisplatin doses of ≥ 120 mg/m^2, and were the focus of the US Bioscience study report. [This report did not describe treatment outcomes in the 5 patients who received initial cisplatin doses of < 120 mg/m^2; however, review of the investigators' journal article of findings in the first 36 patients treated in this study reveals that 4 patients had received initial cisplatin doses of ≤ 100 mg/m^2 because of some level of baseline renal impairment, and none of these 4 had an objective response to treatment.] Of the 48 patients included in the US Bioscience analysis, 25 patients started treatment at a cisplatin dose of 120 mg/m^2; 14 started at 135 mg/m^2;

and 9 started at 150 mg/m^2. A total of 180 courses of cisplatin treatment were administered to these patients (this included 11 courses at 150 mg/m^2, 35 courses at 135 mg/m^2, 115 courses at 120/m^2, 12 courses at 100 mg/m^2, and 7 courses at ≤ 90 mg/m^2). Twenty-six of 47 evaluable patients reportedly had an objective response to treatment (1 CR, 25 Prs). Responses were reportedly observed at visceral sites of metastatic disease (e.g., liver) as well as in skin and soft tissue metastases. Response durations were not reported, but according to the investigators' journal article of findings in the first 36 patients treated in this study, there were 19 responders and the median duration of response was 4 months. Median survival for the 48 analyzed patients was reportedly 5.4 months. Survival duration appeared to be somewhat longer for the 9 patients who received initial treatment at 150 mg/m^2 cisplatin. However, this difference was not statistically significant; and all but one of these 9 patients required cisplatin dose reduction after their first course, so these patients received only slightly more cisplatin than the patients who started treatment at 120 or 135 mg/m^2 cisplatin. Treatment toxicity was reportedly modest. However, antiemetics were rarely used prior to amifostine administration in this study, and nausea/vomiting was reported in 32 patients (67%).

US Bioscience protocol WR-2721-18: This study has been conducted under the direction of M. Avril, MD, The preliminary report of the results of this study that was included in supplement 017 to NDA 20-221 was dated December 1993, and indicated that the study was still ongoing. In this study, patients with metastatic melanoma are treated with amifostine (910 mg/m^2), followed by escalating doses of cisplatin (120, 135, 150 mg/m^2). Treatment was to be repeated at 3 week intervals, unless tumor progression or severe toxicity precluded repeat treatment. At the time of this preliminary report, 21 patients had been enrolled at 120 mg/m^2, 12 at 135 mg/m^2, and 2 at 150 mg/m^2 cisplatin. Doses of cisplatin were not escalated in individual patients; doses were reduced for treatment toxicities. The 21 patients enrolled at the 120 mg/m^2 dose level received a total of 71 cycles of cisplatin treatment at this level and 2 cycles at 90 mg/m^2; two patients were taken off treatment for toxicity (moderate paresthesias after 7 and 8 cycles of treatment, respectively). At the 135 mg/m^2 dose level, the 12 patients enrolled received a total of 21 treatment cycles at this level plus 6 cycles at 108-110 mg/m^2. One patient reportedly withdrew due to ototoxicity. The two patients enrolled at the 150 mg/m^2 dose level each received only 1 cycle of treatment at this level; one of these patients received an additional cycle at 120 mg/m^2. Both went off study due to early disease progression.

Objective responses were reported in 7 of the 35 patients enrolled (2 complete remissions, 5 partial remissions). The median response duration was 8 weeks according to the investigators published abstract of the study results. Time to progression and survival data were not analyzed, but based on the data listings it appears that median time to progression was about 66 days and median survival was about 237 days.

11. Conclusions and recommendations

A. Based on review of the US Bioscience submissions to NDA 20-221, and review of the Oncologic Drugs Advisory Committee deliberations on these data, approval of amifostine is recommended, for the limited indication of reducing the cumulative nephrotoxicity of cisplatin in patients undergoing treatment with cisplatin-based chemotherapy for advanced ovarian cancer.

Limited data are currently available regarding the preservation of anticancer efficacy when amifostine is administered prior to cisplatin chemotherapy in settings other than advanced ovarian cancer. Interference with the efficacy of cancer treatment would be of greatest concern in settings where chemotherapy is often curative. Therefore, labeling should include a warning that amifostine should not be used in conjunction with cisplatin in settings where cisplatin-based chemotherapy is commonly curative (e.g., certain germ cell tumors).

These and other specific comments and recommendations regarding ETHYOL® amifostine labeling are noted on the attached copy of the latest ETHYOL® proposed labeling, which was submitted by U.S. Bioscience in the July 14, 1995 correspondence submission to NDA 20-221.

B. Labeling of ETHYOL for use in a broader spectrum of solid tumors (i.e., all solid tumors except tumors of germ cell origin) can be considered when adequate clinical data have been submitted to demonstrate that ETHYOL does not significantly interfere with efficacy in at least one additional type of solid tumor. Some of the phase 2 study data recently submitted are suggestive that the palliative efficacy of cisplatin in the treatment of certain other types of solid tumors (particularly, head and neck cancers and non-small cell lung cancer) may not be adversely affected by the administration of ETHYOL, but these data are limited in quantity and detail. Additional data (e.g., completion and full analysis of the randomized phase 2 trial in head and neck cancers that was reviewed above; or completion of the applicant's phase 3 trial in non-small cell lung cancer, which has been submitted to IND in submission serial number 210 and other submissions) could provide sufficient data to support such an expansion of ETHYOL® amifostine labeling.

Robert J. DeLap, MD, PhD
Acting Medical Group Leader

cc: NDA 20-221
HFD-150 / R. Justice
HFD-150 / L. McCollum

Medical Officer Review - Amended NDA 20-221
ETHYOL[R] *amifostine to protect from adverse effects of chemotherapy,*
in patients receiving cisplatin/cyclophosphamide for ovarian cancer

Major amendment #017, received July 13, 1994
Reviewing Medical Officer: Robert J. DeLap, MD, PhD
Date of completion of this review: January 30, 1995

Contents

1. Background / Synopsis

Chemotherapy for advanced ovarian cance. substa ial benefit; but this illness is ultimately fatal in most patients, and c. motherapy can cause significant morbidity and occasional mortality. Major goals of current research are to develop more effective treatments for this disease, and to reduce treatment toxicity. The use of ETHYOL[R] amifostine as a chemoprotective agent, to reduce treatment toxicity in women receiving cisplatin plus cyclophosphamide for advanced ovarian cancer, is the subject of this New Drug Application.

Theoretical rationales underlying the development of chemoprotective agents have included (a) some of the toxic effects of cancer chemotherapy may be unrelated to anticancer efficacy, so it may be possible to develop agents that will ameliorate toxicity without compromising efficacy; and/or (b) it may be possible to take advantage of biochemical and physiologic differences between normal tissues and tumor tissues, to develop agents that will selectively protect normal tissues.

In recent years, several potential chemoprotective agents have been evaluated in preclinical and clinical studies. Examples are dexrazoxane to reduce anthracycline-induced cardiomyopathy; diethyldithiocarbamate to reduce toxicities of platinum-based chemotherapy; pyridoxine to reduce chemotherapy-induced neuropathy; and amifostine (ETHYOL[R], also known as WR-2721) to ameliorate toxicities of platinum and alkylating agent chemotherapy. Studies of some of these agents have indicated that reduction of the toxic effects of chemotherapy may be achieved in certain clinical settings. However, there are continuing concerns, based on evidence from preclinical and clinical studies, that effective chemoprotective agents may also protect tumors from the toxic effects of chemotherapy. Thus, while preclinical studies have clearly shown that amifostine may ameliorate the toxic effects of certain chemotherapy drugs (or radiation therapy) on normal tissues, results of some preclinical studies have suggested that amifostine might protect tumor tissues as well.

Absolute selectivity for chemoprotective agents (effective protection of normal tissues with no protection of tumors) may be an unattainable goal. Even if an absolutely selective chemoprotective agent were available, randomized clinical studies to prove the absence of tumor protection (with demonstration of equivalent response rate, time to progression, and survival, in patients randomized to receive the chemoprotective agent) could require very large patient numbers.

Relatively selective chemoprotective agents (agents that provide significant protection to normal tissues, but may also protect tumors to a lesser extent) could have a useful role in cancer treatment. Potentially appropriate uses of such agents could include: (a) to allow more intensive treatment (where a gain in efficacy due to treatment intensification, made possible by the use of the chemoprotective agent, could be shown to exceed any efficacy loss due to tumor protection); (b) to allow continued administration of an effective treatment, which the patient would otherwise be unable to tolerate; or (c) to reduce significant clinical toxicities of a palliative treatment, where a modest loss of therapeutic efficacy might be acceptable.

The US Bioscience New Drug Application for ETHYOL[R] amifostine was originally submitted on September 30, 1991. The original application was based primarily on the results of a single randomized trial, in patients with advanced ovarian cancer. Following debulking surgery, patients received chemotherapy with cyclophosphamide and cisplatin (control arm), or amifostine followed by cyclophosphamide and cisplatin. After treatment of the first 121 of 200 planned patients, an interim analysis performed by the applicant indicated that patients receiving amifostine had fewer episodes of neutropenia with fever ("febrile neutropenia"). Response rates were similar on the two study arms, consistent with a lack of tumor protection by amifostine. Accrual to the study was subsequently interrupted, and these data were submitted to the Agency in the original New Drug Application.

After Agency and Oncologic Drugs Advisory Committee review, the application was determined to be non-approvable. There were several concerns regarding the clinical data and analyses included in the application. The protocol for the applicant's pivotal trial had specified multiple study endpoints, and did not provide for statistical adjustments for multiple analyses. The protocol did not include a precise definition of a "febrile neutropenia" event; and did not specify that such events would be the primary study endpoint. The definition of what constituted a febrile neutropenia event was in fact determined in the course of the applicant's analyses of the study data, (after the data were collected). Reduction in the occurrence of these events was similarly identified as the primary study endpoint only after the study results were in. Also, collection of the data needed to determine whether a patient had experienced a "febrile neutropenia" event was inconsistent and often inadequate. For example, data on patient temperatures during periods of grade 4 neutropenia were often absent. There was also concern that the size of the study (63 amifostine patients and 58 control patients) was inadequate to exclude the possibility of substantial tumor protection by amifostine. Finally, significant deficiencies were also identified in the chemistry and biopharmaceutical parts of the initial application.

For the above reasons, the clinical data provided in the initial application were considered to be interesting, but non-definitive. A second adequate and well controlled study was needed, to prospectively verify whether amifostine has significant efficacy in reducing the frequency of neutropenia-associated clinical events in patients receiving cyclophosphamide-cisplatin chemotherapy for advanced ovarian cancer. More data were also required to better evaluate whether amifostine has significant tumor-protective effects. Subsequently, the applicant and the Agency (with the advice of the Oncologic Drugs Advisory Committee) agreed that the advanced ovarian cancer study would be completed, with accrual of 200 patients as originally planned. The efficacy of amifostine in reducing the incidence of "grade 4 neutropenia with fever (with or without sepsis)", as observed in the applicant's analyses of results in the first 121 patients treated in the study, was defined as the primary study endpoint. The results obtained in patients enrolled in the second half of this study would be analyzed as a second, confirmatory study, to verify whether amifostine pretreatment is effective in reducing the incidence of these clinical events in this study population. Treatment efficacy (response rate, time to disease progression, and survival duration) would also be examined in updated analyses of all 200 patients, to better assess whether amifostine has significant adverse effects on treatment efficacy.

This amended New Drug Application now includes data from 242 patients that have been treated in the applicant's pivotal ovarian cancer study. Separate analyses are provided for the original 121 patient cohort, the new 121 patient cohort, and all 242 patients combined. The definition of the primary study endpoint used in the applicant's analyses has further evolved, and is now "neutropenia with fever and/or infection, requiring antibiotic therapy". As noted above, the principal efficacy hypothesis to be tested was whether the incidence of neutropenia-associated clinical events would be significantly lower in the amifostine study arm in the new patient study cohort, to confirm the observation made in the original 121 patient cohort. According to the applicant's analyses as initially submitted in this amended application, the incidence of neutropenia-associated clinical events was significantly lower in the amifostine treatment arm of the new 121 patient cohort (compared to the control arm), thus confirming the observation made in the original 121 patient cohort.

Unfortunately, the current definition of a neutropenia-associated clinical event ("neutropenia with fever and/or infection, requiring antibiotic therapy") still lacks precision, and determining whether a given episode qualifies as an event often requires substantial subjective judgement. The study results for this primary study endpoint turn out to be highly dependent on the precise definition of "neutropenia-associated clinical event" that is used, and how the definition is applied. On Agency medical officer review of the case records of each of the 242 study patients, it was found that some patients on the amifostine study arm had episodes that were not counted as events in the applicant's analyses of neutropenia-associated clinical events (but should have been, in the reviewer's judgement). Similarly, some patients on the study control arm had episodes that should not have been (but were) counted as events in these analyses. The preliminary medical officer determinations of which episodes should be included as events in the primary study analyses of "neutropenia-associated clinical events" were shared with the applicant, US Bioscience, and additional US Bioscience comments on some of these episodes were obtained and considered. The final medical officer determinations of episodes included and excluded as events, in FDA analyses of neutropenia-associated clinical events, are summarized in appendix II of this review. While it is clearly possible to debate the appropriateness of including or excluding some of these individual episodes, review of the entire content of appendix II is highly recommended, to illustrate the overall patterns of inclusion / exclusion judgements of the applicant, and of the medical officer. Overall, the final medical officer judgements are deemed to lack any significant bias against amifostine (and may be regarded by some as evidencing a bias in favor of this investigational drug).

When analyses of neutropenia-associated clinical events were repeated, using the Agency medical officer's final judgements as to which episodes should be included as events for these analyses, the incidence of neutropenia-associated clinical events was not significantly reduced in the amifostine treatment arm of the new 121 patient cohort (although patients in the amifostine treatment arm did have a slightly smaller total number of events). Thus, the primary efficacy hypothesis was not statistically confirmed in the new 121 patient cohort.

Agency analyses of the study data did confirm that amifostine-treated patients had a significantly reduced incidence of neutropenia-associated clinical events in the original 121-patient cohort. Although statistically weaker than in the original 121-patient cohort, this finding was still significant in the total 242-patient study cohort (p=.065 two-sided, p=.033 one-sided, chi-squared test, with exclusion of 7 amifostine arm patients and 2 control arm patients deemed to have been inappropriately entered in the study, or deemed to be nonevaluable).

Other analyses of this study performed by the applicant indicated that amifostine pretreatment was associated with a lesser frequency of grade 4 neutropenia in later treatment cycles. Also, a trend indicating more rapid recovery from grade 4 neutropenia was observed for patients in the amifostine arm, in some treatment cycles. Of course, such changes in laboratory parameters are regarded as clinically meaningful only if they are found to be useful surrogate markers for clinical benefit (such as a reduction in the occurrence of neutropenia-associated clinical events).

In retrospect, this was probably a difficult patient population in which to evaluate the efficacy of amifostine in reducing neutropenia-associated events. Chemotherapy was initiated early after major abdominal surgery. Many of the study patients were probably at high risk for infection, regardless of the effects amifostine may have had on the severity or duration of chemotherapy-induced neutropenia, for reasons such as their (a) multiple recent invasive procedures (surgery, central lines, catheters); (b) incomplete wound healing; (c) poor nutritional status; and/or (d) disturbed bacterial flora, due to antibiotics administered around the time of surgery, with possible colonization by pathogenic bacteria during the hospital stay for surgery. In fact, most of the neutropenia-associated events that occurred in this study did occur in the first treatment cycle. Conceptually, amifostine might be expected to reduce the incidence of neutropenia-associated events throughout a patient's treatment with chemotherapy, by reducing the extent and duration of neutropenia after each chemotherapy cycle, and reducing cumulative damage to the bone marrow (resulting in less cumulative myelosuppression in later treatment cycles).

For the above reasons, a better study design might have been to evaluate amifostine in a setting where patients had not recently undergone major surgery. A suggestion of how such a study might have turned out can be obtained by exploratory analyses of the current study data, excluding all neutropenia-associated event data from the first chemotherapy cycle. By the time of the second chemotherapy cycle, patients were presumably much more fully recovered from their surgery, and had a lesser underlying tendency to develop infections. Thus, the severity and duration of chemotherapy-induced neutropenia would be of greater relative importance in determining the odds of developing a neutropenia-associated event. Such analyses were performed as a part of the Agency review of this application. Although exclusion of cycle 1 neutropenia-associated event data resulted in exclusion of two-thirds of the total events (markedly reducing the statistical power of comparisons between the study arms), these exploratory analyses revealed trends toward a reduced incidence of neutropenia-associated events in the amifostine study groups, in both the original and the new patient study cohorts. In the 242-patient total study population, this reduction in neutropenia-associated events was statistically significant.

Although the renal toxicity of chemotherapy was relatively mild in this study, some of the applicant's analyses suggested that the cumulative renal toxicity of chemotherapy was reduced in the amifostine-treated patient group. The applicant's analyses indicated that after repeated treatment with cisplatin-cyclophosphamide, prolonged elevation of serum creatinine levels (defined as a serum creatinine level of 1.5 mg/dl or higher, that was still present 21 days or longer after a chemotherapy treatment) occurred with greater frequency among control group patients. Similarly, at the time of initiation of the 5th and 6th cycles of chemotherapy, patients in the control arm were noted to have serum creatinine levels that had increased by a median of 25-31% (compared to pre-study baseline), while serum creatinine levels in patients in the amifostine pretreatment group had increased by only 11-12%. However, in Agency analyses of the time to first occurrence of a serum creatinine > 1.5 mg/dl, no advantage was seen for patients in the amifostine group; in fact, in the new patient study cohort the amifostine arm was statistically inferior to the control arm (possibly because of increased nausea/vomiting/dehydration in the amifostine group, causing more pre-renal azotemia).

Also, over the course of the study, the applicant reported that 6 control arm patients were taken off study because of renal toxicity, compared to none in the amifostine arm (although medical officer review suggested a lesser difference between the two study arms in the incidence of treatment termination related to renal toxicity, as discussed later in this review). In any event, the potentially-protective effects of amifostine on renal function that were observed in this study were of questionable clinical significance, since renal toxicity was relatively mild in both arms of this study. As an example, consider the median 25-31% increase in serum creatine levels reported for control group patients by the time of initiation of the 5th or 6th cycle of chemotherapy (compared to pre-therapy baseline). On medical officer review, this appeared to represent an increase from a baseline average serum creatinine of 0.81-0.82 mg/dl, to an average serum creatinine of 1.02-1.05 mg/dl by the 5th or 6th treatment cycle.

Analyses of the anticancer efficacy of treatment in the total 242 patient study population revealed no differences between the amifostine and control groups. The pathologically confirmed complete response rate (as documented by a negative "second look" laparotomy, revealing no evidence of residual cancer after completion of chemotherapy) was unchanged in the amifostine group, compared to the chemotherapy-alone control group. Analyses of time to disease progression and analyses of survival duration also indicated that treatment efficacy was similar in the amifostine and control groups. However, this study did not have adequate statistical power to exclude the possibility of a substantially inferior time to progression (and/or survival duration) for patients in the amifostine group.

It must be noted that cisplatin-cyclophosphamide regimens are now being supplanted in many centers by cisplatin-paclitaxel regimens, for the front-line chemotherapy of advanced ovarian cancer. Since cyclophosphamide and paclitaxel have substantially different mechanisms of action, it is not possible to use data from the applicant's studies of amifostine with cisplatin-cyclophosphamide to estimate whether amifostine therapy might significantly reduce the hematologic treatment toxicities of cisplatin-

paclitaxel chemotherapy for ovarian cancer. Also, carboplatin is now often used in place of cisplatin in the front-line treatment of ovarian cancer. Again, currently available data do not allow any conclusions to be drawn about the utility of amifostine in patients receiving a carboplatin-based regimen for ovarian cancer.

To summarize, data from the applicant's pivotal study provide support for the clinical efficacy of amifostine, both in reducing the bone marrow toxicity of cisplatin-cyclophosphamide chemotherapy in advanced ovarian cancer patients, and in reducing the renal toxicity of this treatment in these patients (particularly, the cumulative bone marrow and renal injury that may follow repeated treatment cycles). The available clinical data do not demonstrate (but do not rule out) a significant negative effect of amifostine on the anticancer efficacy of this chemotherapy.

The fundamental regulatory concern with this amended application is that there is still only one study that demonstrates an apparent clinical benefit of amifostine, in reducing the incidence of neutropenia-associated clinical events in patients undergoing chemotherapy for ovarian cancer. None of the studies included as supportive studies in the application are suitable for use as a second, confirmatory study. The hope that the new cohort of patients enrolled in the applicant's ovarian cancer study would be able to serve as a second study, that would provide confirmation of the efficacy of amifostine in reducing the incidence of neutropenia-associated clinical events in these patients, has not been realized. Flaws in the design and conduct of this single pivotal study also detract from the credibility of the study results (see comments regarding data collection concerns in the Study Results section, below). However, secondary and exploratory analyses of the study database do provide additional support for the proposed bone marrow and renal protective effects of amifostine pretreatment. Also, the suggestive (albeit generally non-statistically significant) study results regarding possible amifostine protection from chemotherapy-induced neurotoxicity and ototoxicity are intriguing.

From a regulatory viewpoint, the available clinical data do not appear to be adequate to support approval of this New Drug Application in the usual fashion under 21 CFR 314.105. Other possible actions on this application include (a) non-approval; (b) non-approval, but with encouragement that the applicant submit a treatment IND application, while pursuing additional NDA-directed studies; or (c) accelerated approval under 21 CFR 314.510 (accelerated approval based on a surrogate endpoint or on an effect on a clinical endpoint other than survival or irreversible morbidity).

A clinical non-approval decision would be based on the finding that the application does not include two adequate and well controlled studies that demonstrate the safety and effectiveness of amifostine (used as described in the proposed labeling). Approval of a treatment IND application for amifostine (in patients receiving cyclophosphamide-cisplatin chemotherapy for advanced ovarian cancer) could be considered only after a determination by the Agency that the applicant had initiated and was diligently pursuing another study (deemed to be a potentially pivotal, adequate and well controlled study of the effectiveness and safety of amifostine in reducing the treatment toxicity of an acceptable chemotherapy regimen). Patients or

their insurance carriers could be charged for the cost of amifostine provided under a treatment IND. Amifostine distribution under a treatment IND would be limited to patients with the appropriate diagnosis.

Approval under 21 CFR 314.510 (accelerated approval based on a surrogate endpoint or on an effect on a clinical endpoint other than survival or irreversible morbidity) could be based on (i) analyses of granulocyte count nadirs after repeated cyclophosphamide-cisplatin treatment (where there were indications of amifostine benefit, in both halves of the ovarian cancer study), accepting these data as a surrogate marker for clinically significant bone marrow protection from cumulative treatment toxicity, and/or (ii) analyses of changes in serum creatinine levels after repeated cyclophosphamide-cisplatin treatment (where there were again indications of amifostine benefit, as described above, in both halves of the ovarian cancer study), accepting these data as a surrogate marker for clinically significant renal protection from cumulative treatment toxicity. Further, accelerated approval of the current application under 21 CFR 314.510 would be considered only after it was determined that the applicant had initiated and was diligently pursuing another study (deemed to be a potentially pivotal, adequate and well controlled study of the effectiveness and safety of amifostine in reducing the treatment toxicity of an acceptable chemotherapy regimen), that would demonstrate the validity of the surrogate marker data as predictive of clinical efficacy. Although accelerated approval would allow commercial marketing of amifostine in the usual fashion, approval under 21 CFR 314.510 could be withdrawn if the applicant failed to exhibit due diligence in pursuing the required additional research; or in the event that the additional research failed to confirm the validity of the surrogate marker data as predictive of clinical efficacy. Finally, such an approval could be considered only after all remaining significant Agency concerns regarding the chemistry/manufacturing and biopharmaceutical sections of the application have been resolved to the Agency's satisfaction.

Possibilities for an additional, potentially pivotal, adequate and well controlled study of amifostine might include randomized, controlled studies of amifostine with newer ovarian cancer treatment regimens that include (a) paclitaxel as well as cisplatin (and possibly cyclophosphamide), or (b) carboplatin. A randomized, controlled study in another malignancy might also be acceptable. This research would presumably be performed in a study population more suited to detecting a clinical effect of amifostine in reducing neutropenia-associated events (as discussed elsewhere in this review). Also, if this New Drug Application was to be approved under 21 CFR 314.510, initial amifostine labeling should be restricted to the limited indication of reducing treatment toxicity in ovarian cancer patients undergoing cisplatin-cyclophosphamide chemotherapy. Available data are clearly inadequate to assess the benefits and risks, including the risks of tumor protection, in other settings. Finally, labeling would also have to include a warning that amifostine may reduce the survival benefit of chemotherapy, and should not be used in other settings where there is a substantial possibility that chemotherapy may be curative.

Following review of this amended application at the December 12, 1994 meeting of the Oncologic Drugs Advisory Committee, the Committee voted 9-0 that this application should not be approved. The Committee was not persuaded that clinical efficacy of amifostine had been adequately demonstrated in the applicant's studies. The issue of possible tumor protection was deferred by the Committee as it was deemed unnecessary to discuss this issue, absent a finding of amifostine efficacy in protection of bone marrow or other normal tissues.

Subsequent to the Advisory Committee action, a meeting was held between representatives of US Bioscience and the Agency on December 21, 1994. It was agreed that the Agency would accept a resubmission of the applicant's clinical data, focusing more on the potential renal-protective effects of amifostine and including a specific proposal for consideration of amifostine approval under the accelerated approval mechanism.

2. Scope of application, and materials reviewed

Materials reviewed included:

(a) the regulatory history of this application and its prior non-approval in 1992;

(b) Agency guidance to the applicant regarding the recommended medical-statistical content for an amended application (as detailed in Agency letters to the applicant dated December 7, 1993 and March 22, 1994; and in medical officer and statistical officer reviews of a US Bioscience "general correspondence" submission to NDA 20-221, dated May 9, 1994);

(c) volumes 1-52 of the US Bioscience major amendment submission #017 to NDA 20-221 (dated July 12, 1994, received by CDER on July 13, 1994);

(d) volumes 1-74 of the US Bioscience submission of case report forms from the pivotal study of amifostine in advanced ovarian cancer (submission date, May 19, 1994). These volumes contained case report forms for all 242 patients enrolled in this study as of the cutoff date for analysis. As noted elsewhere in this review, each of these case records was examined in detail, in order to (i) verify the accuracy of the patient progression and survival dates used in the applicant's analyses of this study, (ii) determine the accuracy of reporting of antibiotic administration to study patients, and the reasons for antibiotic administration; and (iii) assess the accuracy and precision of identification of "neutropenia-associated clinical events" in study patients (since the primary goal of this pivotal study was to compare the occurrence of neutropenia-associated clinical events in the two study arms).

3. Regulatory History

As noted above, US Bioscience initially submitted a New Drug Application for ETHYOL[R] amifostine on September 30, 1991. The application was based primarily on the results of a single randomized study of cisplatin plus cyclophosphamide, with or without amifostine pretreatment, in women with advanced ovarian cancer (following debulking surgery). While this study was initially planned to enroll 200 patients, an interim look after 121 patients were enrolled reportedly revealed a substantial, statistically-significant reduction in the incidence of neutropenia-associated clinical events (including febrile neutropenia) among patients in the amifostine group; and no apparent difference in anticancer efficacy, as determined by response rates. Accrual was stopped, and these data (as well as other supportive data) were submitted for Agency review in NDA 20-221.

Following review by the Agency and by the Oncologic Drugs Advisory Committee, US Bioscience was advised in a May 15, 1992 Agency letter that the application was not approvable. The Agency letter stated that insufficient clinical evidence had been submitted to demonstrate the efficacy of amifostine in reducing the toxic effects of cisplatin-cyclophosphamide chemotherapy; and the submitted data were also inadequate to rule out a significant decrease of antitumor efficacy, in amifostine-pretreated patients. The letter also specified a number of other deficiencies in the application, including chemistry/manufacturing and biopharmaceutical deficiencies. Appendix I to this review includes a copy of the May 15, 1992 letter.

In examining the results obtained in the initial 121 patients enrolled in the advanced ovarian cancer study, one major concern was the specification of multiple study endpoints in the study protocol. The primary endpoint was not clearly defined, and detailed plans for analyses of the study results were not provided in the study protocol. Hence, the finding of a reduced incidence of neutropenia-associated clinical events among patients randomized to receive amifostine was regarded as an hypothesis that required confirmation, rather than an established fact. Also, given the findings of possible tumor protection in some preclinical studies of amifostine, there was concern that more data were needed to assess the effects of amifostine on the anticancer efficacy of treatment. US Bioscience was subsequently advised to reopen the randomized trial of cyclophosphamide plus cisplatin, with or without amifostine pretreatment, in women with advanced ovarian cancer (following debulking surgery); and to accrue additional patients, to complete the planned accrual of 200 evaluable patients. The results in the second half of the study could be analyzed separately, and (if sufficiently favorable) could serve as a second, confirmatory study of the efficacy and safety of amifostine in this setting.

The study was reopened to accrual, and the protocol was revised in June 1992 to specify that the primary endpoint was "grade 4 neutropenia and fever with or without sepsis". This endpoint has subsequently evolved to the endpoint actually used in this application, "grade 4 neutropenia with fever and/or infection, requiring antibiotic therapy". Unfortunately, this endpoint is still subject to some interpretation. The degree of fever was not specified, nor was the type of antibiotics (IV versus oral or

other route; broad spectrum versus limited spectrum). For example, does administration of oral nitrofurantoin for an uncomplicated urinary infection, to a patient who is neutropenic but has no fever, qualify as an event? Is it necessary to document fever on the same day that neutropenia is documented? And so forth.

US Bioscience and the Agency have subsequently been in ongoing contact, with Agency pre-review of US Bioscience plans for submission of a major amendment to NDA 20-221, providing the required additional clinical and nonclinical data. The revised clinical data package, which now includes randomized study data on 242 patients with advanced ovarian cancer, was formally submitted for Agency review on July 13, 1994 (NDA 20-221, Amendment 017). This revised clinical data package is the focus of this review.

4. Chemistry/Manufacturing (refer to CMC review RE chemistry/environmental issues)

Amifostine (ETHYOL^{R}, also known as WR-2721) is chemically characterized as 2-[(3-aminopropyl)amino]-ethanethiol, dihydrogen phosphate ester. The structural formula for amifostine is :

$$H_2N(CH_2)_3NH(CH_2)_2S{\cdot}PO_3H_2$$

Amifostine has a molecular weight of 214.22, and is supplied as a lyophilized powder formulation (containing 500 mg amifostine plus 500 mg mannitol, for reconstitution to 10 ml), for intravenous administration.

5. Preclinical Pharmacology/Toxicology

Numerous preclinical studies have shown that amifostine (and WR-1065, the dephosphorylated active metabolite of amifostine) can diminish the cellular and tissue damage caused by ionizing radiation or "radiomimetic" chemotherapeutic agents (such as alkylating agents or cisplatin). Mechanisms for this protective effect may include amelioration of free-radical mediated damage by the free thiol metabolite of amifostine (WR-1065), and/or an inactivating reaction between WR-1065 and the active species of alkylating or platinum chemotherapy agents. Selective protection of normal cells and tissues may occur based on physiologic and biochemical differences between normal tissues and tumor tissues. For example, alkaline phosphatase activity (which converts amifostine to its active metabolite, WR-1065) may be much higher in membranes of normal cells and normal capillary beds than in membranes of tumor cells / tumor capillary beds. Also, substantially greater uptake of amifostine and WR-1065 by normal cells may occur as a result of differences between normal and tumor cells in pH and in uptake mechanisms. Numerous in vivo studies (in tumor bearing animals) have demonstrated that amifostine may afford significant protection for normal tissues, without a major negative effect on the anticancer efficacy of chemotherapeutic treatment (i.e., without significant tumor protection). However, evidence of tumor protection has been observed in some preclinical studies[1-4],

suggesting that the protective effects of amifostine may not be absolutely selective for normal tissues (refer to pharmacology/toxicology review).

Limited preclinical studies have suggested that amifostine lacks significant mutagenic activity. No long-term animal studies have been conducted to assess the carcinogenic potential of amifostine. Given its pharmacologic mechanisms of action, pretreatment with amifostine could in theory reduce the *in vivo* mutagenic and carcinogenic effects of alkylating agents and ionizing radiation on normal tissues. Embryotoxicity, but not teratogenicity, has been observed in rat studies.

6. Clinical Pharmacology/Pharmacokinetics (see also biopharmaceutical review)

Amifostine is stated to be rapidly cleared from plasma, with an α half-life of less than 1 minute and a β half-life of approximately 8 minutes. Plasma clearance is > 90% by 6 minutes after administration. By 1 hour after IV administration of 740-910 mg/m^2 amifostine over 15 minutes, renal elimination of amifostine, plus WR-1065 (the active free thiol metabolite), plus WR-33278 (a less active disulfide metabolite) totalled about 5 % of the administered dose. Pretreatment with dexamethasone or metoclopramide was not observed to affect amifostine pharmacokinetics; and limited studies suggest that amifostine pretreatment has no effect on the pharmacokinetics of cisplatin, administered 15 minutes after completion of the amifostine infusion.

7. Related IND submissions

US Bioscience has conducted and is conducting numerous studies of the potential radioprotective and chemoprotective effects of amifostine, under IND Also, the US National Cancer Institute has an open IND for studies of amifostine (IND . NCI studies listed as ongoing in the August 15, 1994 annual report to IND included study T90-0189 (amifostine followed by cisplatin using a daily X 5 schedule, with escalation of the amifostine dose); CALGB-9160 (a three arm randomized study evaluating high dose cyclophosphamide - 4.5 grams/m^2 - in solid tumor patients, with GM-CSF, amifostine, or both GM-CSF and amifostine to ameliorate toxicity); and T89-0132 (a study evaluating possible preservation of salivary gland function by amifostine, in patients with head and neck cancer who undergo radiation therapy). Finally, IND 40,795 is held by an individual investigator for studies of the possible chemoprotective effects of amifostine in children undergoing treatment for primitive neuroectodermal tumors (PNET).

8. Non-US post-marketing experience

No non-US post-marketing experience is reported. Amifostine was not marketed in any country at the time this NDA amendment was filed.

9. Overview: Chemotherapy of Advanced Ovarian Cancer

Ovarian cancer is the fourth leading cause of cancer death in American women, and accounts for more cancer deaths in our population than any other form of gynecologic cancer. It is estimated that approximately 24,000 US women were found to have ovarian cancer in 1994, with approximately 13,600 deaths from this disease[5].

Unfortunately, early detection of ovarian cancer is uncommon; disease that has spread outside the pelvis is found in about two-thirds of women at the time of initial diagnosis. Early diagnosis is hampered by the fact that early symptoms are often minimal and vague. While the CA-125 tumor marker is used in some settings to screen for early ovarian cancer, available data are insufficient to recommend its routine use as a screening tool; it may be relatively ineffective in detecting early stage ovarian cancers[6,7]. From a public health viewpoint, much more significant findings have come from epidemiologic studies, that have revealed a significant reduction in ovarian cancer risk among women with a history of oral contraceptive use[8].

Progress in the management of advanced ovarian cancer has been limited. For patients with stage III disease, the standard therapeutic approach has been aggressive surgical removal of as much tumor as possible, followed by combination chemotherapy. While a substantial proportion of patients can be rendered free of detectable disease by this approach (often for periods of many months to several years), the number of women cured by this approach may be relatively small (and limited primarily to women with the most favorable prognostic indicators). In larger studies with longer periods of follow-up, relapses and deaths from disease have continued to occur in the 5 to 10 year window among women who had no evidence of disease at 5 years[9].

The conventional chemotherapeutic approach in initial treatment of ovarian cancer is use of a cisplatin- or carboplatin-containing regimen. Commonly used regimens in past years have included cisplatin-cyclophosphamide-doxorubicin, supplanted more recently by cisplatin-cyclophosphamide (typically at higher dosage) after a major study suggested that comparable results might be obtained with the two-drug regimen. Carboplatin-based regimens are also widely used, based on the lesser non-hematologic toxicities of this drug, and generally comparable efficacy[9]. At this point in time, paclitaxel is being incorporated in the front-line treatment of advanced ovarian cancer, but it is too early to fully assess the value of this drug in front-line treatment.

Response to front-line chemotherapy for ovarian cancer is frequently difficult to assess. The substantial majority of women enrolled in research studies typically have no measurable disease after their debulking surgery, and thus cannot be evaluated for objective response. Time to progression is also often difficult to precisely determine, as symptoms of progression can be initially vague, and imaging studies can be non-diagnostic even in women with extensive recurrent disease. While survival can be precisely measured, randomized clinical trials to date have generally demonstrated only a modest survival benefit in patients given chemotherapy for this disease. In a recent meta-analysis of worldwide randomized trials of chemotherapy in

advanced ovarian cancer, a 7%-9% improvement in the hazard ratio for survival was noted when platinum-containing regimens were compared to treatment with non-platinum agents; and an 11-15% improvement in the hazard ratio for survival was noted in comparisons of platinum-containing combination regimens to single-agent platinum[10]. Some study data suggest that better results may be attainable with higher cisplatin doses[11]. The survival contribution of cyclophosphamide as a component of front-line chemotherapy for ovarian cancer is unknown, although it is generally considered to be less significant than the platinum benefit.

The adverse effects of ovarian cancer front-line chemotherapy regimens are considerable. Cisplatin often causes significant nephrotoxicity and neurotoxicity (including paresthesias, ototoxicity, and possibly more subtle problems such as reduced color vision). While marrow suppression is not a prominent finding after single-agent cisplatin therapy (using standard doses), cisplatin can substantially contribute to marrow suppression (including thrombocytopenia and anemia, as well as neutropenia), when used at high dosage or when combined with other myelotoxic drugs such as cyclophosphamide. Carboplatin has qualitatively similar adverse effects, but quantitatively more myelosuppression and less nephro- and neurotoxicity. Some randomized studies have suggested that response rates may be slightly less for carboplatin-containing regimens, but no significant differences have been observed in progression-free or overall survival rates in studies comparing cisplatin and carboplatin in advanced ovarian cancer [9]. Cyclophosphamide is primarily toxic to the bone marrow, but can sometimes cause other significant adverse effects. Longer-term survivors may develop treatment-related leukemia or bladder cancer.

Clearly, a primary goal of drug development in advanced ovarian cancer must be the development of more effective treatment regimens for this disease. The contribution of paclitaxel to front-line treatment is still being defined, but it appears that it may turn out to be significant. Other promising drugs and biologically-derived agents in development offer new and novel mechanisms of action. At the same time, given the substantial adverse effects of treatment on patients' health and quality of life, research aimed at making existing therapies more tolerable (without substantially reducing any efficacy they may have) is clearly warranted. Therefore, the current submission endeavors to demonstrate that amifostine treatment, immediately prior to each cycle of cyclophosphamide-cisplatin treatment, can significantly reduce some of the toxic effects of treatment, without substantially compromising treatment efficacy.

10. Pivotal Clinical Study (sponsor study no. WR-2721-01): **study design**.

As noted above, the pivotal clinical study included in this amended application was a randomized study of cisplatin plus cyclophosphamide, with or without amifostine pretreatment, in women with advanced ovarian cancer (following debulking surgery). The design of this study was as follows:

A. Eligibility criteria included:

- Histologically proven stage III or IV epithelial ovarian cancer.
- Cytologic confirmation of a malignant pleural effusion (if protocol entry was to be based on this finding).
- Optimal debulking surgery, no more than 6 weeks prior to entry.
- Measurable or non-measurable disease.
- WBC ≥ 3,000/cmm; granulocyte count ≥ 2,000/cmm; platelet count ≥ 100,000/cmm; serum creatinine ≤ 1.5 mg/dl, serum bilirubin and SGOT ≤ 2 times normal.
- Gynecologic Oncology Group performance status of 0, 1, or 2.
- No prior chemotherapy.
- Recovered from the effects of major surgery.
- Informed consent.

B. Exclusions were:

- Diagnosis of low malignant potential (borderline) carcinoma.
- No biopsy proven histologic disease following debulking surgery.
- Brain or meningeal metastases.
- Prior chemotherapy or radiotherapy.
- Septicemia, severe infection, acute hepatitis, symptomatic heart disease.
- Age ≥ 70 years
- Severe gastrointestinal bleeding.
- Any circumstances that would prevent completion of study treatment or required follow-up.
- Unstaged/unclassified ovarian cancer (patients believed to have ovarian cancer, but not explored).
- Prior or concomitant diagnosis of another invasive malignancy (except non-melanoma skin cancers).
- Inability to tolerate the fluid load required as a part of protocol therapy; or presence of urinary outlet obstruction that cannot be managed by catheterization.

C. Stratification/randomization:

- Stratification by center and by extent of residual disease after debulking surgery (estimated largest dimension of largest mass left behind < 2 cm, versus ≥ 2 cm).
- Central randomization (Clinical Research Department, US Bioscience).

D. Treatment:

- All patients received IV hydration beginning prior to treatment.

- Patients randomized to arm A (amifostine plus cyclophosphamide-cisplatin):

  Amifostine, 910 mg/m^2 IV infusion over 15 minutes (patient supine; blood pressure monitored at least every 5 minutes, with interruption of infusion for significant hypotension; if blood pressure recovers within 5 minutes, restart infusion). *COMMENT: The original protocol specified an amifostine dose of 740 mg/m^2; an early amendment, dated 5/17/88, increased the amifostine dose to 910 mg/m^2*

  Cyclophosphamide 1000 mg/m^2 IV infusion over 20 minutes, to start 5 minutes after completion of amifostine infusion.

  Immediately after completion of cyclophosphamide, mannitol bolus, then cisplatin, 100 mg/m^2 IV infusion over 30 minutes, followed by 6 hours of IV hydration / mannitol. *COMMENT: The original study protocol specified administration of cisplatin starting 15 minutes after amifostine, with cyclophosphamide administered last. This was changed to administration of cyclophosphamide after amifostine, and before cisplatin (as above), in the 5/17/88 protocol amendment. Only three patients received the lower starting dose of amifostine, and cisplatin prior to cyclophosphamide, before this protocol amendment was implemented.*

- Patients randomized to arm B were not to receive amifostine, but otherwise received the same treatment as arm A patients.

- All patients were to receive dexamethasone as an antiemetic, pretreatment and 4, 8, and 12 hours after cisplatin. Other recommended antiemetics included metoclopramide, diphenhydramine, lorazepam, and ondansetron. *COMMENT: dexamethasone was excluded as an antiemetic early in the study, due to concern about possible confounding effects on neutrophil counts and on cisplatin-related neurotoxicity. After five patients withdrew from the study because of intolerable nausea (four in the amifostine group, and one in the control group), the protocol was amended 10/26/88 to specify that all patients would receive dexamethasone.*

E. Criteria for re-treatment:

- Recovery of granulocyte count to ≥ 1,500/cmm; platelet count to ≥ 100,000/cmm; and serum creatinine to ≤ 1.5 mg/dl or creatinine clearance to ≥ 65 was required prior to administration of each subsequent treatment course. If blood counts had recovered, but serum creatinine had not, cisplatin was to be withheld; the patient was to be treated with cyclophosphamide alone.

Cisplatin was also to be withheld in patients who developed grade 3 or 4 peripheral neuropathy (until resolution to grade 1 or 0).

- Patients were to be taken off study if (i) their subsequent treatment was delayed for more than 2 weeks due to delayed recovery from a prior treatment; or (ii) cisplatin had to be omitted from 2 sequential courses of treatment because of persistent elevation of serum creatinine; or (iii) treatment-related grade 3 or 4 peripheral neuropathy did not improve to grade 1 or 0 within 6 weeks.

- Patients were also to be taken off study if they developed significant hearing loss, defined by audiogram as >56 dB at 6 kHz; >35 dB at 4 kHz; or >25 dB at 2 kHz.

F. Dose reductions for treatment toxicity:

- Amifostine: patients who were unable to receive their full scheduled dose of amifostine due to hypotension (that did not resolve within 5 minutes to a level that would allow resumption and completion of the amifostine infusion) had their amifostine dose reduced in the next cycle of treatment, to a dose slightly less than the dose they had received when the hypotensive event occurred.

- Cisplatin and cyclophosphamide dose reductions were based on nadir counts:

Percentage of Cisplatin Dose to be Administered				
Platelet Nadirs (mm^3)	Granulocyte Nadirs (mm^3)			
	≥ 1500	1000 - 1499	500 - 999	< 500
≥ 75,000	100%	100%	100%	100%
50,000 - 74,999	100%	100%	100%	75%
<50,000	75%	75%	75%	50%

Percentage of Cyclophosphamide Dose to be Administered				
Platelet Nadirs (mm^3)	Granulocyte Nadirs (mm^3)			
	≥ 1500	1000 - 1499	500 - 999	< 500
≥ 75,000	100%	100%	100%	75%
50,000 - 74,999	75%	75%	75%	50%
< 50,000	50%	50%	50%	0%

- If a peak serum creatinine value was recorded after a treatment that was more than 1.0 mg/dl higher than the baseline value (the value prior to the first cycle of therapy), the cisplatin dose was to be reduced by 20% in the next treatment cycle.

- If grade 2 peripheral neuropathy (other than loss of deep tendon reflexes) was observed, the dose of cisplatin was to be reduced to 60 mg/m^2.

- Moderate hearing loss was to require a 25% reduction in subsequent cisplatin dose. Moderate hearing loss was defined as 41-55 dB at 6 kHz; 26-34 dB at 4 kHz; or 16-24 dB at 2 kHz.

G. Schedule of study efficacy and toxicity assessments:

- CBC with granulocyte and platelet counts twice weekly for the first two treatment cycles, then weekly for the remaining 4 cycles.

- Serum bilirubin, alkaline phosphatase, calcium, magnesium, and phosphate; urinalysis; and 12-hour creatinine clearance, prior to each course of therapy. Repeat creatinine clearance if a serum creatinine > 1.5 mg/dl is recorded.

- Physical exam (including ophthalmoscopic exam) and assessment of performance status every 3 weeks, to coincide with each course of therapy.

- Audiogram prior to the first course of therapy, and at the conclusion of therapy. Repeat audiogram prior to each course of therapy, when clinically indicated.

- Standardized neurological assessment prior to 1st, 4th, 5th, and 6th courses of therapy, and post-treatment (to be performed by an observer who was unaware of the patient's treatment group assignment).

- Tumor assessment by physical exam every 3 weeks, to coincide with each course of therapy. Chest X-rays and CT scans "will be performed when clinically indicated".

- Second look laparotomy to assess tumor response to treatment, when clinically indicated. To be performed within 8 weeks of the last course of chemotherapy.

- Follow-up every 3 months after initial post-therapy evaluation, to monitor for subsequent treatment, response, dates of disease progression, complications, and date of death.

H. Response criteria:

- Clinical response (in the minority of patients with measurable disease) was defined conventionally; complete response was to be complete disappearance of all evidence of disease for at least 4 weeks. Partial response was to be at

least 50% reduction in the sum of the cross-sectional areas of all measurable lesions, for at least 4 weeks.

- Complete remission at peritoneoscopy was defined as no evidence of residual disease at restaging peritoneoscopy (in patients who originally had positive intraperitoneal washings and/or biopsy specimens).

- Second look laparotomy response categorizations were: (i) pathologically complete remission (no tumor seen, washings and biopsy specimens all negative for residual tumor); and (ii) microscopic residual disease (positive washings and/or biopsies without macroscopic tumor). The protocol did not specifically provide definitions of partial remission, stable disease, or progressive disease for the second-look laparotomy setting (although it can be assumed that an experienced surgeon's assessment of progressive disease at second-look surgery would be reliable).

I. Criteria for removal of patients from study:

- Toxicity (physician assessment).
- Patient request due to toxicity.
- Patient request (reason other than toxicity).
- Non-compliance.
- Disease progression.
- Death.

J. Statistical plan:

Efficacy endpoints as specified in the original study protocol included:

(i) incidence and duration of hematologic toxicity, defined as granulocyte nadir < 500/cmm, leukopenia related fever, or the need to reduce or delay the dose of cyclophosphamide for safety reasons.

(ii) incidence of nephrotoxicity defined by the need to delay or reduce the dose of cisplatin.

(iii) incidence of neurotoxicity defined by the occurrence of grade 1 or worse neuropathy (GOG criteria), and the cumulative dose of cisplatin at the onset of neuropathy.

(iv) incidence of ototoxicity defined by moderate or severe hearing loss or the need to reduce or delay the dose of cisplatin.

(v) incidence of dose limiting toxicity - defined as the need for dose reduction, delay, or discontinuation of cisplatin or cyclophosphamide due to toxicity.

(vi) tumor response rate

Statistical methods, quoted verbatim from the original study protocol, included:

(i) The objective of this study is to establish that pretreatment with WR 2721 reduces the toxicity of cisplatin and alkylating agents without reducing their antitumor effect, therefore one-sided tests of significance will be utilized.

(ii) A sample size of 200 (100 patients per arm) will provide > 90% power to detect a decrease in the incidence of hematologic toxicity from 50% to 25% (p=.05, one-sided). This sample size will also provide 80% power to detect a decrease in the incidence of renal toxicity from 30% to 15% (p=.05, one-sided).

(iii) Assuming the response rate to be 70%, one hundred patients per treatment arm will enable the calculation of a 95% confidence interval within 10% of the observed response rate. Additionally, this sample size provides 90% power to detect a reduction in response rate of 20% (p=.05, one-sided).

(iv) The incidence of toxicities will be compared using a χ^2 statistic. Any potential differences by center will be examined using a Mantel-Haenzel χ^2 statistic.

(v) Tumor response rates, duration of hematologic toxicity, and the cumulative dose of cisplatin at the onset of neuropathy will be analyzed using a covariance model adjusting for differences in prognostic variables and an effect due to investigators. If normality assumptions are not met, non-parametric procedures will be used to compare the two treatment arms.

(vi) An interim analysis will be performed approximately halfway through the study. If there is a statistically significant reduction ($p < .03$, one-sided) in the incidence of toxicity in the WR-2721 arm and no significant reduction in tumor response rate, no additional patients will be enrolled.

As noted previously, accrual to this study was interrupted after interim analyses performed by the applicant revealed an apparent reduction in the occurrence of neutropenia-associated events, among patients treated with amifostine. However, in accordance with Agency and Oncologic Drugs Advisory Committee findings that this study should be completed, and the statistical design should more precisely identify the primary study endpoint, the study protocol was reopened for continued accrual in 1992 with the following paragraph added to the Objectives section (protocol section 1.1 (1), as amended 6/16/92):

> "As the use of G-CSF is now permitted (see section 3.4.3.2.2), this will affect the duration of neutropenia and hospitalization for this toxicity. Therefore, the primary endpoint will be the incidence of grade 4 neutropenia and fever with or without sepsis. The incidence and duration of hospitalization for neutropenic fever will also be measured, as will the use of G-CSF and antibiotics. These will be compared as secondary endpoints."

COMMENTS: As noted above, the applicant's definition of a neutropenia-associated clinical event has subsequently evolved further, to the endpoint actually used in this amended New Drug Application, which is "grade 4 neutropenia with fever and/or infection, requiring antibiotic therapy". Also, while use of G-CSF was permitted in the latter part of this study (in patients who had experienced grade 4 neutropenia), only 13 patients actually received G-CSF while on study, generally after a neutropenic event. Amifostine patients who received G-CSF were patient numbers (in treatment cycle 1), (cycle 1), (cycle 1), (cycle 1), (cycle 1), (cycles 3,4,5), (cycle 2), and (cycles 1,4). Control arm patients who received G-CSF were patient numbers (cycle 5), (cycles 1,2,4), (cycle 1), (cycles 1,2,3,4,5), and (cycle 4).

11. Pivotal Clinical Study (sponsor study no. WR-2721-01): **study results**.

A total of 242 patients had been enrolled and treated in this study at the time the study database was frozen for analysis. As recommended by the Agency, the sponsor has provided separate analyses of (i) the "original" 121-patient cohort, (ii) the subsequent "new" cohort of 121 patients, and (iii) all 242 patients combined. As noted above, the intent was to determine whether the findings in the second "new" cohort would confirm a chemoprotective effect of amifostine (with the primary endpoint being a reduction in the incidence of neutropenia-associated clinical events among patients randomized to receive amifostine). To assess the possibility of tumor protection by amifostine, anticancer efficacy results (response rate, including pathologic CR at second-look surgery; time to progression of ovarian cancer; and survival duration) were also compared in the control and amifostine treatment study arms. Again, the sponsor has provided separate analyses for (i) the original 121-patient cohort, (ii) the subsequent, new 121 patient cohort, and (iii) all 242 patients combined.

Medical reviewer comments on key aspects of the study results are as follows:

A. Patient demographics

The two study treatment arms were well balanced for all baseline characteristics examined, as demonstrated by table 4C from the applicant's report on this study (reproduced on the following page). This was true individually for the old 121 patient cohort and for the new 121 patient cohort, as well as for the combined (242 patient) all patients cohort. There were minimal differences in the proportion of patients with performance status 0 (more in the control arm), and in the proportion of patients with ≥ 2 cm residual disease after debulking surgery, but these small differences were not statistically significant.

TABLE 4C

Baseline Patient Characteristics: All Patients (N=242)

Parameters	Amifostine + CP (N = 122) Number	(%)	CP (N = 120) Number	(%)	χ^2	p-value[a] 2-sided	1-sided
Age (years)					1.490	0.475	0.238
Median	55		55				
Range	21-75		25-78				
<50	40	(32.8)	35	(29.2)			
50-59	39	(32.0)	47	(39.2)			
≥60	43	(35.2)	37	(30.8)			
Not Specified	0		1	(0.8)			
Race					2.141	0.544	0.272
Black	11	(9.0)	9	(7.5)			
Caucasian	103	(84.4)	104	(86.7)			
Oriental	2	(1.6)	4	(3.3)			
Other	5	(4.1)	2	(1.7)			
Not Specified	1	(0.8)	1	(0.8)			
FIGO Stage					0.005	0.945	0.473
III	103	(84.4)	100	(83.3)			
IV	19	(15.6)	18	(15.0)			
Not Specified	0		2	(1.7)			
Extent of Residual Disease					0.801	0.371	0.186
<2 cm	79	(64.8)	71	(59.2)			
≥2 cm	43	(35.2)	49	(40.8)			
Measurable Disease					0.471	0.492	0.246
Measurable	25	(20.5)	29	(24.2)			
Non-measurable	97	(79.5)	91	(75.8)			
% Tumor Removed					0.586	0.444	0.222
Median	95.0		95.0				
Range	0-100		0-100				
<90%	28	(23.0)	32	(26.7)			
≥90%	86	(70.5)	78	(65.0)			
Not Specified	8	(6.6)	10	(8.3)			
Histology[b,c]					4.720	0.787	0.394
Clear-Cell A.	2	(1.6)	2	(1.7)			
Endometrioid A.	14	(11.5)	16	(13.3)			
Mixed Epithelial C.	2	(1.6)	4	(3.3)			
Moderately Different. C.	1	(0.8)	0				
Mucinous A.	3	(2.5)	5	(4.2)			
Papillary Cyst A.	0		1	(0.8)			
Poorly Different. Papi.	0		1	(0.8)			
Serous A.	93	(76.2)	85	(70.8)			
Undifferentiated C.	7	(5.7)	6	(5.0)			
GOG Performance Status					1.674	0.433	0.217
0 Fully Active	46	(37.7)	53	(44.2)			
1 Ambulatory	63	(51.6)	52	(43.3)			
2 Self Care	13	(10.7)	15	(12.5)			

[a] Based on Pearson Chi-Square test.
[b] A. = adenocarcinoma; C. = ca cinoma.
[c] Some patients had more than one type of histologically-confirmed ovarian malignancy.

B. Patient treatment - amifostine

Per the study protocol, patients in the amifostine treatment group were to receive 910 mg/m^2 amifostine in each treatment course, with dose reductions when necessitated by intolerance of this dose. Dose reductions, when they were necessary, were almost always related to hypotension during the amifostine infusion. The following table summarizes the doses of amifostine actually administered to study arm A patients in each treatment cycle.

Reviewer Table 1

Amifostine doses actually administered to study arm A patients

Cycle number	Total patients treated	910 mg/m^2 amifostine	800-909 mg/m^2 amifostine	700-799 mg/m^2 amifostine	600-699 mg/m^2 amifostine	500-599 mg/m^2 amifostine	< 500 mg/m^2 amifostine
1	122	116	1	3*	0	0	0
2	113	103	5	4	0	0	1
3	104	93	4	3	1	3	0
4	89	81	3	2	0	3	0
5	79	70	3	1	0	3	2
6	74	66	3	1	0	3	1

*the first 3 patients started treatment at 740 mg/m^2, and one of these three had a second treatment at 740 mg/m^2, before the protocol was amended to specify a starting dose of 910 mg/m^2.

As can be seen, most of the patients were able to tolerate continued treatment with amifostine at 910 mg/m^2.

C. Patient treatment - cisplatin and cyclophosphamide

Most patients had cyclophosphamide dose reductions over the course of their study participation. The incidence of cyclophosphamide dose reductions among all 242 study participants was summarized in table 17C of the applicant's report on this study. Some patients had cisplatin dose reductions as well. The incidence of cisplatin dose reductions among all 242 study participants was summarized in table 15C of the applicant's study report. The applicant also analyzed cyclophosphamide and cisplatin dose intensity over the 6 cycles of study treatment, for all 242 study participants; these findings were summarized in tables 18C and 16C of the applicant's study report. Tables 17C, 15C, 18C, and 16C are reproduced below.

TABLE 17C

Incidence of Cyclophosphamide Dose Reductions
All Patients (N=242)

Cycle	Regimen	Not Reduced	Reduced	Total	χ^2	p-value[a] 2-sided	p-value[a] 1-sided
1	Amifostine + CP	122	0	122			
	CP	120	0	120			
2	Amifostine + CP	54	59	113	0.004	0.949	0.475
	CP	54	58	112			
3	Amifostine + CP	31	73	104	0.408	0.523	0.262
	CP	33	64	97			
4	Amifostine + CP	17	72	89	1.442	0.230	0.115
	CP	24	66	90			
5	Amifostine + CP	14	65	79	1.154	0.283	0.142
	CP	20	61	81			
6	Amifostine + CP	11	63	74	1.524	0.217	0.109
	CP	15	50	65			
Overall	Amifostine + CP	32	90	122	0.626	0.429	0.215
	CP	37	83	120			

[a] Based on Pearson Chi-Square test.

TABLE 15C

Incidence of Cisplatin Dose Reductions
All Patients (N=242)

Cycle	Regimen	Not Reduced	Reduced	Total	χ^2	p-value[a] 2-sided	p-value[a] 1-sided
1	Amifostine + CP	122	0	122			
	CP	120	0	120			
2	Amifostine + CP	99	14	113	0.174	0.676	0.338
	CP	96	16	112			
3	Amifostine + CP	91	13	104	0.646	0.422	0.211
	CP	81	16	97			
4	Amifostine + CP	76	13	89	0.330	0.566	0.283
	CP	74	16	90			
5	Amifostine + CP	67	12	79	1.290	0.256	0.128
	CP	63	18	81			
6	Amifostine + CP	61	13	74	0.134	0.715	0.358
	CP	52	13	65			
Overall	Amifostine + CP	96	26	122	0.142	0.706	.353
	CP	92	28	120			

[a] Based on Pearson Chi-Square test.

TABLE 18C

Analysis of Cyclophosphamide Dose Intensity:
Percentages of Cyclophosphamide Protocol Dose
All Patients (N=242)

	Amifostine + CP			CP		
Cycle	# Patients	Cumulative Dose	Percent Dose	# Patients	Cumulative Dose	Percent Dose
1						
Mean		1000	100.0		1000	100.0
Median		1000	100.0		1000	100.0
Min		1000	100.0		1000	100.0
Max		1000	100.0		1000	100.0
2						
Mean		1854	92.7		1842	92.1
Median		1750	87.5		1750	87.5
Min		1000	50.0		1000	50.0
Max		2000	100.0		2000	100.0
3						
Mean		2611	87.0		2613	87.1
Median		2500	83.3		2500	83.3
Min		1000	33.3		1500	50.0
Max		3000	100.0		3000	100.0
4						
Mean		3321	83.0		3324	83.1
Median		3250	81.3		3250	81.3
Min		2252	56.3		1750	43.8
Max		4000	100.0		4000	100.0
5						
Mean		3925	78.5		4014	80.3
Median		3875	77.5		4000	80.0
Min		2628	52.6		2100	42.0
Max		5000	100.0		5000	100.0
6						
Mean		4531	75.5		4752	79.2
Median		4469	74.5		4750	79.2
Min		3000	50.0		3125	52.1
Max		6000	100.0		6000	100.0
Overall						
Mean		3740	62.3		3770	62.8
Median		3999	66.7		3792	63.2
Min		1000	16.7		1000	16.7
Max		6000	100.0		6000	100.0

TABLE 16C

Percentages of Cisplatin Protocol Dose Received
All Patients (N=242)

	Amifostine + CP			CP		
Cycle	# Patients	Cumulative Dose	Percent Dose	# Patients	Cumulative Dose	Percent Dose
1						
Mean		100	100.0		100	100.0
Median		100	100.0		100	100.0
Min		100	100.0		100	100.0
Max		100	100.0		100	100.0
2						
Mean		195	97.7		195	97.6
Median		200	100.0		200	100.0
Min		100	50.0		100	50.0
Max		200	100.0		200	100.0
3						
Mean		294	97.9		292	97.3
Median		300	100.0		300	100.0
Min		200	66.7		225	75.0
Max		300	100.0		300	100.0
4						
Mean		392	97.9		387	96.8
Median		400	100.0		400	100.0
Min		325	81.3		281	70.3
Max		400	100.0		400	100.0
5						
Mean		487	97.3		480	96.0
Median		500	100.0		500	100.0
Min		375	75.0		325	65.0
Max		500	100.0		500	100.0
6						
Mean		582	96.9		578	96.4
Median		600	100.0		600	100.0
Min		425	70.8		450	75.0
Max		600	100.0		600	100.0
Overall						
Mean		461	76.8		452	75.3
Median		555	92.5		500	83.3
Min		100	16.7		100	16.7
Max		600	100.0		600	100.0

If amifostine substantially reduced chemotherapy toxicity, cyclophosphamide and cisplatin dose reductions for treatment toxicity might be less frequent, and it might be possible to deliver these drugs at a higher dose intensity. However, inspection of the above tables indicates that cyclophosphamide and cisplatin dose reductions, and the delivered dose intensities of these drugs, were virtually identical in the two arms in this study. One further possibility is that amifostine pretreatment would allow fewer treatment delays for toxicity, allowing more rapid delivery of the same cumulative drug doses (and thus a higher dose intensity). However, medical reviewer calculation of the rate of drug delivery to study patients (total dose of each drug actually delivered, divided by the time the patient was on study, arbitrarily assigning a length of 3 weeks to the patient's last treatment cycle) yielded the following results:

Reviewer Table 2

Delivered dose intensity of cyclophosphamide and cisplatin

Treatment group	Cohort	Average Cisplatin dose intensity (mg/m^2/week)	Average Cyclophosphamide dose intensity (mg/m^2/week)
Amifostine	Original (n=63)	28.1	230.1
	New (n=59)	26.3	230.1
	Total (n=122)	**27.2**	**230.1**
Control	Original (n=58)	27.6	235.7
	New (n=62)	27.3	237.8
	Total (n=120)	**27.5**	**236.8**

In conclusion, in this study, amifostine pretreatment had no discernable effect on the need to reduce cyclophosphamide or cisplatin doses for treatment toxicities; and it did not appear that amifostine pretreatment allowed delivery of significantly higher doses or dose intensities of cyclophosphamide or cisplatin.

D. Reasons for early treatment termination

Of the 122 patients in the amifostine arm of the study, 48 (39%) discontinued therapy prematurely (prior to completion of the planned 6 cycles of treatment). Of the 120 control arm patients, 55 (46%) discontinued therapy prematurely. Control arm patients reportedly were taken off study more frequently for various manifestations of renal toxicity or hematologic toxicity; amifostine patients went off treatment more frequently because of nausea/vomiting. Reasons for early treatment termination were summarized in tables 12C and 14C of the applicant's study report (reproduced on the following pages).

TABLE 12C

Discontinuation of Chemotherapy for Study Endpoints
- All Patients (N=242) -

Reasons for Discontinuation	Amifostine + CP (N=122)		CP (N=120)			p-value[a]	
	Number	(%)	Number	(%)	χ^2	2-sided	1-sided
Hematologic Toxicity	1	(1%)	8	(7%)	5.752	0.016	0.008
Anemia		0		1			
Death (Pancytopenia)		0		1			
Neutropenia		1		3			
Neutropenia w/ Infection		0		1			
Neutropenia and Thrombocytopenia		0		1			
Thrombocytopenia		0		1			
Renal Toxicity	0	(-%)	6	(5%)	6.229	0.013	0.007
Magnesium Wasting		0		1			
Decr. Creatinine Clearance		0		1			
Prolonged Incr. Creatinine		0		4			
Neurotoxicity	0	(-%)	2	(2%)	2.042	0.153	0.077
Ototoxicity	10	(8%)	15	(13%)	1.204	0.272	0.136
TOTAL	11	(9%)	29[b]	(24%)	10.023	0.002	0.001

[a] Based on Pearson Chi-Square test.

[b] Patient 2519 (CP arm) discontinued protocol therapy for hematologic toxicity and ototoxicity and Patient 521 (CP arm) discontinued protocol therapy for renal toxicity and ototoxicity.

Appendix III

Patient (amifostine)	This patient was taken off study therapy after the third treatment cycle, apparently due to treatment toxicity, and then received treatment with carboplatin and cyclophosphamide (beginning on 5/30/90). Censor patient for time to progression analyses, as of 5/30/90.
Patient (control)	This patient was taken off study after her fourth treatment cycle, due to hearing loss. Therapy with carboplatin plus cyclophosphamide was started on 7/6/90. Censor patient for time to progression analyses, as of 7/6/90.
Patient (amifostine)	This patient was taken off study after her fourth treatment cycle, due to hearing loss. Therapy with carboplatin plus cyclophosphamide was started on 12/26/90. Censor patient for time to progression analyses, as of 12/26/90.
Patient (control)	This patient was taken off study after her second treatment cycle, due to hearing loss. Four cycles of carboplatin plus cyclophosphamide were then given, starting on 12/28/90. Censor patient for time to progression analyses, as of 12/28/90.
Patient (control)	This patient was taken off study after her fourth treatment cycle, due to hearing loss. Two cycles of carboplatin plus cyclophosphamide were then given, starting on 1/10/92. Censor patient for time to progression analyses, as of 1/10/92.
Patient (control)	This patient received second line therapy with carboplatin beginning on 4/7/93, because of a rise in CA-125 to 91 (but no evidence of disease progression was recorded on physical exam, or on radiologic studies). The CA-125 subsequently normalized. Censor patient for time to progression analyses, as of 4/7/93.

Appendix III

Patient (amifostine)	This patient was taken off study after her fourth treatment cycle, due to hearing loss. Therapy with carboplatin plus cyclophosphamide was then given, starting on 5/27/92. Censor patient for time to progression analyses, as of 5/27/92.
Patient ((control)	This patient was taken off study after her second treatment cycle, due to hearing loss. Four cycles of carboplatin plus cyclophosphamide were then given, starting on 5/7/92. Censor patient for time to progression analyses, as of 5/7/92.
Patient (control)	This patient was removed from study because of severe nausea and vomiting after her 1st cycle of treatment. She subsequently received carboplatin-cyclophosphamide, beginning on 4/29/92. Censor patient for time to progression analyses, as of 4/29/92.
Patient (amifostine)	Second-line chemotherapy (hexamethylmelamine) was administered to this patient starting on 4/28/93, after residual disease was detected at second-look laparoscopy (performed on 4/20/93). Censor patient for time to progression analyses, as of 4/28/93.
Patient (control)	This patient was removed from study after the 4th cycle of treatment, because of persistent nausea and vomiting after each treatment cycle, and weight loss. She subsequently received carboplatin-cyclophosphamide, beginning on 8/8/92. Censor patient for time to progression analyses, as of 8/8/92.

Appendix III

Patient (amifostine)	This patient received only one cycle of protocol chemotherapy (amifostine, cyclophosphamide, cisplatin), was hospitalized with nausea, vomiting, dehydration, renal failure, and developed neutropenic fever. She recovered from these problems rather promptly, but the treating physician elected to remove her from the study, and treated her with carboplatin-cyclophosphamide (first dose, 6/23/93). Censor patient for time to progression analyses, as of 6/23/93.
Patient (amifostine)	This patient was taken off study after her second treatment cycle, due to hearing loss. Four cycles of carboplatin plus cyclophosphamide were then given, starting on 11/17/93. Censor patient for time to progression analyses, as of 11/17/93.
Patient (amifostine)	Second-line chemotherapy (cisplatin-mitoxantrone, then carboplatin) was administered to this patient starting on 6/13/90, after residual disease was detected at second-look surgery (performed on 5/16/90). Censor patient for time to progression analyses, as of 6/13/90.
Patient (amifostine)	Second-line chemotherapy (carboplatin) was administered to this patient starting on 10/22/90, after residual disease was detected at second-look surgery (performed on 10/2/90). Censor patient for time to progression analyses, as of 10/22/90.
Patient (amifostine)	The patient was treated with intraperitoneal ^{32}P on 9/18/89, after her second look surgery. Censor patient for time to progression analyses, as of 9/18/89.

Appendix III

Patient (amifostine)	This patient was treated with intraperitoneal cisplatin and fluorouracil starting on 11/8/89, after her second look surgery (performed 10/23/89). Censor patient for time to progression analyses, as of 11/8/89.
Patient (control)	Second-line therapy (an investigational immunotoxin protocol) was administered to this patient beginning on 12/18/89. Censor patient for time to progression analyses, as of 12/18/89.
Patient (amifostine)	This patient was treated with carboplatin starting on 2/2/90, after her second look surgery (performed 1/19/90). Censor patient for time to progression analyses, as of 2/2/90.
Patient (control)	This patient was treated with carboplatin starting on 3/20/90, after her second look surgery (performed 3/13/90). Censor patient for time to progression analyses, as of 3/20/90.
Patient (amifostine)	This patient was treated with etoposide starting 3/1/91, after her second look surgery (performed 1/23/91). Censor patient for time to progression analyses, as of 3/1/91.
Patient (amifostine)	This patient was treated with IP Yttrium-90 on 7/9/91, after her second look surgery (performed 6/17/91). Censor patient for time to progression analyses, as of 7/9/91.
Patient (amifostine)	This patient was treated with paclitaxel starting on 8/12/91, after her second look surgery (performed 6/24/91). Censor patient for time to progression analyses, as of 8/12/91.

Appendix III

Patient (control)	Per the case report forms, the date of progression should be 12/22/89 for this patient.
Patient (control)	The patient was treated with intraperitoneal cisplatin and thiotepa, starting 10/8/91, after her second look surgery (performed 9/30/91). A later third look operation revealed a complete remission. Censor patient for time to progression analyses, as of 10/8/91.
Patient (amifostine)	Per the case report forms, the date of progression should be 9/22/92 for this patient.
Patient (control)	This patient was taken off study after her second treatment cycle, due to hearing loss. Carboplatin was then administered, starting 11/12/91. Censor patient for time to progression analyses, as of 11/12/91.
Patient (amifostine)	This patient received second line therapy with intraperitoneal cisplatin and interferon, starting 4/8/92 at her second look surgery (which revealed residual disease). Censor patient for time to progression analyses, as of 4/8/92.
Patient (amifostine)	This patient received second line therapy with intraperitoneal cisplatin and interferon, starting 6/12/92 (after residual disease was documented at second look surgery, on 6/1/92). Censor patient for time to progression analyses, as of 6/12/92.
Patient (control)	The patient received second line therapy with intraperitoneal cisplatin and interferon, starting 6/25/92 (after residual disease was documented at second look surgery, on 6/17/92). Censor patient for time to progression analyses, as of 6/25/92.

Appendix III

Patient (control)	This patient received second line therapy with intraperitoneal interferon, starting 11/11/92 (after residual disease was documented at second look surgery, on 11/4/92). Censor patient for time to progression analyses, as of 11/11/92.
Patient (amifostine)	This patient was taken off study after her third treatment cycle. Carboplatin was then administered, starting 12/28/92. Censor patient for time to progression analyses, as of 12/28/92.
Patient (amifostine)	Second-line therapy with carboplatin was started 5/18/90, based on a rising CA-125 level. No findings of progression were documented on examination or CT scan. Censor patient for time to progression analyses, as of 5/18/90.
Patient (control)	This patient was taken off study 3/6/91, after her second treatment cycle, due to tinnitus. Carboplatin was then administered. Censor patient for time to progression analyses, as of 3/6/91.
Patient (control)	This patient went off study after 2 cycles; she changed health insurance plans and had to change physicians. Follow-up data is sketchy. Evidently she received 3 more cycles cisplatin-cyclophosphamide, then refused further therapy. She was NED per Kaiser physicians 5/91 with a normal CA-125, but relapsed at some later time. Censor patient for time to progression analyses, as of 5/1/91.
Patient (amifostine)	Follow-up of this patient for progression was sketchy. She received paclitaxel 7/5/93; progression was evidently documented at some time between 1/6/93 (when last known not to have a diagnosis of progression) and 7/5/93. Use 7/5/93 as date of progression, in time to progression analyses.

Appendix III

Patient (control)	This patient refused further protocol therapy after her 4th treatment cycle. She went on to receive one cycle of carboplatin-cyclophosphamide, on 3/7/91. Censor patient for time to progression analyses, as of 3/7/91.
Patient (amifostine)	This patient received second-line therapy with intraperitoneal cisplatin and etoposide, starting 8/28/91 (after residual disease was documented at second look surgery, on 8/5/91). Censor patient for time to progression analyses, as of 8/28/91.
Patient (control)	Case records indicate "peritoneal progression 10/93", with second line therapy (paclitaxel, carboplatin) starting 10/13/93. The patient is currently censored in the database (no progression). Use 10/13/93 as progression date in time to progression analyses..
Patient (control)	The last follow-up date recorded in the case report forms for this patient is 7/6/92 (lost to follow up). Absent data on disease progression, censor this patient for the time to progression analyses as of 7/6/92.
Patient (amifostine)	This patient received second line therapy with epirubicin and ifosfamide, starting on 8/19/92 (after residual disease was documented at second look surgery, on 7/19/92). Censor patient for time to progression analyses, as of 8/19/92.
Patient (control)	This patient was taken off study after her second treatment cycle, due to hearing loss. Carboplatin was then administered, starting 8/17/92. Censor patient for time to progression analyses, as of 8/17/92.

Appendix III

Patient (amifostine)	This patient received abdominal radiation therapy (in November-December 1992), after completing the planned 6 cycles of protocol treatment with no evidence of disease. Censor patient for time to progression analyses, as of 11/1/92.
Patient (control)	This patient received second line therapy with epirubicin and ifosfamide starting on 8/18/93, after residual disease was documented at second look surgery (performed on 7/6/93). Censor patient for time to progression analyses, as of 8/18/93.
Patient (amifostine)	This patient received second line therapy with carboplatin and cyclophosphamide 12/22/93, after residual disease was documented at second look surgery (performed on 11/29/93). Censor patient for time to progression analyses, as of 12/22/93.
Patient (amifostine)	This patient received abdominal radiation therapy (beginning 8/19/93) for "consolidation of CR" after her negative second look operation. Censor patient for time to progression analyses, as of 8/19/93.
Patient (control)	This patient received 3 cycles of carboplatin plus cyclophosphamide as "consolidation" after her negative second look surgery, starting 7/5/93. Censor patient for time to progression analyses, as of 7/5/93.
Patient (amifostine)	This patient received abdominal radiation therapy (beginning 7/12/93) after her second look operation (performed 5/19/93). Censor patient for time to progression analyses, as of 7/12/93.

Appendix III

Patient (amifostine)	This patient received second line treatment with Navelbine starting 3/29/93 after her second look operation (performed 2/24/93). Censor patient for time to progression analyses, as of 3/29/93.
Patient (amifostine)	This patient received second line treatment with carboplatin starting 3/2/93 after her second look operation (performed 1/25/93). Censor patient for time to progression analyses, as of 3/2/93.
Patient (amifostine)	This patient was removed from study after 1 cycle, because of transient impaired renal function (she could have continued on study). Follow-up therapy with carboplatin started 8/25/92. Censor patient for time to progression analyses, as of 8/25/92.
Patient (amifostine)	This patient had hearing loss after her first treatment, and her therapy was changed to doxorubicin-cyclophosphamide (started 6/10/92). Censor patient for time to progression analyses, as of 6/10/92.
Patient (control)	This patient received second line treatment with carboplatin starting 5/19/92 after her second look operation (performed 4/23/92). Censor patient for time to progression analysis, as of 5/19/92.
Patient (amifostine)	This patient took herself off study after 2 cycles, because of nausea and transient impaired renal function (she could have continued on study). Follow-up therapy with carboplatin plus cyclophosphamide started 5/4/93. Censor patient for time to progression analyses, as of 5/4/93.

Appendix III

Patient (control)	This patient received second line treatment with hexamethylmelamine, starting 3/6/93, after her second look operation (performed 1/19/93). Censor patient for time to progression analyses, as of 3/6/93.
Patient (amifostine)	This patient received second line treatment with AD-32, starting 9/15/93, after her second look operation (performed 8/10/93). Censor patient for time to progression analyses, as of 9/15/93.
Patient (control)	This patient received second line treatment with paclitaxel, starting 8/23/93, after her second look operation (performed 7/27/93). Censor patient for time to progression analyses, as of 8/23/93.
Patient (control)	This patient had hearing loss after her third treatment, and therapy was changed to carboplatin-cyclophosphamide (started 10/19/92). Censor patient for time to progression analyses, as of 10/19/92.
Patient (control)	This patient went off study after 2 cycles due to poor compliance. She received additional treatment with cisplatin-cytoxan through 12/92; but has not been seen by study investigators since 1/25/93. Censor patient for time to progression analyses, as of 1/25/93.
Patient (control)	Oral etoposide was started 8/26/93 for a rising CA-125 level. No objective evidence of disease was reported. Censor patient for time to progression analyses, as of 8/26/93.
Patient (amifostine)	This patient had hearing loss after her fourth treatment, and her therapy was changed to carboplatin (started 11/12/92). Censor patient for time to progression analyses, as of 11/12/92.

The major clinical studies will be reviewed in the clinical section of this review. Generally WR-2721 induced hypotension occasionally associated with fainting, dizziness, but without cardiovascular complications. The drug also may induce nausea, vomiting, sneezing, a warm or flushed feeling, mild somnolence, and hypocalcemia due to an inhibition of parathyroid hormone secretion and a direct inhibition of bone resorption.

b. Pharmacokinetics (see pharmacology review)

c. Toxicology (see pharmacology review)

4. Clinical Background

a. Previous similar human studies.

The Armed Forces Radiological Institute embarked on a systematic search for compounds that could protect normal tissues against radiation damage. WR-2721 was the most effective and least toxic of a multitude of compounds tested. Several pharmacokinetic evaluations were performed in man (Shaw et.al. Chromatogr. 7:2447, 1986; Shaw et.al. J. Liq Chromatogr.9: 845, 1986; Shaw et.al. J. Liq Chromatogr.10:439, 1987, and Swynnerton et.al. Int. J. Rad. Oncol.Biol. Phys.10: 521, 1984). These studies provided much of the early human pharmacokinetic data as demonstrated in the table below (Chabner et.al., Cancer Chemotherapy, 1990)

Additionally the human pharmacokinetics was well presented by the sponsor. Fig.1 demonstrates the concentration of WR-2721 in human plasma after a single dose of 3.4 mg/kg. The majority of the drug is cleared form the plasma within approximately 5 minutes. The distribution half life (T1/2a) was 0.84 minutes and the clearance from the central compartment was 0.977 L/hr/kg.

Fig.2 indicates the concentrations of WR-2721 and WR-1065 in human blood after multiple i.v. doses. minutes after the last dose was given, the concentration of WR-1065 exceeded that of WR-2721.

The 2nd Table provided by the sponsor summarizes the pharmacokinetics of WR-2721 administered to 13 patients at a dose of 150 mg/m2. The low volume of distribution at steady-state Vss was 6.44 L. That low value indicates that the unmetabolized drug is largely confined to the intravascular system and to a small volume of extravascular space. A two-compartment pharmacokinetic model best describes the plasma concentration data for all but 2 patients.

Shaw's data in 10 patients given 740 mg/m2 over 15 minutes is presented in Fig.3. The results demonstrate a rapid decline in plasma level of the drug.

There was some concern the combination of WR-2721 and either metoclopropamide or dexamethasone might alter the pharmacokinetics

of WR-2721. Tables 3 and 4 indicate that the pharmacokinetics of WR-2721 is not effected by either anti-emetic drug.

The conclusions of the sponsor are replicated below.

CONCLUSIONS

"Results suggest that WR-2721 exits in bloodstream rapidly and enters normal tissues where it is rapidly converted to its metabolites and exerts its protective effects. These properties of WR-2721 are evidence for its administration immediately prior to chemotherapy or radiation therapy and suggest the potential need for multiple doses of WR-2721 to protect against the toxicities of drugs with long half-lives such as carboplatin.

Furthermore, the pharmacokinetics of WR-2721 is not affected by pretreatment with either of the antiemetic drugs, metoclopramide or dexamethasone."

Single Dose

The following results were observed following administration of WR-2721 as an intravenous bolus dose or as an intravenous infusion.

- WR-2721 is rapidly cleared from plasma (clearance was greater than 90% within approximately 5 minutes following a 10-second intravenous bolus dose).
- The distribution phase of WR-2721 was rapid and was the predominant behavior of the drug.
- WR-2721 has a small volume of distribution, indicating that the unmetabolized drug is largely confined to the intravascular system (rather than to the extravascular space). This is consistent with the concept that WR-2721 is rapidly dephosphorylated and enters normal tissues as WR-1065.
- Renal excretion of WR-2721 was low; average loss was 0.69% for patients who received an intravenous bolus dose and 1.05% for patients who received a 15-minute intravenous infusion.

Multiple Doses

The following points were observed in one patient following five multiple intravenous injections of WR-2721 (the first four injections were given every 4 minutes, the fifth injection was administered 3 minutes after the fourth injection).

- The concentration of WR-1065 in blood increased steadily during the time interval of the first four doses and reached a plateau minutes after the first dose.

- Conversely, the level of WR-2721 decreased rapidly. WR-2721 appeared in the bloodstream of the patient shortly after administration of WR-2721 for a longer period of time.

Drug - Drug Interactions

- Pretreatment with either metoclopramide or dexamethasone did not alter the pharmacokinetics of WR-2721 (740 mg/m2 as a 15-minute i.v. infusion).

e. Overall evaluation and Conclusions.

(1) Pharmacology - The Pharmacology review is submitted separately; however, the summary evaluation and recommendations to the medical officer are replicated below.

Evaluation

WR2721 (ethyol) protects against some of the toxicities of some of the platinum, alkylating agent, and antibiotic cancer chemotherapeutic compounds by preferential uptake into normal tissue, where cytotoxins can be bound to the sulfhydryl group of WR1065 (the active dephosphorylated form of the drug) and free radicals scavenged. Levels of drug in the CNS and tumor is generally low, with the exception of the Morris hepatoma, indicating the possibility that some tumors may be protected from cytotoxicity by this compound. Kidney damage with the platinum compounds cisplatin and ormaplatin, measured both by serum levels of BUN and creatinine and histopathologically was decreased with WR2721 administration. Depletion of peripheral WBC's by cisplatin, CBDCA, and mitomycin C was decreased with WR2721. Toxicity to colony forming units of the bone marrow with nitrogen mustard, cyclophosphamide, BCNU, melphalan, cisplatin, and adriamycin were all decreased with WR2721 administration. Finally, pulmonary toxicity seen with cyclophosphamide was decreased with WR2721 administration.

WR2721 is rapidly cleared from the blood, as is its dephosphorylated metabolite, WR1065 (the half-life of both compounds is less than 10 minutes for all species observed). Excretion is via the kidneys, with over half of the dose removed within the first 12 hours (smallest time increment studied).

Toxicities seen with the drug include neurologic-related clinical signs (mydriasis, watery salivation, emesis from an i.v. administered drug, ptosis of the eyelid and gait abnormalities) elevations of some liver enzymes (less than 2 fold), marrow toxicity in the rat but not the dog, and kidney damage. Paradoxically, protection of hematopoietic units when WR2721 is combined with other marrow-toxic agents is well documented. Hence, liver and kidney function, and hematological parameters should be monitored closely during therapy. Reproductive toxicity was not well characterized (studies are currently in progress), but, the compound will be used with known teratogens, so pregnancy issues are somewhat irrelevant. A similar argumen: can be used for carcinogenicity testing, as the compound will be administered with known carcinogens; however, animal studies have shown some protection against delayed tumor formation with radiotherapy and tissue culture studies have shown a protective effect on HGPRT locus mutation.

Note: previous pharmacologic deficiencies, lack of multiple dose and reproductive toxicologic studies, have been corrected.

Labelling Issues

1. Eliminate the sentence in the second paragraph of the clinical pharmacology section reading "Other studies suggest a facilitated..."
as this experiment was not performed in an appropriate system.

2. The final sentence in the second paragraph of the clinical pharmacology section should begin with "In a cell-free system..."

3. In the precautions section under "Carcinogenesis, mutagenesis, and impairment of fertility" the third sentence should read: "Data from in vitro studies demonstrate that ethyol decreases mutations at the HGPRT locus in Chinese hamster cells by 60 to 95% compared to mutations with the cytotoxic agent alone using cisplatin, bleomycin, or nitrogen mustard (Nagy et al., 1986). In vivo rodent experiments show a 60% reduction in tumor incidence following X-ray irradiation of the leg with ethyol therapy (Hunter et al., 1991)."

4. In the pregnancy section under precautions, the study the sponsors quote is woefully inadequate and the conclusion of nonteratogenic unfounded. The reproductive toxicity experiments are being repeated, so this may be acceptable once that data is submitted. As currently submitted, the labelling should read "Adequate teratogenicity testing has not been performed with WR2721".

Notes to the Medical Officer on Labelling Issues

The largest question in the labelling is whether to allow the vague description of uses and agents: i.e. should their indications and usage read "as a chemoprotective agent against the serious toxicities associated with intensive regimens of platinum and alkylating agent chemotherapy" or should it be more specific as to which toxicities and which drugs, especially as some of these agents have not actually been tested with WR2721 in well-controlled human trials. If specificity is desired, the first sentence of the clinical pharmacology section and the indications and usage section could read as follows.

From the evidence in rodents, ethyol is indicated as a chemoprotective agent against some of the toxicities (bone marrow and nephrotoxicity) of platinum compounds (cisplatin, CBDCA, and ormaplatin), and the marrow toxicities of cyclophosphamide and alkylating agents (nitrogen mustard, cyclophosphamide, BCNU, and melphalan).

There are no significant pharmacological issues that would result in a clinical hold, and the preclinical data justifies the clinical conduct of the studies submitted.

Chemistry consultation discloses multiple chemistry deficiencies that have not been adequately addressed by the sponsor. Their consultation will be submitted separately, but their criticisms involved issues of purity and acceptable standards of synthesis. Their comments are replicated below.

NDA 20-221:
Amifostine for Injection

The Chemistry, Manufacturing, and Controls (CMC) portion of the NDA submission, 20-221, Amifostine for Injection, was found to be very deficient. Among the most significant of the deficiencies are the failure to provide adequate methods and specifications to control for manufacturing and degradative impurities and the failure to provide meaningful stability data. What little stability information which is provided for the drug product suggests an extreme instability of the formulated drug product compared to extraordanary stability of the bulk drug substance, which remains unexplained. In general, the CMC information submitted fails to assure the identity, strength, quality, and purity of the drug substance and the drug product.

'linical Evaluation.

Five "adequate and well-controlled" clinical studies with WR-2721 were submitted as pivotal studies. Study WR-2721-B001 (201c, 201a) was a retrospectively controlled study comparing the protective effect of WR-2721 on cyclophosphamide at a dose of 1500 mg/m2. Patients acted as their own control. In the first study, cyclophosphamide was given first and in the later study WR-2721 was given first. This pivotal study suffers from small numbers of patients in the study groups (a total of 21 patients in both groups), the lack of a sequence and period effects established by utilizing a retrospective control, and the lack of a prospective cohesive protocol. The study is weakly supportive of WR-2721 efficacy.

The second pivotal study WR-2721-1 was a randomized controlled parallel group study performed in women with Stage III or IV ovarian cancer. The study compared a combination of cyclophosphamide and cisplatin to WR-2721 and the same regimen. It is the most important study. When preliminarily submitted, after interim analysis, the FDA's and the advisory chairman's recommendation was to continue the study to its completion. Nevertheless, another unplanned interim analysis was performed after accruing more patients, and the results were submitted for NDA regulatory review. As a major pivotal study, multiple methodological and statistical problems are present. The study was not blinded, some of the appraised endpoints were arbitrarily chosen and deviated from those specified in the protocol, he confidence intervals did not provide assurance that the complete response rate was not impaired by WR-2721, the analysis of neurotoxicity utilized post-hoc definitions of neurotoxicity and did not agree with the FDA analysis suggesting no protection from neurotoxicity, study endpoints were poorly defined, the protection from nephrotoxcity and ototoxicity were not statistically significant as defined prospectively in the protocol, there was poor compliance in the accumulation of data points, particularly as the study related to the evaluation of ototoxicity and neurotoxicity, and the plethora of protocol amendments. As the most important randomized study submitted it is supportive for the indication of protecting against cytoxan and cisplatin induced leukopenia but should be completed before regulatory approval, particularly in light of the study's failure to demonstrate conclusively other protective actions.

The third randomized controlled parallel group study utilized mitomycin-c with and without WR-2721 in men and women previously treated with 5-FU or 5-FU + leukovorin for carcinoma of the colon or rectum with metastases. The sponsor was not able to demonstrate a protective effect of WR-2721 with respect to granulocyte nadirs, platelet count nadirs 75,000, fever, infections or bleeding complications, or the need to reduce or delay a repeat dose of mitomycin-c. The sponsor's demonstration of protective effect on platelets utilizing and endpoint of a 150,000 platelet count does not meet the sponsor's objectives or efficacy criteria. The study is of borderline regulatory and clinical significance.

The other two "pivotal" studies are not pertinent to the approval process, as one of the studies addresses the protective efficacy of WR-2721 against radiotherapy (an indication not requested in this NDA), and the other study addresses the in-vitro use of WR-2721 in counteracting the potential toxicity of 4-HC in the ex-vivo treatment of bone marrow for patients with metastatic or locally recurrent breast cancer, lymphoma, or leukemia. 4-HC is not an approved drug and an indication for this use has not bee requested.

The last pivotal study is an uncontrolled study utilizing WR-2721 with high dose cisplatin for the treatment of patients with metastatic melanoma. Historical controls are utilized for comparison of toxicity. It is this reviewer's opinion that the historically selected control groups were not comparable and that inappropriately selected parts of historical studies were utilized to make apparent claims of protection against toxicity. In addition, other studies utilizing the combination of high dose cisplatin and WR-2721 have not shown a protective effect from WR-2721, and in point of fact have suggested greater toxicity from the combination. The study does not support the indication requested.

Several other small completed, terminated, or small ongoing studies were submitted as uncontrolled but supportive. None of those studies submitted provided significant support for the proposed indications.

The data for a protective effect of WR-2721 against cisplatin and or cisplatin and cyclophosphamide is borderline but clearly no significant human data has been submitted to justify the indication of "protection of patients who are at risk for serious hematologic toxicities from [all] alkylating agents".

Chemistry issues will need to be addressed prior to approval.

Gerald H. Sokol, M.D., M.S., FCP

Addendum to Medical Officer Review.

The questions presented to the Oncology Drugs Advisory Committee are replicated below.

QUESTIONS FOR THE FDA
ONCOLOGY DRUGS ADVISORY COMMITTEE
JANUARY 31, 1992

NDA 20221 ETHYOL

Randomized study WR-2721-1 compares CP with or without Ethyol in patients with stage III or IV ovarian cancer.

1.Does this study show that Ethyol decreases the toxicities caused by the CP regimen? If so, which toxicities are decreased? hospitalization for febrile granulocytopenia less than 500? hospitalization for febrile granulocytopenia less than 500 plus hospitalization for infection with either granulocytopenia less than 500 or fever? median granulocyte nadir? granulocyte count less than 1500 on day 25? serum creatinine higher than 1.5 on day 25? neurotoxicity? ototoxicity? other toxicity?

2.Based on complete response rates, do the results of this study provide sufficient assurance that Ethyol does not decrease the antitumor effect of the chemotherapy?

3.There are a number of issues regarding the interim analysis of this study. The protocol indicated the interim analysis would be done "halfway through". "Halfway through" is subject to a wide range of definitions. There are multiple endpoints for stopping the study without provisions for P value adjustment. There are two unplanned analyses since the planned interim analysis. Are these issuses sufficiently serious to have an important impact on interpretation of the study results?

4.The planned interim analysis was done when 97 of the planned 200 patients had been accrued. Presently 121 patients have been accrued. Accrual is temporarily on hold awaiting the advice of this Committee. Should this study be completed?

Study 201C with 21 patients compares Cytoxan in cycle #1 with Cytoxan plus Ethyol in cycle #2 in the same patient. In a separate study (201A) with 15 patients the treatments are administered in the reverse order.

5.Do these studies show that Ethyol decreases the toxicity of Cytoxan? If so, which toxicities are decreased? granulocytes less than 500? duration of granulocytes less than 500? other?

6. Do the design of these studies and the fact that the patients had several different tumor types permit an assessment of whether Ethyol decreases the antitumor effect of the Cytoxan?

An uncontrolled study WR-2721-B001: 201D evaluates cisplatin 120-150 mg/M2 with Ethyol in 48 patients with metastatic malignant melanoma.

7.Are cisplatinum toxicities decreased compared to historical experience with cisplatinum alone? If so, which toxicities are decreased? hematologic? renal? neurologic? other?

Randomized ECOG study #1686 compares cisplatinum 120 mg/M2 with cisplatinum 150 mg/M2 plus Ethyol in a total of 94 patients with metastatic malignant melanoma. This study was conducted to confirm the results of the above uncontrolled study. ECOG study #1686 was closed to accrual on July 15, 1991. Preliminary toxicity data are available, but not tumor response data.

Applicant has agreed as of two weeks ago to limit labeling and advertising claims for Ethyol to decreasing toxicity of cisplatinum at doses of 100 mg/M2 or less. However, there is only one study submitted with cisplatinum at this dose level.

8.Does the Committee agree that a decision on the indication for decreasing the toxicities of cisplatinum should await availability of the final results of ECOG study #1686?

General Questions

9. Is it essential to have data showing whether Ethyol affects the pharmacokinetics of cisplatin and cyclophosphamide?

10. Are additional studies needed to show that Ethyol decreases the neutropenia related toxicity of cyclophosphamide?

11. Are additional studies needed to show that Ethyol decreases the toxicities of cisplatinum?

12. In the randomized ovarian cancer study WR-2721-1 dexamethasone had to be added to the Ethyol plus CP regimen because Ethyol adds to the emetic effect of the CP. The dexamethasone was given intravenously prior to the Ethyol and at 4, 8 and 12 hours after the cisplatin. If this NDA is approved, should the package insert be specific in this regard? Oncologists may be inclined to substitute odansetron for the complicated antiemetic regimen used in the ovarian cancer study. What should the package insert say about this?

The votes of the committee are indicated below in the order of the question.

1.The committee voted that Ethyol decreases the hematological toxicities caused by CP but it was not clear to the committee which hematological parameter was the appropriate value to utilize. The committee indicated that there was not sufficient evidence that renal, neuro-toxicity, and oto-toxicity weredecreased by Ethyol.

2. The wording of the second question was changed to "....do the results of this study provide (sufficient changed to preliminary) assurance that Ethyol does not decrease the anti-tumor effect of the chemotherapy?" The committee voted 7 yes to 1 no to the second question.

3. The committee agreed unanimously but for 1 vote that the issues of multiple " looks " were sufficiently serious to have an important impact on interpretation of the study results.

4. The committee voted 8 to 0 that the study should be completed.

5. The committee- without a formal vote felt that Ethyol decreases the toxicity of Cytoxan but it was not clear that there was a clinical benefit.

6.The committee without a formal vote-indicated that the design of the studies and the fact that patients had several types of tumors did not permit an assessment of whether Ethyol decreases the anti-tumor effect of the Cytoxan.

7. Concerning the uncontrolled B001,201D study the committee deferred, that as they were uncontrolled studies the question could not be answered.

8. The question was withdrawn.

9. The committee voted no as to whether there was the need to obtain pharmacokinetic data on the effects of Ethyol on cisplatin and Cytoxan.

10. The committee voted 4 yes and 4 no as to whether additional studies were needed to show whether Ethyol decreases the neutropenia related toxicities of Cytoxan.

11. The committee voted 7 yes vs. 1 no as to whether additional studies were needed to show that Ethyol decreases the toxicities of cisplatinum.

12. With respect to labeling and to the use of decadron as an anti-emetic the committee generally felt that the package insert should reflect the regimen that was used without additional comment.

The committee felt that the drug had promise and were encouraged about ultimate approval but did not recommend approval at this time.

A meeting was held on 3/9/92 with the applicant. The applicant suggested that only the indication for hematological protection against the combination of cytoxan and cisplatin would be requested at this time by the sponsor pending additional data on the other protective effects of Ethyol on neuro-toxicity, oto-toxicity, and renal toxicity.

The agency indicated another area of concern. When anticancer agents are used in combination it is not always clear what percentage of the response rate is contributed by each agent. Specifically in the CP regimen if cyclophosphamide contributes 20% of the antitumor effect, one might neutralize half of it with Ethyol and see only a 10% decrease in antitumor effect. In other words, the CP regimen would not be suitable for studying the effect of Ethyol on the efficacy of this anticancer drug

It was requested by the agency that U.S. Bioscience conduct a second randomized study in ovarian cancer with the CP regimen +/- Ethyol to confirm a favorable effect on hematologic toxicity. This would be sufficient to take the application back to the ODAC with the prcposed indication being limited to the prevention of hematological toxicity caused by the CP regimen in patients with ovarian cancer. The response rates and survival results from the second study may be combined with data from the first randomized ovarian cancer study to demonstrate lack of interference by Ethyol with the anti-tumor effect of the CP regimen. This is consistent with the recommendation by ODAC that the randomized ovarian cancer study be completed as originally planned. Originally 200 patients were planned and the current suggestin would require a total of 200-220 patients. 121 patients had been studied at the time of the NDA submission on Sept. 30, 1991. It was agreed that the protocol may need to be modified and to add new endpoints e.g., the need for the administraion of CSF. They would need to incorporate specific criteria for each endpoint. Realizing that recruitment might be difficult because of competing protocols the agency stressed the need to vigorously recruit, [illegible] endpoints that correspond to current practices, and provide follow-up on all patients.

Final Recommendation- Non-approval. See above for agency's recommendations.

Gerald H. Sokol M.D.,M.S.,F.C.P.
3/20/92

I concur with the not approvable recommendation, See also my written Comments dated Jan 9, 1992 and the Memorandum of the FDA/US Bioscience meeting on March 9, 1992. See also my written Comments dated 4-2-92.

John R Johnson MD
4-2-92

ONCOLOGY DRUGS GROUP LEADER COMMENTS
ON NDA 20221

Date January 9, 1992

Drug Ethyol (amifostine) (WR-2721)

Applicant U.S. Bioscience

Indication "Protection of patients who are at risk for serious hematologic toxicities from alkylating agents and cisplatin and/or the neurologic and nephrotoxicity from cisplatin"

NOTE: Three versions of the Indication have been proposed by the Applicant. The Indication submitted initially in the NDA was for protection against all alkylating agents and all platinum agents. The second version, proposed by the Applicant on its own initiative by telephone, was protection against cyclophosphamide and cisplatin. The third and current version of the proposed Indication was submitted to the NDA on 1-6-92 and is as stated above.

Background

This is not intended to be a complete written review of the NDA. Its purpose is to convey the questions and concerns I have about the NDA.

This NDA was submitted on 9-30-91. Unfortunately there was no Pre NDA Meeting to discuss formating and analysis of the data. This has resulted in many wasted weeks for the FDA reviewers. There have been numerous telephone conversations with the applicant resulting in clarifications and reanalyses and new analyses and submission of three new volumes, the most recent of which came in on 1-7-92. There must now be a cutoff date, so we can complete our reviews and get them to the Advisory Committee in a timely manner.

Clinical Study
Ovarian Cancer Stage III or IV CP + or - WR-2721

Study Design

This RCT was designed with a single planned interim analysis "halfway through" that provided for stopping patient accrual to the study if there was a statistically significant difference ($p < .03$, one sided) in the "incidence of

toxicity" and no statistically significant difference in "tumor response rate" .

There are several problems with this.

1."Halfway through" is subject to a wide range of definitions.
2."Incidence of toxicity" provides multiple endpoints without P value adjustment.
3.P <.03 (one sided) on the difference in non fatal reversible toxicity may not be a sufficient basis for stopping study accrual. There is also need to demonstrate that WR-2721 does not interfere with the antitumor effect of the chemotherapy.
4.An interim analysis was submitted to the FDA in November 1991 when 97 patients had been accrued. After consultation with the Chairmen of ODAC, Dr. Craig Henderson, the FDA advised the applicant to continue the study. An additional 24 patients were accrued and then the analysis presented in this NDA was done. This constitutes an unplanned statistical analysis. Since submission of the NDA, the Applicant has added 3 pCRs to the WR + CP arm and 1 pCR to the CP arm resulting in still another unplanned analysis.

Study Results

1. The most impressive result regarding toxicity was a decrease in hospitalizations for granulocytopenia and fever and a decrease in hospitalizations for infection with either granulocytopenia or fever. These are endpoints of some importance, but are not hard endpoints. For example, the protocol does not define fever or granulocytopenia as applied to these endpoints.

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Following are the Applicant's and the FDA Reviewer's analyses of these endpoints. I believe the total of the patients hospitalized in these two categories is the most important comparison. In the patients hospitalized for febrile granulocytopenia there were documented infections in 4/15 in the CP arm and 4/7 in the CP+WR-2721 arm.

Applicant's Analysis

	CP	CP+WR-2721	
Hosp. for fever and granulocytopenia	16/58	5/63	p=.003, one sided
Hosp. for infection with fever and/or granulocytopenia	17/58	9/63	p=.022, one sided

FDA Reviewer's Analysis

Hosp. for fever and granulocytopenia	15/58	7/63	p=.031, one sided
Hosp. for infection with fever or granulocytopenia	1/58	2/63	
Total	16/58	9/63	p=.057, one sided

2.The number of patients with granulocyte nadir less than 500 was a key endpoint in the protocol, but is not reported in the NDA.

3.The FDA agreed prior to the study that a demonstration that the complete response rate on the WR=2721 arm was at least as good as the non WR=2721 arm (using two sided 95% CI on the difference in complete response rates) would be accepted as adequate evidence that WR-2721 does not impair the antitumor efficacy of the chemotherapy regimen. Duration of complete response, time to progression and survival data will be reported, but the WR-2721 arm need not be shown to be at least as good statistically for NDA approval.

Following are the Applicant's and the FDA Reviewer's analyses of these endpoints. I believe the "patients hospitalized for infection with either granulocytopenia or fever" is the most important comparison.

Applicant's Analysis

	CP	CP+WR-2721	
Hosp. for fever and granulocytopenia	16/58	5/63	p=.003, one sided
Hosp. for infection with fever and/or granulocytopenia	17/58	9/63	p=.022, one sided

FDA Reviewer's Analysis

Hosp. for fever and granulocytopenia	15/58	7/63	p=.031, one sided
Hosp. for infection with fever and/or granulocytopenia	16/58	9/63	p=.057, one sided

2.The number of patients with granulocyte nadir less than 500 was a key endpoint in the protocol, but is not reported in the NDA.
3.The FDA agreed prior to the study that a demonstration that the complete response rate on the WR=2721 arm was at least.as good as the non WR=2721 arm (using two sided 95% CI on the difference in complete response rates) would be accepted as adequate evidence that WR-2721 does not impair the antitumor efficacy of the chemotherapy regimen. Duration of complete response, time to progression and survival data will be reported, but the WR-2721 arm need not be shown to be at least as good statistically for NDA approval.

Following are the analyses of complete response by the Applicant and the FDA Reviewer. The difference in pCR numbers between the Applicant and the FDA Reviewer appears to be that the Applicant has added four pCRs since the cutoff date for analysis (3 in the WR+CP arm and 1 in the CP arm). I do not believe the Applicant's analysis of pCR + cCR + NED is very important because NED status is quite different from complete response.

Applicant's Analysis

All Patients

	N = 58	N = 63		
	CP	CP+WR	DIFF.	95% CI TWO SIDED
pCR	11 (19.0%)	14 (22.2%)	3.2%	-11.1 to 17.7
pCR + cCR + NED	15 (25.9%)	23 (36.5%)	10.6%	-5.7 to 27.0

FDA Reviewer's Analysis

pCR	10 (17.3%)	11 (17.5%)	0.2%	-13.3 to 13.7
cCR	4 (6.9%)	3 (4.8%)	-2.1%	-10.5 to 6.2
pCR + cCR	14 (24.1%)	14 (22.2%)	-1.9%	-17.0 to 13.1

Clinical Study #201C Cytoxan first cycle, Cytoxan + WR second cycle

Clinical Study #201A Cytoxan + WR first cycle, Cytoxan second cycle

Advanced Cancer-Multiple Tumor Types

1.These are not randomized controlled studies.
2.There is apparently no prospective protocol for study #201A. The protocol for study # 201C consists of an undated one paragraph addendum to a Phase I protocol.

3.The principal endpoint is number of patients with granulocyte nadirs less than 500 on cycle #1 compared to cycle #2. In study 201C this is 14 patients versus 5 (p=.004) favoring cycle #2 with Cytoxan + WR over cycle #1 with Cytoxan alone. This endpoint is not as clinically important as the number of patients with hospitalization for granulocytopenia and fever or with hospitalization for infection with either granulocytopenia or fever as used in the ovarian cancer RCT.
4.We are unable to determine if WR impairs the antitumor effect of Cytoxan in this small, uncontrolled study in patients with multiple tumor types.

Clinical Study
Advanced Colorectal Cancer Mitomycin-C + or - WR

This is a RCT in a total of 96 patients.

1.There was no statistically significant effect on serious or life threatening hematological toxicity.
2.It is not possible to assess for impairment of mitomycin-C antitumor effect because there was no mitomycin antitumor effect. There was only one partial responder in the entire study.

Clinical Study
Breast Cancer-Marrow Purging with 4HC + or - WR Prior to ABMT

In this RCT a clinically and statistically significant difference was seen in time to engraftment and other related parameters. This is especially interesting to the FDA because ABMT investigators have adamently maintained that 4-HC has no adverse effect on ABMT engraftment. This study does not seem very important to the FDA decision on whether to approve WR for the proposed indication in this NDA.

Clinical Study
Metastatic Melanoma Cisplatin 120-150mg/m2 + WR

This uncontrolled study reports a decrease in cisplatinum toxicity compared to historical experience. Partial responses were reported in 26/47 patients (55%).

The FDA has obtained additional information having a bearing on this study.

1.This study was published in the JCO in April 1987. Objective responses were reported in 19/36 patients (53%). In an NCI audit of this study 6 of the 19 reported responses could not be confirmed. The NCI audit report is attached to this document.
2. conducted a RCT comparing P 120mg/m2 with P150mg/M2 + WR-2721, each given Q3 weeks to patients with metastatic melanoma. Tumor response data is not available. Toxicity data in the first 86 patients raises questions as to whether WR-2721 protects against renal and neurologic toxicities of high dose cisplatinum. This study was closed early. The ECOG summary of this study is attached.
3.The NCI is conducting a study in patients with advanced ovarian cancer using a high dose regimen of cyclophosphamide, cisplatinum and carboplatin. Patients were randomized to receive or not receive WR-2721. Sixteen patients were treated with a total of 58 cycles. There were neutropenic fevers on 20 and 21% of the cycles in the respective study arms. On this basis WR-2721 was dropped from the study and G-CSF was added. The carboplatin dose was also reduced. The NCI summary of this study is attached.

All of the inquiries FDA has received from oncologists concerning WR=2721 have involved using it to allow administration of high dose chemotherapy regimens. There have been no inquiries regarding standard dose regimens. Thus the results of the ECOG and NCI studies, although not conclusive, are of concern.

Issues to be Resolved

The issues are reflected in the FDA's questions for the ODAC. A copy is attached. In addition to the issuses detailed in the comments above on the individual clinical studies, the main issues are as follows.

1.Is there sufficient basis for approving the NDA and related to this should the RCT in ovarian cancer be completed as originally planned?
2.Is there sufficient assurance that WR=2721 does not impair the antitumor effect of the cancer chemotherapy?
3.If the NDA is approved, should the indication be for protection against the hematologic toxicity of all alkylating agents or restricted to cyclophosphamide?
4.This "outpatient" regimen requires approximately 19 hours to administer, starting with prehydration 6 hours prior to WR-2721 and ending with dexamethasone i.v. 12 hours after the cisplatin. Dexamethasone is necessary because of the additive emetic effect when WR-2721 is added to CP and it

adds 4 hours to the administration time. It is predictable that many oncologists will use odansetron to simplify the regimen and shorten the administration time. What should the package insert say in this regard as there is no experience using odansetron with this regimen?

Conclusion

This NDA will be presented to the ODAC on 1-31-92. We will await the advice of the ODAC before deciding on the disposition of this NDA.

NOTE: The FDA chemists indicate that there are many deficiences in the chemistry amd manufacturing section of this NDA. They estimate that it will take US Bioscience and the companies doing the manufacturing for US Bioscience a year to resolve these deficiencies and make the NDA approvable from a chemistry and manufacturing standpoint.

John R Johnson, MD

John R. Johnson, M.D.
Oncology Drugs Group Leader

cc NDA 20221
HFD-150 Div File
Dr. Burke
Dr. Johnson
Dr. Sokol

QUESTIONS FOR THE FDA
ONCOLOGY DRUGS ADVISORY COMMITTEE
JANUARY 31, 1992

NDA 20221 ETHYOL

Randomized study WR-2721-1 compares CP with or without Ethyol in patients with stage III or IV ovarian cancer.

1.Does this study show that Ethyol decreases the toxicities caused by the CP regimen? If so, which toxicities are decreased? hospitalization for febrile granulocytopenia less than 500? hospitalization for febrile granulocytopenia less than 500 plus hospitalization for infection with either granulocytopenia less than 500 or fever? median granulocyte nadir? granulocyte count less than 1500 on day 25? serum creatinine higher than 1.5 on day 25? neurotoxicity? ototoxicity? other toxicity?

2.Based on complete response rates, do the results of this study provide sufficient assurance that Ethyol does not decrease the antitumor effect of the chemotherapy?

3.There are a number of issues regarding the interim analysis of this study. The protocol indicated the interim analysis would be done "halfway through". "Halfway through" is subject to a wide range of definitions. There are multiple endpoints for stopping the study without provisions for P value adjustment. There are two unplanned analyses since the planned interim analysis. Are these issuses sufficiently serious to have an important impact on interpretation of the study results?

4.The planned interim analysis was done when 97 of the planned 200 patients had been accrued. Presently 121 patients have been accrued. Accrual is temporarily on hold awaiting the advice of this Committee. Should this study be completed?

Study 201C with 21 patients compares Cytoxan in cycle #1 with Cytoxan plus Ethyol in cycle #2 in the same patient. In a separate study (201A) with 15 patients the treatments are administered in the reverse order.

5.Do these studies show that Ethyol decreases the toxicity of Cytoxan? If so, which toxicities are decreased? granulocytes less than 500? duration of granulocytes less than 500? other?

6. Do the design of these studies and the fact that the patients had several different tumor types permit an assessment of whether Ethyol decreases the antitumor effect of the Cytoxan?

An uncontrolled study WR-2721-B001: 201D evaluates cisplatin 120-150 mg/M2 with Ethyol in 48 patients with metastatic malignant melanoma.

7.Are cisplatinum toxicities decreased compared to historical experience with cisplatinum alone? If so, which toxicities are decreased? hematologic? renal? neurologic? other?

Randomized ECOG study #1686 compares cisplatinum 120 mg/M2 with cisplatinum 150 mg/M2 plus Ethyol in a total of 94 patients with metastatic malignant melanoma. This study was conducted to confirm the results of the above uncontrolled study. ECOG study #1686 was closed to accrual on July 15, 1991. Preliminary toxicity data are available, but not tumor response data.

Applicant has agreed as of two weeks ago to limit labeling and advertising claims for Ethyol to decreasing toxicity of cisplatinum at doses of 100 mg/M2 or less. However, there is only one study submitted with cisplatinum at this dose level.

8.Does the Committee agree that a decision on the indication for decreasing the toxicities of cisplatinum should await availability of the final results of ECOG study #1686?

General Questions

9.Is it essential to have data showing whether Ethyol affects the pharmacokinetics of cisplatin and cyclophosphamide?

10.Are additional studies needed to show that Ethyol decreases the neutropenia related toxicity of cyclophosphamide?

11.Are additional studies needed to show that Ethyol decreases the toxicities of cisplatinum?

12.In the randomized ovarian cancer study WR-2721-1 dexamethasone had to be added to the Ethyol plus CP regimen because Ethyol adds to the emetic effect of the CP. The dexamethasone was given intravenously prior to the Ethyol and at 4, 8 and 12 hours after the cisplatin. If this NDA is approved, should the package insert be specific in this regard? Oncologists may be inclined to substitute odansetron for the complicated antiemetic regimen used in the ovarian cancer study. What should the package insert say about this?

Pharm/
Tox

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 6

NDA No. 20221

Serial No(s)., AL **Date(s) of Submission:** Oct. 2[illegible] 1995

Information to be Conveyed to Sponsor: Yes (X), No ( )

Reviewer: Wendelyn J. Schmidt, Ph.D.

Date Review Completed: 12/7/95

Sponsor: USBioscience **Manufacturer (if different):**

Drug Name: Primary: ethyol Other Names: amifostine, WR2721, ethiofos

Related INDs/NDAs/DMFs: IND

Class: chemoprotectant

Indication: chemoprotective agent that selectively protects against the serious toxicities associated with intensive regimens of platinum and alkylating agent chemotherapy.

Route of Administration: i.v.

Studies Reviewed for this submission:
1. In vitro studies of the effect of WR1065 on the antitumor activity of standard anticancer drugs against human MCF-7 breast cancer and human A2780 Ovarian cancer
2)Effect of ethyol and paclitaxel on survival of SCID mice bearing human ovarian cancer Xenografts:
3) Investigation of cisplatin cytotoxic activity in sensitive and resistant human embryonic carcinoma cell lines, in the presence and absence of amifostine
4) New Labeling

Pharmacology
1) In vitro studies of the effect of WR1065 on the antitumor activity of standard anticancer drugs against human MCF-7 breast cancer and human A2780 Ovarian cancer

The study was performed by
Cell cultures of either MCF-7 breast or A2780 ovarian cells were exposed to a single dose of WR1065 for 15 minutes at the concentrations shown below. After rinsing, cells were exposed continuously for 3 or 7 days to multiple doses of antineoplastics to allow calculation of an IC50. Assays were performed in sixtuplicate with triplicate replications. An approximate Cmax with 910 mg/m2 ethyol in humans is 230 uM which calculates to roughly 50 ug/ml. Cytotoxicity was assessed with a 96 well colorimetric assay.

Conc WR1065	3 day	7 day
A2780	0.5 ug/mL	0.05 ug/mL
MCF-7	50 ug/mL	5 ug/mL

The data is presented in the tables below. As shown, it is unclear whether the cells were incubated for 3 or 7 days. No statistical difference was noted between +/- WR1065. However, maximum difference in MCF-7 was 80%

increase with WR1065 with melphalan. The maximal decrease in IC50 in the MCF7 line with WR1065 was 88% with cisplatin. With the A2780 cells, similar results were noted.

Problems:

lack of dose response with ethyol (single dose)
lack of explanation for different doses between cell lines
what was the degree of reproducibility between replications
is a 15 minute exposure to WR1065 adequate
no protection of normal cells for comparison

Positive:

the concentration of ethyol used in the MCF-7 3 day experiment correlated roughly with the Cmax in humans with 910 mg/m2.
Used the dephosphorylated WR1065

***In Vitro* Growth Inhibitory Effects of Standard Anticancer Drugs With and Without WR-1065 (Pre-Exposure) Against Human [redacted] reast Cancer Cells**

Anticancer Drugs	I.C.50 (M) (-) WR-1065	I.C.50 (M) (+) WR-1065	P-value
ARA-C	1.39 x 10-7	0.71 x 10-7	0.083
Bleomycin	3.43 x 10-6	1.59 x 10-6	0.33
Carboplatin	9.04 x 10-2	9.52 x 10-2	0.97
Cisplatinum	8.91 x 10-3	1.07 x 10-3	0.36
Daunorubicin	7.5 x 10-7	8.64 x 10-7	0.74
Doxorubicin	1.05 x 10-6	0.47 x 10-6	0.33
5-FU	1.54 x 10-6	1.08 x 10-6	0.51
Idarubicin	1.81 x 10-7	1.8 x 10-7	0.995
Melphalan	0.96 x 10-3	1.73 x 10-3	0.78
Mitomycin-C	1.55 x 10-5	1.42 x 10-5	0.94
Mitoxantrone	0.78 x 10-6	1.16 x 10-6	0.37
Taxol	1.15 x 10-9	1.00 x 10-9	0.73
Taxotere	1.04 x 10-9	0.97 x 10-9	0.74
Vinblastine	1.19 x 10-9	0.74 x 10-9	0.36
Vincristine	1.24 x 10-9	0.81 x 10-9	0.69
VP-16	6.39 x 10-7	6.69 x 10-7	0.86

***In Vitro* Growth Inhibitory Effects of Standard Anticancer Drugs With and Without WR-1065 (Pre-Exposure) Against Human [redacted] Ovarian Cancer Cells**

Anticancer Drugs	I.C.50 (M6) (-) WR-1065	I.C.50 (M) (+) WR-1065	P-value
ARA-C	0.91 x 10-8	1.4 x 10-8	0.19
Bleomycin	3.4 x 10-9	5.98 x 10-9	0.16
Carboplatin	1.31 x 10-4	1.12 x 10-4	0.84
Cisplatinum	1.1 x 10-5	3.2 x 10-5	0.72
Daunorubicin	1.1 x 10-7	1.08 x 10-7	0.98
Doxorubicin	1.94 x 10-7	1.27 x 10-7	0.33
5-FU	1.17 x 10-6	1.18 x 10-6	0.995
Idarubicin	1.87 x 10-8	2.23 x 10-8	0.83
Melphalan	4.44 x 10-5	3.31 x 10-5	0.83
Mitomycin-C	1.42 x 10-6	0.75 x 10-6	0.48
Mitoxantrone	1.26 x 10-7	0.56 x 10-7	0.73
Taxol	5.81 x 10-9	7.91 x 10-9	0.89
Taxotere	4.17 x 10-9	3.68 x 10-9	0.86
Vinblastine	2.22 x 10-9	3.01 x 10-9	0.47
Vincristine	0.73 x 10-8	1.19 x 10-8	0.74
VP-16	2.93 x 10-7	1.65 x 10-7	0.56

2)Effect of ethyol and paclitaxel on survival of SCID mice bearing human ovarian cancer Xenografts:

The study was performed by

The Sponsor stated that pilot studies established dose of ethyol in SCID mice that did not cause splenomegaly and hypothermia (data not submitted). Two doses of ethyol, 100 and 200 mg/kg were administered ip 30 minutes prior to 27 mg/kg taxol on days 1, 3, 5, 7, and 9 to SCID mice (n=6) injected on day 0 with A2780/s cells. Tumor volume was monitored through day 66. Alternatively, WR2721/taxol treatment was begun when tumors were 0.2 X 0.2 cm on days 1, 3, 5, 7, and 9 (n=9).

The data on tumor size was not presented in the tumors treated on the first day. Survival time in these mice was increased with ethyol alone and in combination with drug as compared to the controls (see table below). In the treatment of established tumor, the tumor size and tumor-free survival rate at 90 days were reported. In the taxol alone group, 57% (4/7) of the mice were tumor-free, while 33% and 55% of the mice at 100 and 200 mg/kg were tumor free. Thus, there was not a significant dose dependent effect on the tumor regrowth with ethyol in a model where the antineoplastic agent had appreciable activity.

Positive features:

multiple doses of ethyol
In vivo model using proposed schedule
Human tumor line
Monitored animals for 90 days with dosing thru d9 (adequate regrowth antitumor agent with appreciable activity in the model

Negative

how do doses correlate with effective doses for preventing toxicity (ie despite hypothermia/splenomegaly what happens? Why wasn't the tumor volume data presented for the newly established tumor?

3) Investigation of cisplatin cytotoxic activity in sensitive and resistant human embryonic carcinoma cell lines, in the presence and absence of amifostine. *Preliminary data*

The study was performed by

Cisplatin sensitive and resistant embryonal carcinoma cells (H12.1 and H12.1DDP) which express alkaline phosphatase were exposed to 150 uM WR2721 for 30 minutes or 2 hours, then cDDP at 0.01-100 uM for 3, 6, or 24 hours. Nude mice implanted with the H12.1 tumor were treated i.p. with 200 mg/kg/day amifostine 15 minutes prior to 3 mg/kg/day cisplatin i.p. on days 1-5.

The 200 mg/kg dose of WR2721 dropped body temperature of mice by 1-2° C approximately 2 hours after administration. Tumor volume did not differ significantly between treated and controls over days 1-30.

Positive features: in vivo experiment coupled with in vitro data

Negative features: single dose level of WR2721. Insufficient data. Not clear if WR2721 or WR1065 was used in culture systems.

Summary and Evaluation of Preclinical Pharmacology:

The in vitro studies investigating protection from the actions of antineoplastic agents are summarized below. With the exception of the A2780/MCF-7 studies reviewed above, all of the drug concentrations used were well above clinically relevant levels. Only two of the summarized experiments studied the dose-response effects of WR2721 on antineoplastic activity. The MCF-7/A2780 experiment is problematic in the duration of exposure to WR1065, the actual length of exposure of cells to antineoplastics was not clearly specified, and although the changes in IC50 were not statistically significant as analyzed, there were increases in IC50 (protection) of up to almost 90% above the "without" WR1065 cultures.

In the overall context of the tumor protection data, there is still

evidence of tumor protection in some animal models. This data is discussed in previous reviews.

Thus, the labeling suggested in the previous review should remain.

Cell Lines	WR2721/WR1065	Conc.	Antineoplastic
Morris hepatoma FAO/FTO	WR2721	mg/ml	4-HC
Human ovarian A2780, A2780CP, OVCAR-3, OVCAR-10	WR1065	mg/ml	cDDP
Human ovarian BG-1	WR2721, WR1065	mg/ml	cDDP, HN2, araC
Human melanoma SK-MEL	WR2721	mg/ml	MitoC, DOX, carboplat, VP-16, 4-HC, cDDP
Human ovarian PA-1	WR2721	mg/ml	MitoC, DOX, carboplat, VP-16, 4-HC, cDDP
Fibrosarcoma HT1080	WR1065	mM	radiation
Human ovarian A2780	WR1065	ug/ml	All types
Human breast MCF-7	WR1065	ug/ml	All types

Labeling review:

Clinical Pharmacology:

In the second paragraph, the sentence "As a result of protection against cisplatin-induced nephrotoxicity, mice were able to tolerate larger doses of cisplatin which resulted in improved antitumor efficacy against human ovarian carcinoma implanted into nude mice." should be omitted.

Warnings: The sentence "Some animal data suggest interference is possible, although in most tumor models the effects of chemotherapy are not altered by amifostine." should remain in the label.

Carcinogenesis, Mutagenesis and impairment of fertility:

The sponsor argues that the sentence "The free thiol metabolite, however, was positive in the Ames test with S9 microsomal fraction in the TA1535 *Salmonella typhimurium* strain and at the TK locus in the mouse L5178Y cell assay." be deleted as the experiments were not performed with catalase in the medium. They noted that thiols *in vitro* mediate production of peroxides and superoxides, free radicals and oxygen depletion of the medium in the absence of catalases. Other experiments by Grdina with catalase show no mutagenesis at the HPRT locus in the CHO line and TK locus in human lymphoblastoid line TK6 at concentrations up to 4 mM.

From the literature (Leff et al., J.Lab Clin Med. 1991, 118(4):352-358 and Am. J. Physiol 1995 268(5 pt1):L809-817), rat plasma has between 10 and 20 IU/ml catalase. In the Grdina experiments which USBioscience discusses, it is unclear how much catalase was present with respect to activity, but it was likely between 200 and 3000 IU/mL , depending on which catalase preparation was bought from Sigma. Human plasma catalase levels were unavailable. Thus, it is unclear that the catalase model tested is relevant to the human situation.

In conclusion, the sentence on mutagenicity should be included in the label. This is confirmed in an "expert report" from Anthony Dayan included in vol 22.4 which agreed that WR2721 has a "weak and not entirely reproducible effect...which was manifested in the TA1535...and which occurred at high doses in vitro."

Wendelyn J. Schmidt, Ph.D.
Pharmacologist/Toxicologist

c.c. Original IND/NDA/DMF
/Division File
/JDeGeorge, Supervising Pharmacologist
/Medical Officer
/C.S.O.
/WSchmidt, Reviewer

12/7/95

JAN 4 1995

~~IND #~~

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 5

NDA No. 20221

Serial No(s)., BZ **Date(s) of Submission:** Aug. 30, 1993

Information to be Conveyed to Sponsor: Yes ( ), No (X)

Reviewer: Wendelyn J. Schmidt, Ph.D.

Date Review Completed: 1/3/95

Sponsor: USBioscience **Manufacturer (if different):**

Drug Name: Primary: ethyol Other Names: amifostine, WR2721, ethiofos

Related INDs/NDAs/DMFs: IND

Class: chemoprotectant

Indication: chemoprotective agent that selectively protects against the serious toxicities associated with intensive regimens of platinum and alkylating agent chemotherapy.

Route of Administration: i v.

Overall Summary and Evaluation

The submission contains the sponsor's evaluation of the data pertaining to tumor protection. The sponsor's interpretation does not adequately reflect all of the data available. The absolute test of tumor protection, particularly micro-metastases, will be obtained from clinical experience due to the inadequacy of some of the models to reflect the human situation (normothermia, prevalence of metastases, size of tumor, blood supply to tumor, drug delivery to tumor). No additional animal studies are requested at this time on this issue.

Wendelyn J. Schmidt, Ph.D.
Pharmacologist/Toxicologist

c.c. Original NDA
/Division File
/Joseph DeGeorge
/C.S.O.
/WSchmidt

1/4/95

NDA 20221-WR2721-ethyol

JUL 18 1995

Subject: labelling for Ethyol

To: Division file
J. DeGeorge
R. DeLap

From: W. Schmidt

Date: 7/18/95

Comments on labelling from the 1/31/95 submission (not annotated):
1. CLINICAL PHARMACOLOGY Section
4th sentence currently reads

> In addition, cell culture studies have shown that amifostine uptake by normal tissues occurs through facilitated diffusion whereas potential entry into tumors is via a slower passive diffusion.

should read:

> In addition, a cell culture study has shown that amifostine uptake by normal tissues may occur through facilitated diffusion....

The original experiment suggesting facilitated uptake of WR2721 by normal tissues was performed by Yuhas using liver cells, a source of alkaline phosphatase, while other studies by Millar et al (Am J. Clin. Oncol. 5:321, 1982, included in original NDA submission) suggested no difference in rate and extent of WR2721 uptake by marrow and Lewis lung cells.

2. CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY
Currently reads:

> No long term animal studies have been performed to evaluate the carcinogenic potential of ETHYOL. The Ames *Salmonella typhimurium* test revealed no mutagenic activity. Data from both *in vitro* and *in vivo* studies demonstrate that ETHYOL protects against the mutagenicity and genotoxicity of chemotherapeutic agents such as cisplatin bleomycin and nitrogen mustard and against the carcinogenicity associated with radiation therapy.

Should read:

> No long term animal studies have been performed to evaluate the carcinogenic potential of ETHYOL. Ethyol was negative in the Ames test and in the mouse micronucleus test. The dephosphorylated metabolite, WR1065, was positive for mutagenicity in the Ames test with S9 microsomal fraction in the TA1535 *Salmonella typhimurium* strain and at the TK locus assay in mouse L5178Y cells. WR1065 was negative in the mouse micronucleus test and negative for clastogenicity in human lymphocytes.

The human/CHO mutagenicity studies at the TK and HPRT locus by Grdina et al., were not included as no information was available on the cloning efficiency etc. for the doses used (up to 4 mM, which the author stated were relevant to achievable plasma levels).

3. PREGNANCY
Currently reads:

> Pregnancy Category C. While ETHYOL has been shown to have dose-related embryotoxicity in rats at doses greater than 200 mg/kg, it is not teratogenic. There are no adequate and well-controlled studies in

pregnant women. If this drug is used during pregnancy or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Should read:

Pregnancy Category C. ETHYOL has been shown to be embryotoxic in rabbits at doses of 50 mg/kg, approximately sixty percent of the recommended dose in humans on body surface area basis. There are no adequate and well-controlled studies in pregnant women. ETHYOL should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

As a post-marketing agreement, the sponsor should repeat the rat teratogenicity assay with a dose which shows maternotoxicity (>60 mg/kg, but less than 200 mg/kg).

Note: in the OVERDOSAGE section, the maximum single dose was misprinted as 130 mg/m2 when it should be 1300 mg/m2.

Notes from discussion with MO:
Chemoprotection Issue
1) Although most tumor cell lines take up WR2721/WR1065 to a lesser extent than in plasma/blood, there are a few lines (e.g. Morris hepatoma, RIF-1) which do concentrate WR2721.

2) There does not appear to be much evidence for large tumor protection with WR2721. However, a body of evidence exists on the protection of small, well-oxygenated tumors (or micrometastases) from the effects of chemotherapy in conjunction with WR2721.

Wendelyn J. Schmidt, Ph.D.
Pharmacologist/Toxicologist

c.c. Original NDA
/Division File
/Joseph DeGeorge
/Medical Officer
/C.S.O.
/W. Schmidt

7/18/95

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review # 4

IND 31157, NDA 20221

Serial No(s):		**Letter date:**
IND 31157,	#162	4/22/93
	#173	4/18/94
NDA 20221,	AM	7/12/94

Information to be relayed to Sponsor: Yes (X)/ No ( )

Reviewer: Wendelyn J. Schmidt, Ph.D.

Date Review Completed: 12/19/94

Sponsor: US Bioscience

Drug manufacturer: US Bioscience

Drug Name: Primary: ethyol, amifostine, WR2721, ethiofos

Chemical Name: ethanethiol, 2-[(3-aminopropyl)amino] dihydrogen phosphate

Molecular weight (Formula weight): 214.22 g/mol

Related IND/NDA's or DMF's: NDA 20221

Class: chemoprotectant

Indication: "cytoprotective agent against both the acute and cumulative hematologic and renal toxicities associated with alkylating agents such as cyclophosphamide and platinum agents such as cisplatin in patients with ovarian cancer.

Dosage Forms and Route of Administration: i.v.

Previous Review(s), Date(s), and Reviewer(s): original: A.W. Coulter, Original NDA and amendments 1-3, W. Schmidt

Studies Reviewed:

I. Pharmacodynamics:
   1. RCC Group, Project 359065- General Pharmacology of WR2721: effect on isolated ileum of guinea pigs. NDA 20221, AM 7/12/94
   2. RCC group, Project 359076- general pharmacology of WR2721: effect on isolated uterus of female rats. NDA 20221, AM 7/12/94
   3. Rcc groups, project 359087-general pharmacology of WR2721: effects on isolated aorta of the rabbit. NDA 20221, AM 7/12/94
   4. RCC group, project 359100-general pharmacology of WR2721: effects on inferior eyelid contractions, cardiovascular and respiratory systems of the rat. NDA 20221, AM 7/12/94

II. Reproductive Toxicology:
   1. 621-001 Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ethyol administered intravenously to CRL:CDBR VAF/plus presumed pregnant rats. IND
      Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ethyol administered intravenously to New Zealand white rabbits. IND

III. Genotoxicology
   1. Study to determine the ability of WR1065 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan

requiring strains of Escherichia Coli. (Report 1185/4) IND
2. Study to determine the ability of WR2721 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan requiring strains of Escherichia Coli. (Report 1185/5) IND
3. Study to evaluate the chromosome damaging potential of WR1065 by its effects on cultured human peripheral blood lymphocytes using an in vitro cytogenetics assay (report # 1185/2) IND
4. Study to evaluate the potential of WR2721 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (report #1185/3) IND

5. Study to evaluate the potential of WR1065 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (report # 1185/7) IND

Study to determine the ability of WR1065 to induce mutations at the thymidine kinase (tk) locus in mouse lymphoma L5178Y cells using a fluctuation assay (report # 1185/1) IND
Amifostine report to US Bioscience by Dr. David J. Grdina. Human lymphoblastoid tk test and CHO cell hprt test of WR2721 and WR1065. IND

Studies Not Reviewed: none

Note - Portions of this review were excerpted directly from the sponsor's submission.

I. Pharmacodynamics:

1. RCC Group, Project 359065- General Pharmacology of WR2721: effect on isolated ileum of guinea pigs. NDA 20221, AM 7/12/94

The study was performed according to Swiss and French GLP at RCC research and Consulting Company Ltd., Itingen, Switzerland in 1993, but no signatures accompanied the data. The isolated ileum from 2 male guinea pigs was used to investigate the effects of WR2721 (batch # R104-EH) on contractility at concentrations between mg/ml. Positive controls were histamine and acetylcholine ug/ml).

WR2721 alone at concentrations up to mg/ml had no effect on the contractility of guinea pig ileum; the presence of WR2721 may have increased the response to histamine of the guinea pig ileum.

2. RCC group, Project 359076- general pharmacology of WR2721: effect on isolated uterus of female rats. NDA 20221, AM 7/12/94

The study was performed according to Swiss and French GLP at RCC research and Consulting Company Ltd., Itingen, Switzerland in 1993, but no signatures accompanied the data. The uterus horns from 4 female HAN Wistar rats, age 18 weeks were dissected and hung in organ baths; WR2721 (batch # R104-EH) was added in ascending concentrations between mg/ml alone and in the presence of positive agonists. Oxytocin mU/ml) and serotonin ug/ml) were used as standard agonists.

WR 2721 alone had no effect on the contractility of isolated rat uterus. WR2721 had no effect on oxytocin induce contractions, but increased contractility of rat uterus to serotonin at concentrations above 1.0 mg/ml.

3. RCC groups, project 359087-general pharmacology of WR2721: effects on isolated aorta of the rabbit. NDA 20221, AM 7/12/94

The study was performed according to Swiss and French GLP at RCC research and Consulting Company Ltd., Itingen, Switzerland in 1993, but no signatures accompanied the data. The aorta was isolated from 2 male New Zealand white rabbits, hung in an organ bath, and exposed to increasing concentrations of WR2721 (batch # R-104-EH) at concentrations between mg/ml alone and in the presence of adrenaline (positive agonist, concentrations from ug/ml).

WR2721 did not affect the contractility of aorta either alone or in the presence of adrenaline.

4. RCC group, project 359100-general pharmacology of WR2721: effects on inferior eyelid contractions, cardiovascular and respiratory systems of the rat. NDA 20221, AM 7/12/94

The study was performed according to Swiss and French GLP at but no signatures accompanied the data. Six male HAN Wistar rats were intubated and connected to a pneumotachograph (respiratory parameters including respiratory rate, tidal volume and minute volume, cannulated in the left carotid artery (blood pressure, heart rate), the right inferior eyelid was fixed and connected to a force displacement transducer, and the right cervical sympathetic nerve was attached to an electrode. WR2721 (batch r-104-EH(A)) was administered i.v. via another catheter in the left femoral vein at concentrations of 0, 3, 10, 30, or 100 mg/kg. Adrenaline and McNeal A-343 were used to attempt to separate direct stimulation of the muscle from receptor mediated stimulation.

WR2721 reduced the amplitude of electrically stimulated eyelid contraction dose dependently to a maximum of 90% at 100 mg/kg 28 minutes after drug administration. Recovery occurred by 2-4 hours later. WR2721 in combination with adrenaline did not significantly alter contractions, while contractions induced by McN-343 were increased with 30 and 100 mg/kg WR2721.

Systolic and diastolic blood pressure were reduced by approximately 10% at 10 mg/kg WR2721 but resolved within 30 minutes. Thirty mg/kg WR2721 resulted in a 12-18/20-36% drop in diastolic/systolic blood pressure, while 100 mg/kg reduced pressure by 33%/46%. Heart rate was also significantly decreased (17%) at 100 mg/kg. Only transient changes in respiratory parameters were noted.

Summary and Evaluation of Preclinical Pharmacodynamics:

WR2721 alone had no effect on the contractility of guinea pig ileum, rat uterus or rabbit aorta. However, there was a suggestion that WR2721 may increase the response to serotonin or histamine in these organs. WR2721 alone decreased the amplitude of electrically stimulated eyelid contractions, and increased the stimulation with a post-ganglionic stimulatory agent McN-343. Blood pressure was decreased in the rat with doses above 30 mg/kg (180 mg/m2) although heart rate was not affected significantly until 100 mg/kg (600 mg/m2).

The sponsor has not submitted a recent literature search to the NDA.

II. Reproductive Toxicology:

1. 621-001 Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ethyol administered intravenously to CRL:CDBR VAF/plus presumed pregnant rats. IND

The study was performed according to GLP at Twenty-five presumed pregnant Crl:CDBR/VAF/plus (Sprague Dawley) female rats were administered i.v. ethyol (lot #1905) in sterile saline daily on days 6-15 of pregnancy at 0, 10, 30 or 60 mg/kg/day (maximum volume 1.2 ml/kg; 0, 60, 180, or 360 mg/m2). Rats were sacrificed on day 20.

Measurements and Observations:

Twice daily: mortality and clinical signs.

Daily: body weight (after day 6)

Day 20: Maternal: gross necropsy, corpora lutea, implantation sites, early and later resorptions.

Fetal: # live and dead, weight, sex, gross external anomalies, half of each litter for either skeletal or soft tissue anomalies.

Note: dead and late resorptions were not counted in statistical analysis.

Maternal observations:

All dams survived to scheduled sacrifice. There were no significant clinical observations and no observations at gross necropsy.

Maternal body weight and food consumption:

While there were statistically significant differences in body weight at day 15, the difference between the treated and controls was less than 10%. Body weight gain was decreased by 68% as compared to control in the 60 mg/kg group between days 6-9. Food consumption was decreased by approximately 10% in the 60 mg/kg (HD) group as compared to controls between days 8 and 16, which was not reflected in the maternal weight.

Litter data:

The number of pregnant rats did not differ significantly in any of the treated groups. Likewise, the number of corpora lutea, implantations, litter sizes and live/dead fetuses did not differ significantly with treatment. The percentage of males/litter and the body weights of the fetuses did not differ significantly between treated and controls.

There were no dose-dependent increases in anomalies. While there was 1 HD fetus with a threadlike tail, other anomalies noted mostly consisted of incomplete ossification of ribs and was not dose-dependent.

PROTOCOL 621-001: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF ETHYOL® ADMINISTERED INTRAVENOUSLY TO Crl:CD®BR VAF/Plus® PRESUMED PREGNANT RATS

TABLE 6 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 10	III 30	IV 60
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	24(96.0)	23(92.0)	25(100.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 20 OF GESTATION	N	24	24	23	25
CORPORA LUTEA	MEAN±S.D.	16.7 ± 2.0	16.4 ± 2.5	16.9 ± 3.1	16.3 ± 2.6
IMPLANTATIONS	MEAN±S.D.	16.4 ± 1.7	16.6 ± 1.9	16.1 ± 1.8	16.1 ± 1.5
LITTER SIZES	MEAN±S.D.	16.0 ± 1.6	15.5 ± 2.3	15.3 ± 2.0	15.3 ± 1.6
LIVE FETUSES	N MEAN±S.D.	383 16.0 ± 1.6	373 15.5 ± 2.3	353 15.3 ± 2.0	382 15.3 ± 1.6
DEAD FETUSES	N MEAN±S.D.	1 0.0 ± 0.2	0 0.0 ± 0.0	0 0.0 ± 0.0	0 0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.5 ± 0.6	1.0 ± 1.1	0.7 ± 1.1	0.8 ± 1.1
EARLY RESORPTIONS	N MEAN±S.D.	13 0.5 ± 0.6	25 1.0 ± 1.1	17 0.7 ± 1.1	20 0.8 ± 1.1
LATE RESORPTIONS	N MEAN±S.D.	0 0.0 ± 0.0	0 0.0 ± 0.0	0 0.0 ± 0.0	1 0.0 ± 0.2
DAMS WITH ANY RESORPTIONS	N(%)	11(45.8)	15(62.5)	10(43.5)	12(48.0)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	24(100.0)	23(100.0)	25(100.0)
LIVE MALE FETUSES	N	199	166	176	195
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	51.9 ± 11.1	45.4 ± 14.0	49.7 ± 15.5	51.8 ± 14.3
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	3.62 ± 0.17	3.70 ± 0.19	3.62 ± 0.25	3.64 ± 0.24
MALE FETUSES	MEAN±S.D.	3.72 ± 0.17	3.81 ± 0.19	3.73 ± 0.26	3.76 ± 0.25
FEMALE FETUSES	MEAN±S.D.	3.52 ± 0.16	3.62 ± 0.28	3.51 ± 0.23	3.51 ± 0.23
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.4 ± 3.9	6.4 ± 6.7	6.5 ± 6.5	5.1 ± 6.4

a. Dosage occurred on days 6 through 15 of gestation.

PROTOCOL 621-001: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF STATOL® ADMINISTERED INTRAVENOUSLY TO Crl:CD®BR VAF/Plus® PRESUMED PREGNANT RATS

TABLE 11 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	60
Litters Evaluated	N	24	24	23	25
Fetuses Evaluated	N	199	192	183	197
Live Fetuses	N	198	192	183	197
Dead Fetuses	N	1[b]	0	0	0
STERNEBRAE (CONTINUED):					
Not Ossified					
Litter Incidence	N(%)	0	1(4.2)	1(4.3)	0
Fetal Incidence	N(%)	0	1(0.5)[a]	1(0.5)	0
PELVIS SUMMARIZATION (Includes incompletely ossified pubes and ischia and unossified pubes):					
Litter Incidence	N(%)	3(12.5)	4(16.7)	6(26.1)	2(8.0)
Fetal Incidence	N(%)	5(2.5)	6(3.1)	7(3.8)	2(1.0)
PELVIS:					
Pubes, Incompletely Ossified					
Litter Incidence	N(%)	2(8.3)	1(4.2)	6(26.1)	2(8.0)
Fetal Incidence	N(%)	4(2.0)c,d	3(1.6)i,j,k	7(3.8)m,o	2(1.0)
Pubes, Not Ossified					
Litter Incidence	N(%)	1(4.2)	2(8.3)	0	0
Fetal Incidence	N(%)	1(0.5)	2(1.0)k	0	0
Ischia, Incompletely Ossified					
Litter Incidence	N(%)	1(4.2)	2(8.3)	1(4.3)	0
Fetal Incidence	N(%)	1(0.5)d	4(2.1)e,i,j,k	2(1.1)m,o	0

a. Dosage occurred on days 6 through 15 of gestation.
b. The dead fetus (4476-5) was excluded from all statistical analyses. Observations for this fetus are cited on Table 20.
c. Fetus 4477-3 also had other skeletal alterations.
d. Fetus 4477-11 also had other skeletal alterations.
e. Fetus 4490-1 also had other skeletal alterations.
f. Fetus 4490-7 also had other skeletal alterations.
g. Fetus 4490-15 also had other skeletal alterations.
h. Fetus 4491-1 also had other skeletal alterations.
i. Fetus 4492-3 also had other skeletal alterations.
j. Fetus 4492-5 also had other skeletal alterations.
k. Fetus 4492-13 also had other skeletal alterations.
l. Fetus 4525-5 also had other skeletal alterations.
m. Fetus 4525-7 also had other skeletal alterations.
n. Fetus 4525-9 also had other skeletal alterations.
o. Fetus 4525-15 also had other skeletal alterations.

PROTOCOL 621-001: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF STATOL® ADMINISTERED INTRAVENOUSLY TO Crl:CD®BR VAF/Plus® PRESUMED PREGNANT RATS

TABLE 11 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0(VEHICLE)	10	30	60
Litters Evaluated	N	24	24	23	25
Fetuses Evaluated	N	199	192	183	197
Live Fetuses	N	198	192	183	197
Dead Fetuses	N	1[b]	0	0	0
VERTEBRAE:					
Thoracic, Centrum, Bifid					
Litter Incidence	N(%)	0	1(4.2)	0	2(8.0)
Fetal Incidence	N(%)	0	1(0.5)	0	2(1.0)
Lumbar, Arch, Incompletely Ossified					
Litter Incidence	N(%)	0	1(4.2)	1(4.3)	0
Fetal Incidence	N(%)	0	1(0.5)[e]	1(0.5)	0
RIBS:					
Incompletely Ossified (Hypoplastic)					
Litter Incidence	N(%)	0	1(4.2)	3(4.3)	1(4.0)
Fetal Incidence	N(%)	0	3(1.6)e,f,g	3(1.6)l,m,n	1(0.5)
Wavy					
Litter Incidence	N(%)	1(4.2)	1(4.2)	1(4.3)	0
Fetal Incidence	N(%)	1(0.5)	3(1.6)e,f,g	5(2.7)l,m,n,o	0
STERNEBRAE SUMMARIZATION (Includes incompletely or unossified sternebrae):					
Litter Incidence	N(%)	2(8.3)	4(16.7)	1(4.3)	0
Fetal Incidence	N(%)	2(1.0)	4(2.1)	1(0.5)	0
STERNEBRAE:					
Incompletely Ossified					
Litter Incidence	N(%)	2(8.3)	3(12.5)	0	0
Fetal Incidence	N(%)	2(1.0)[c]	3(1.6)[k]	0	0

NOTE: the dose ranging study for the rat was included as an appendix
Dose-ranging study of WR2721 in the rat.

Eight presumed pregnant rats/dose were administered i.v. ethyol at 0, 12.5, 25, 50, 100 or 200 mg/kg/day on days 6-15 of pregnancy. All rats were sacrificed on day 20 of pregnancy.

Observations and measurements:

Daily: mortality, clinical signs, abortions, premature delivery, body weight (days 6-20).

Day 20: gross necropsy, corpora lutea, implantations, live/dead fetuses, resorptions, sex of fetuses, and gross external anomalies.

Maternal observations:

On days 11 and 12, 7/8 rats at 200 mg/kg/day were found dead (6/7 were pregnant). Clinical signs in these rats included ptosis, decreased motor activity, chromodacryorrhea, chromorhinorrhea, urine stained fur, and bradypnea were observed after 3-7 doses and persisted for 1-3 days. A black substance (presumed blood) was found in the stomachs at necropsy. Each of these dams had 16-18 fetuses which appeared to be of normal development for their gestational age. At 100 mg/kg/day, clinical observations included decreased motor activity, ptosis, chromorhinorrhea and urine stained fur. No other dose showed abnormal signs. At necropsy, isolated findings included enlarged spleen with yellow adherent areas in 1 50 mg/kg rat, and slight dilation of the pelvis of each kidney in 1 100 mg/kg rat.

Maternal body weight was decreased by 12% beginning on day 8 of pregnancy in the 200 mg/kg/day group. Body weight as decreased by >10% in the 100 mg/kg/day group on day 10 of gestation. No other dose group was affected to a biologically significant level. Changes in body weight were paralleled by food consumption.

Litter parameters:

There were no significant differences in the number of corpora lutea, or implantation sites in the treated rats. All fetuses were resorbed in the sole surviving 200 mg/kg dam. There were no statistically significant differences in the number of early resorptions between treated and control rats; no late resorptions or dead fetuses were observed. Although there was a trend towards decreased litter size; no statistical significance was reached at the 100 mg/kg dose. No change in the male/female ratio was noted. Fetal body weight was decreased by 14% at the 100 mg/kg dose only. No gross external anomalies were reported.

PROTOCOL 621-001P: [illegible] ADMINISTERED INTRAVENOUSLY TO Crl:CD(TM)BR VAF/Plus(TM) RATS

TABLE 7 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 12.5	III 25	IV 50	V 100	VI 200
RATS TESTED	N	8	8	8	8	8	8
PREGNANT	N(%)	7(87.5)	8(100.0)	8(100.0)	8(100.0)	7(87.5)	7(87.5)
YOUNG DEAD	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(85.7)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 20 OF GESTATION	N	7	8	8	8	7	1
CORPORA LUTEA	MEAN±S.D.	18.0 ± 2.7	19.0 ± 0.3	17.4 ± 1.5	18.2 ± 2.4	18.1 ± 2.0	21.0 ± 0.0
IMPLANTATIONS	MEAN±S.D.	17.4 ± 2.1	17.4 ± 2.7	16.6 ± 1.4	16.4 ± 1.6	15.6 ± 1.1	20.0 ± 0.0
LITTER SIZES	MEAN±S.D.	16.3 ± 1.8	16.6 ± 2.1	15.4 ± 1.2	14.9 ± 1.4	14.1 ± 1.5	0.0 ± 0.0
LIVE FETUSES	N	114	133	123	119	99	0
	MEAN±S.D.	16.3 ± 1.8	16.6 ± 2.1	15.4 ± 1.2	14.9 ± 1.4	14.1 ± 1.5	0.0 ± 0.0
DEAD FETUSES	N	0	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	1.1 ± 1.2	0.8 ± 1.0	1.2 ± 0.8	1.5 ± 1.2	1.3 ± 1.8	20.0 ± 0.0
EARLY RESORPTIONS	N	8	6	10	12	9	20
	MEAN±S.D.	1.1 ± 1.2	0.8 ± 1.0	1.2 ± 0.8	1.5 ± 1.2	1.3 ± 1.8	20.0 ± 0.0
LATE RESORPTIONS	N	0	0	0	0	0	0
DAMS WITH ANY RESORPTIONS	N(%)	4(57.1)	4(50.0)	6(75.0)	6(75.0)	4(57.1)	1(100.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0	0	0	0	0	1(100.0)
DAMS WITH VIABLE FETUSES	N(%)	7(100.0)	8(100.0)	8(100.0)	8(100.0)	7(100.0)	0

a. Dosage occurred on days 6 through 15 of gestation.

2. 621-002: Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ethyol administered intravenously to New Zealand white rabbits. IND

The study was performed according to GLP at
Twenty New Zealand presumed pregnant white rabbits were administered i.v. ethyol in saline (batch 1905) at 0, 25, 50, or 75 mg/kg/day (max. volume 1.5 ml/kg) on days 6-18 of gestation. Rabbits were sacrificed on day 29 of gestation.

Measurements and Observations:

Daily: clinical observations, mortality, abortion, premature delivery, body weight (days 6-29).

Day 29: Maternal: gross necropsy, corpora lutea, implantation sites, early and later resorptions.

Fetal: # live and dead, weight, sex, gross external anomalies, skeletal and soft tissue anomalies.

Maternal Observations:

Eight of 20 does in the 75 mg/kg group were found dead; an additional two HD rabbits were sacrificed moribund. All of the early death does were pregnant. Deaths occurred on days 16 through 24 of gestation. Four of the 8 rabbits found dead had no clinical signs prior to death. In the other predecedents, observations included no/decreased feces, decreased motor activity, loss of righting reflex, labored breathing, muscle flaccidity, perioral brown substance, ataxia, and bradypnea. In the rabbits at HD which survived to scheduled sacrifice, only changes in feces and decreased motor activity were noted.

At the MD (50 mg/kg/day), the one rabbit delivered prematurely (day 29). Other observations included dried/soft liquid feces, and red substance found in cage pan. There were no abnormal observations in the LD rabbits.

All tissues appeared normal at necropsy with the exception of a parovarian cyst in 1 50 mg/kg rabbit and pale kidneys in 2/20 HD rabbits which died prior to schedule.

Maternal body weight:

At the end of the dosing period, there were no significant differences in body weight between the control and 25 or 50 mg/kg rabbits. The 75 mg/kg/day rabbits showed a 16% reduction in weight as compared to controls, which persisted through sacrifice. Food consumption was decreased by more than 60% as compared to controls in the 75 mg/kg group during the dosing period, while food consumption was decreased by 15-20% from that of controls in the 25 and 50 mg/kg rabbits.

Litter parameters:

The litter data from both the predecedents and those rabbits which survived to scheduled sacrifice are shown in the following tables. Of the 10 does in the HD group which survived to sacrifice, 2 were not pregnant, 6 had all fetuses resorbed and 2 dams had 7 and 6 viable fetuses respectively. No dead fetuses were present in any group. Resorptions were increased to a statistically significant extent at both 50 and 75 mg/kg. Fetal weight was decreased by 15% as compared to controls in the 75 mg/kg group only.

There were no litters in either the treated or control groups without some fetal alterations; although there were no statistically significant differences in the number of fetuses with alterations between treated and control rabbits. With the exception of 1 fetus at 50 mg/kg and 2 fetuses a t 75 mg/kg with medially rotated paw, 1 fetus at 75 mg/kg/ with left ectopic kidney and 1 fetus at 50 mg/kg with moderate dilatation of pelvis, there were minimal differences in treated and control fetuses. One fetus at 75 mg/kg also had hemivertebrae, unilateral ossification/bifid/asymmetric/misaligned vertebrae. The number of alterations were within the range of historical controls.

PROTOCOL 621-002: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF ETHYOL® ADMINISTERED INTRAVENOUSLY TO NEW ZEALAND WHITE RABBITS

TABLE 3 (PAGE 1): UTERINE CONTENTS AND LITTER DATA FOR INDIVIDUAL RABBITS THAT DELIVERED, WERE FOUND DEAD OR WERE MORIBUND SACRIFICED

DOSAGE GROUP / DOSAGE (MG/M2/DAY)a	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R.	L.	T.	IMPLANTATIONS R.	L.	T.	EMBRYOS OR FETUSES b R.	L.	D.	T.	RESORPTIONS c R.	L.	T.
I 0(VEHICLE)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II 25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III 50	30584	DELIVERED AND SACRIFICED ON DAY 29 OF GESTATION	4	2	6	2	2	4	0	0	2	2d	0	0	2(LR)
IV 75	20562	FOUND DEAD ON DAY 20 OF GESTATION	6	7	13	5	3	8	1	1	0	2	4	2	6
	20563	FOUND DEAD ON DAY 16 OF GESTATION	5	4	9	4	4	8	3	3	0	6	1	1	2
	20567	FOUND DEAD ON DAY 18 OF GESTATION	8	5	13	7	3	10	4	1	0	5	3	2	5
	20568	FOUND DEAD ON DAY 24 OF GESTATION	6	4	10	5	2	7	1	0	0	1	4	2	6
	20569	FOUND DEAD ON DAY 16 OF GESTATION	7	4	11	5	4	9	4	4	0	8	1	0	1
IV (CONT.) 75	20570	FOUND DEAD ON DAY 17 OF GESTATION	7	3	10	5	3	8	4	3	0	7	1	0	1
	20572	MORIBUND SACRIFICED ON DAY 18 OF GESTATION	5	5	10	5	5	10	0	0	0	0	5	5	10
	20575	MORIBUND SACRIFICED ON DAY 20 OF GESTATION	8	4	12	5	3	8	4	1	0	5	1	2	3
	20577	FOUND DEAD ON DAY 23 OF GESTATION	3	4	7	2	2	4	0	0	0	0	2	2	4
	20579	FOUND DEAD ON DAY 18 OF GESTATION	6	6	12	3	4	7	3	4	0	7	0	0	0

R = RIGHT L = LEFT T = TOTAL D = DELIVERED LR = LATE RESORPTION
a. Dosage occurred on days 6 through 18 of gestation.
b. Appeared normal for their developmental ages at the time maternal death occurred, unless noted otherwise.
c. Early resorptions unless noted otherwise.

PROTOCOL 621-002: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF ETHYOL® ADMINISTERED INTRAVENOUSLY TO NEW ZEALAND WHITE RABBITS

TABLE 6 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP / DOSAGE (MG/SQ/DAY)a		I 0(VEHICLE)	II 25	III 50	IV 75
RABBITS TESTED	#	20	20	20	20
PREGNANT	#(%)	17(85.0)	18(90.0)	20(100.0)	18(90.0)
FOUND DEAD	#(%)	0(0.0)	0(0.0)	0(0.0)	8(44.4)**
MORIBUND SACRIFICED	#(%)	0(0.0)	0(0.0)	0(0.0)	2(11.1)
DELIVERED PREMATURELY	#(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	#	17	18	19	8
CORPORA LUTEA	MEAN±S.D.	9.8 ± 1.6	9.6 ± 1.8	9.5 ± 2.4	8.8 ± 2.2
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.8	8.3 ± 1.6	7.8 ± 2.3	7.5 ± 3.0
LITTER SIZES	MEAN±S.D.	8.6 ± 1.6	8.1 ± 1.7	6.2 ± 3.1	1.6 ± 3.0**
LIVE FETUSES	#	136	146	117	13
	MEAN±S.D.	8.0 ± 1.8	8.1 ± 1.7	6.2 ± 3.1	1.6 ± 3.0**
DEAD FETUSES	#	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.8 ± 0.2	0.2 ± 0.4	1.7 ± 2.7**	5.9 ± 4.3**
EARLY RESORPTIONS	#	1	3	27	47
	MEAN±S.D.	0.8 ± 0.2	0.2 ± 0.4	1.4 ± 2.8	5.9 ± 4.3**
LATE RESORPTIONS	#	0	0	6	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.7	0.0 ± 0.0
DOES WITH ANY RESORPTIONS	#(%)	1(5.9)	3(16.7)	11(57.9)**	7(87.5)**
DOES WITH ALL CONCEPTUSES RESORBED	#(%)	0(0.0)	0(0.0)	2(10.5)	6(75.0)**
DOES WITH VIABLE FETUSES	#(%)	17(100.0)	18(100.0)	17(89.5)	2(25.0)**

a. Dosage occurred on days 6 through 18 of gestation.
** Significantly different from the vehicle control group value (P≤0.01).

PROTOCOL 621-602: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF ETHYOL® ADMINISTERED INTRAVENOUSLY TO NEW ZEALAND WHITE RABBITS

TABLE 10 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 25	III 50	IV 75
Litters Evaluated	N	17	18	17	2
Fetuses Evaluated	N	136	146	117	13
Live Fetuses	N	136	146	117	13
Dead Fetuses	N	0	0	0	0
Litters with Fetuses with any Alteration Observed	N(%)	17(100.0)	18(100.0)	14(82.4)	2(100.0)
Fetuses with any Alteration Observed	N(%)	52(38.2)	62(42.5)	36(30.8)	5(38.5)
% Fetuses with any Alteration/Litter	X̄±S.D.	38.03 ± 15.02	42.83 ± 22.63	31.45 ± 19.54	40.50 ± 37.85

a. Dosage occurred on days 6 through 18 of gestation.

PROTOCOL 621-602: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF ETHYOL® ADMINISTERED INTRAVENOUSLY TO NEW ZEALAND WHITE RABBITS

TABLE 9 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

% SAGE GROUP DOSAGE (MG/KG/DAY)a		I 0(VEHICLE)	II 25	III 50	IV 75
LITTERS WITH ONE OR MORE LIVE FETUSES	N	17	18	17	2
IMPLANTATIONS	MEAN±S.D.	8.0 ± 1.8	8.3 ± 1.6	8.8 ± 1.8	7.5 ± 0.7
LIVE FETUSES	N	136	146	117	13
	MEAN±S.D.	8.0 ± 1.8	8.1 ± 1.7	6.9 ± 2.3	6.5 ± 0.7
LIVE MALE FETUSES	N	91	73	66	4
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	59.5 ± 21.5	50.2 ± 18.1	57.4 ± 17.4	31.0 ± 3.3
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	40.98 ± 4.16	42.59 ± 4.48	44.98 ± 4.20*	34.84 ± 7.76
MALE FETUSES	MEAN±S.D.	41.02 ± 4.14	43.38 ± 4.29	44.60 ± 4.94*	37.23 ± 1.40
FEMALE FETUSES	MEAN±S.D.	40.99 ± 5.76	41.88 ± 5.19	43.59 ± 3.52 [16]b	33.54 ± 10.80
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	8.6 ± 2.7	2.3 ± 5.6	14.8 ± 30.6**	12.5 ± 17.7

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 18 of gestation.
b. Litter 78559 had no female fetuses.
* Significantly different from the vehicle control group value (P≤0.05).
** Significantly different from the vehicle control group value (P≤0.01).

Summary and Evaluation of Reproductive Toxicology:

While the rat study should have been performed at a higher dose, the rabbit study was adequate. In the rabbit study, a dose with less than 10% decrease in maternal weight and minimal maternal toxicity yielded an increase in the number of resorbed fetuses, both as complete litters resorbed and individual fetuses. Thus, ethyol can be classed as fetotoxic, but not necessarily teratogenic, as there were no significant increases in the number of anomalies above historical controls at doses where minimal maternal toxicity was noted. Further, even at doses with maternal toxicity and death, there were no elevations of anomalies above those noted in historical controls. It will be necessary to repeat the rat reproductive toxicity study including doses between 60 and 200 mg/kg/day.

III. Genotoxicology

1. Study to determine the ability of WR1065 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan requiring strains of Escherichia Coli. (Report 1185/4) IND

The study was performed according to the UK GLP requirements at

Salmonella typhimurium, strains TA98, TA100, TA1535, and TA1537, and Escherichia coli strains WP2 pKM101 and WP2 uvrA pKM101 were used to screen concentrations of up to 5000 ug/plate of WR1065 (batch # AP-X-61) with or without S9 microsomal fraction for revertants. Positive controls included 2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline 1-oxide, and 2-aminoanthracene.

Positive controls were increased over controls by 6-36 fold. There were no significant increases in revertant numbers without S9 fraction. With S9 activation, the number of revertants in the TA1535 strain were increased dose dependently to a maximum of 2.3 fold above controls at 5000 ug/plate.

2. Study to determine the ability of WR2721 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan requiring strains of Escherichia Coli. (Report 1185/5) IND

The study was performed according to the UK GLP requirements at

Salmonella typhimurium, strains TA98, TA100, TA1535, and TA1537, and Escherichia coli strains WP2 pKM101 and WP2 uvrA pKM101 were used to screen concentrations of up to 5000 ug/plate of WR2721 (Sipsy Lot 1) with or without S9 microsomal fraction for revertants. Positive controls included 2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline 1-oxide, and 2-aminoanthracene.

Positive controls were increased by 6-36 fold as compared to controls. In the TA1535 strain, revertants were increased by 1.9 and 1.8 fold at 5000 ug/plate without S9 in two experiments; with addition of S9, revertants were increased by 1.6 and 2.0 fold in two experiments.

3. Study to evaluate the chromosome damaging potential of WR1065 by its effects on cultured human peripheral blood lymphocytes using an in vitro cytogenetics assay (report # 1185/2) IND

The study was performed according to the UK GLP requirements at

Cultured human peripheral lymphocytes were exposed in duplicate to concentrations of WR1065 (batch AP-X-61) of up to 5000 ug/ml with (20 or 44 hours)or without S9 microsomal enzymes (3 hours incubation + 17 hours recovery or 3 + 41 hours). Cyclophosphamide and 4-nitroquinoline-1-oxide were positive controls.

The mean mitotic index was decreased with all doses of WR1065 without S9 by approximately one half. With S9 fraction, mitotic index decreased dose dependently to a maximum of one half at 5000 ug/ml. The number of cells with chromosomal aberrations were within the historical control range, although they were above the controls (5 cells maximum with drug, 0 with solvent control). The maximal number of cells with aberrations in the positive controls were 9. Similar results were obtained with addition of S9. Again, positive controls were between 2-3 fold elevated above the solvent controls.

Cells with structural aberrations: Experiment 1

20+0 hours treatment, - S-9 Test chemical: NR-1065
Donor sex: female

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	0	0		9.1
	B	100	0	0		8.1
	Totals	200	0	0		(8.6)
880.1	A	100	5	3		3.1
	B	100	2	3		4.3
	Totals	200	7	6	p ≤0.05	(3.7)
892.5	A	100	4	2		3.4
	B	100	5	4		4.4
	Totals	200	9	6	p ≤0.01	(3.9)
1373	A	100	4	0		4.3
	B	100	2	1		4.9
	Totals	200	6	1	NS	(4.6)
MQO, 2.5	A	25	[illegible]	[illegible]		
	B	25	[illegible]	[illegible]		
	Totals	50	16	13	p ≤0.001	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 6)

Cells with structural aberrations: Experiment 1

3+17 hours treatment, - S-9 Test chemical: NR-1065
Donor sex: female

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	4	3		6.5
	B	100	1	0		7.2
	Totals	200	5	3		(6.9)
2113	A	100	3	2		5.1
	B	100	2	1		6.1
	Totals	200	5	3	NS	(6.6)
2250	A	100	5	3		6.2
	B	100	3	2		6.6
	Totals	200	8	5	NS	(6.4)
5000	A	100	3	2		3.7
	B	100	2	1		3.8
	Totals	200	5	3	NS	(3.7)
CPA, 25	A	25	8	[illegible]		
	B	25	9	[illegible]		
	Totals	50	17	15	p ≤0.001	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 6)

Cells with structural aberrations: Experiment 1

20+0 hours treatment, - S-9 Test chemical: NR-1065
Donor sex: male

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	0	0		4.9
	B	100	0	0		5.9
	Totals	200	0	0		(5.4)
889.9	A	100	3	2		4.9
	B	100	3	2		5.4
	Totals	200	6	4	NS	(5.2)
1187	A	100	0	0		6.0
	B	100	3	0		3.6
	Totals	200	3	0	NS	(3.9)
1562	A	100	4	4		6.3
	B	94	3	2		6.4
	Totals	194	7	6	p ≤0.01	(6.3)
MQO, 3.5	A	25	8	8		
	B	25	9	9		
	Totals	50	14	[illegible]	p ≤0.001	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 6)

Cells with structural aberrations: Experiment 1

3+17 hours treatment, - S-9 Test chemical: NR-1065
Donor sex: male

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	0	0		4.5
	B	100	4	1		4.4
	Totals	200	4	1		(4.5)
2813	A	100	1	1		2.1
	B	100	2	2		2.0
	Totals	200	3	3	NS	(2.5)
3750	A	100	1	0		2.2
	B	100	4	1		2.3
	Totals	200	5	1	NS	(2.3)
5000	A	100	2	0		2.1
	B	100	3	2		1.1
	Totals	200	4	2	NS	(1.6)
CPA, 12.5	A	25	12	9		
	B	25	7	3		
	Totals	50	19	12	p ≤0.001	

§ Statistical significance (Appendix 5b)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 6)

4. Study to evaluate the potential of WR2721 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (report #1185/3) IND

The study was performed according to the UK GLP requirements at Cd-1 mice (10-15/sex/group were administered i.v. WR2721 (Sipsy batch #1, purity stated as approximately 79.61%, thus doses were adjusted to 100%). A range finding test established the LD50 at 730 mg/kg (no deaths at 633 mg/kg, 6/6 deaths at the next dose of 844 mg/kg). Thus, the doses tested were 0, 118.8, 237.5 and 475 mg/kg WR2721 and 40 mg/kg cyclophosphamide as positive control. Mice were sacrificed at 24 or 48 hours post-injection and the number of PCE's in the marrow counted.

The highest dose used was 65% of the calculated LD50. There were no deaths prior to scheduled sacrifice. There were no significant differences in body weight between treated and control mice at any time. There were no statistically significant increases in the number of micronucleated PCE in any of the WR2721 treated mice at 24 or 48 hours. Micronuclei were increased by 40 fold above controls with 40 mg/kg cyclophosphamide.

Test chemical, vehicle and positive control...

Data for WR-2721

Treatment group (mg/kg)	Kill time (hours)	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000)	
				per sex	per treatment group
Vehicle control	24	M	0.89	0.30	0.30
		F	0.93	0.30	
	48	M	1.03	0.50	0.64
		F	0.97	0.79	
118.8	24	M	0.86	0.49	0.55
		F	0.94	0.60	
	48	M	0.92	0.70	0.70
		F	0.97	0.69	
237.5	24	M	0.89	0.70	0.55
		F	0.93	0.40	
	48	M	0.91	0.60	0.45
		F	1.06	0.30	
475	24	M	0.81	0.50	0.52
		F	0.92	0.55	
	48	M	0.92	0.69	0.84
		F	1.03	0.99	
CPA. 40	24	M	1.02	11.64	12.33
		F	0.91	13.02	

5. Study to evaluate the potential of WR1065 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (report # 1185/7) IND

The study was performed according to the UK GLP requirements at Cd-1 mice (10-15/sex/group were administered i.v. WR1065 (batch # AP-X-61). In a dose ranging experiment (3 mice /sex at 11, 22, 44, 36, 89, 178 and 356 mg/kg, the LD50 was calculated at 56 mg/kg (1/6 deaths at 44.5, 6/6 @ 89 mg/kg). Thus, the doses chosen were 0, 8.75, 17.5 or 35 mg/kg WR1065 and 40 mg/kg cyclophosphamide as a positive control (10-15 mice/sex/dose). Mice were sacrificed at 24 or 48 hours following administration of drug.

Two females @ 35 mg/kg died immediately following dosing and were replaced. Body weight did not differ significantly at sacrifice between treated and control mice. There were no dose-dependent and statistically significant increases in the number of micronuclei in WR1065 treated mice. Micronuclei in the positive controls were increased by approximately 40 fold over controls.

Summary of group mean data for test chemical, vehicle and positive controls

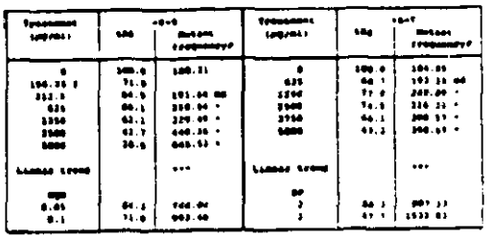
Data for WR-1065

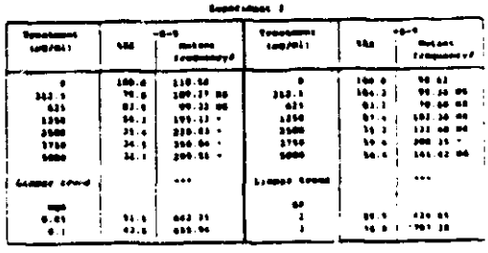
Treatment group (mg/kg)	Kill time (hours)	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000)	
				per sex	per treatment group
Vehicle control	24	M	1.26	0.50	0.45
		F	1.35	0.40	
	48	M	1.11	0.50	0.30
		F	1.26	0.10	
8.75	24	M	1.23	0.20	0.20
		F	1.05	0.20	
	48	M	1.35	0.50	0.60
		F	1.87	0.70	
17.5	24	M	1.46	1.10	0.85
		F	1.68	0.60	
	48	M	1.81	0.40	0.30
		F	1.32	0.00	
35	24	M	1.60	0.30	0.65
		F	1.40	0.80	
	48	M	1.11	0.20	0.30
		F	0.95	0.20	
CPA, 40	24	M	1.18	17.20	14.98
		F	1.11	12.67	

6. Study to determine the ability of WR1065 to induce mutations at the thymidine kinase (tk) locus in mouse lymphoma L5178Y cells using a fluctuation assay (report # 1185/1) IND

The study was performed according to the UK GLP requirements at Using mouse L5178Y lymphoma cells in the presence and absence of S9 metabolizing fraction, the mutagenic activity of WR1065 (batch AP-X-61) for 3 hours at concentrations between 0 and 5000 ug/plate were analyzed. Positive controls were 4-nitroquinoline-1-oxide and benzo(a)pyrene.

The survival and mutation frequencies in the presence and absence of S9 are summarized in the following tables. Neither pH nor osmolarity was affected significantly at doses other than 5000 ug/ml, where mutation was noted. The number of mutant colonies were doubled in the positive controls.





Raw plate count and % relative survival for WR-1065 in the cytotoxicity range-finder

Treatment ug/ml	In the absence of S-9		In the presence of S-9	
	Survival* at day 0**	% Relative survival	Survival* at day 0	% Relative survival*
0	14	100.0	18	100.0
156.25	18	143.7	22	140.7
312.5	18	143.7	16	83.8
625	15	109.9	17	91.7
1250	13	90.6	20	116.6
2500	5	29.5	18	100.0
5000	2	11.2	11	81.0

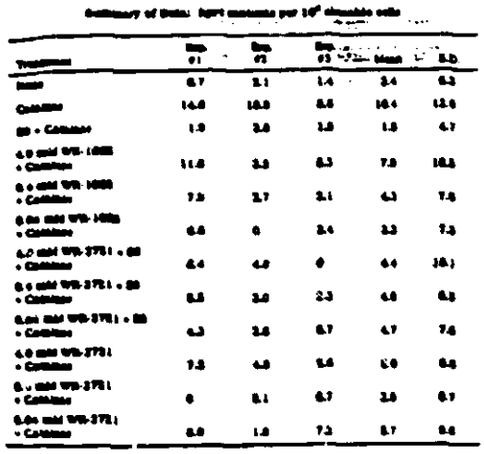
* 1.6 cells/well plated

** 32 wells scored

7. Amifostine report to US Bioscience by Dr. David J. Grdina. Human lymphoblastoid TK test and CHO cell HPRT test of WR2721 and WR1065. IND

No GLP statement accompanied the data, which was collected in the labs of The effects of WR2721 (US Bioscience/Sipsy lot #6) and WR1065 (lot AP-X-61) in PBS on the HPRT locus in CHO cells and the TK locus in human lymphoblastoid line TK6 were investigated at concentrations between mM in the presence of 100 ug/ml catalase for 3 hours (20 hours for WR2721 without S9).

There were no statistically significant increases in HPRT or TK mutation frequency with concentration of WR2721 or WR1065 up to 4 mM in CHO or TK6 cells with or without addition of S9 fraction microsomes.



Summary and Evaluation of Genotoxicology:

WR 2721 and WR1065 were weakly mutagenic in only 1/5 bacterial cell lines tested (TA1535) in the Ames test (maximum 2.3 fold increase above solvent controls). Interpretation of a chromosomal aberration study was equivocal as the positive controls were not elevated to an appreciable extent above the controls; however, both WR2721 and WR1065 may be weak clastogens. Mouse micronuclei studies were negative, as they lacked dose response and were increased by a maximum of 2 fold in the treated mice. Mouse lymphoma TK assay was increased both with and without S9 fraction by 2-3.5 fold above solvent controls; human HPRT and TK mutation assays were negative.

Overall Summary and Evaluation

The sponsor has not submitted a recent search of the published literature on ethyol. However, the agency has been quoted as stating that the proof of tumor protection will arise from the clinical findings. In this submission, the sponsor demonstrated lack of receptor stimulation (contractility) of guinea pig ileum, rabbit aorta, or rat uterus, although ethyol gave some evidence of potentiating the activity of histamine and serotonin in these systems. Clinical signs in the previous toxicity studies in the rat and dog suggested neurologic stimulation. Blood pressure was decreased in the rat at doses above 30 mg/kg (180 mg/m2), which correlates with clinical observations in the human.

The sponsor has performed Segment II reproductive toxicity tests in both the rat and rabbit. The rabbit showed embryo/fetotoxicity at doses in excess of 50 mg/kg (550 mg/m2). Doses of 75 mg/kg (825 mg/m2) in the rabbit and 200 mg/kg (1200 mg/m2) in the rat were maternotoxic. No evidence of an increase in variations or malformations were noted in either rat or rabbit. In a previously submitted 28 day toxicity study in rats, a 1/20 rats at 100 mg/kg on day 32 (next lowest dose was 50 mg/kg/day). The rat study was performed at doses up to 60 mg/kg, which did not produce significant maternal toxicity. Thus, the rat study is not be valid to predict teratogenicity. However, ethyol will only be used in combination with known teratogens, so the findings may be moot.

In the mutagenicity and clastogenicity assays, WR2721 and WR1065 were weak mutagens and clastogens. No significant mutagenicity was noted in the mouse micronucleus assay or in the human HPRT or TK mutation assays, although the mouse TK locus assay was positive. In long term assays of mutation by Grdina, ethyol provided protection against mutation/ carcinogenicity in murine models.

Recommendations

1. It may be necessary to repeat the rat teratogenicity assay with a dose which shows maternotoxicity (>60 mg/kg, but less than 200 mg/kg).

2. An updated literature search for ethyol should be provided, with particular emphasis on evidence (or lack thereof) for tumor protection.

3. The Investigator's Brochure should be updated to include information on the weakly positive data in the Ames test in the TA1535 strain of *Salmonella typhimurium* with both WR 2721 and WR1065, the statistically significant positive dose response in the TK locus mouse lymphoma assay, and the inconclusive results in the human lymphocyte chromosomal aberration assay.

Wendelyn J. Schmidt, Ph.D.

cc:
/IND
/NDA 20221
/Div. file HFD-150

/WSchmidt
/CSO
/MO
/JDeGeorge

Draft letter to sponsor:

In the review of IND (genotoxicity experiment) and submission #162 (reproductive toxicity experiments), as well as the amendment to NDA 20221 dated 7/12/94, the following problems have been noted.

1. It may be necessary to repeat the rat teratogenicity assay with a dose which shows maternotoxicity (>60 mg/kg, but less than 200 mg/kg).

2. An updated literature search for ethyol should be provided, with particular emphasis on evidence (or lack thereof) for tumor protection.

3. The Investigator's Brochure should be updated to include information on the weakly positive data in the Ames test in the TA1535 strain of *Salmonella typhimurium* with both WR 2721 and WR1065, the statistically significant positive dose response in the TK locus mouse lymphoma assay, and the inconclusive results in the human lymphocyte chromosomal aberration assay.

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Amendment 3

NDA 20221

Reviewer: Wendelyn J. Schmidt, Ph.D.
Received by reviewer: 11/27/92 and 12/27
Completed: 3/8/93

Applicant: U.S. Bioscience, Inc.

Drug Name: Ethyol (WR2721, amifostine, ethiofos)

Indication: Protection from myelosuppression and renal damage with cyclophosphamide and cisplatin.

Related Drugs and IND/NDAs: IND

Dosage Forms and Route of Administration: i.v.

Note - Portions of this review were excerpted directly from the applicant's submission.

Overall Summary and Evaluation

In response to the data on tumor protection with WR2721 in conjunction with either radiotherapy or chemotherapy, the applicant emphasized the following points.

1) The authors of the papers cited by the FDA, but not included in the NDA submission, on the tumor protection data stated that there was a great deal of variability in the data between individual animals and strains of mice.
2) WR2721 caused a species specific (mouse) hypothermia, which the applicant speculated could decrease the metabolism and limb perfusion of drugs. Cyclophosphamide (CY) was of particular concern.
3) WR2721 also caused splenomegaly and peritoneal congestion in the mouse, which may affect the distribution of chemotherapeutic agent at the tumor site, especially with i.p. administration.

1) The applicant provided no direct evidence or alterations in CY metabolism/distribution with prior WR2721 administration. However, Yuhas performed tissue distribution studies with i.p. CY and melphalan (L-PAM) -/- pretreatment with WR2721 in BALB/c mice bearing the MCa-11 tumor (Yuhas, Cancer Res. 40: 1519, 1980). Yuhas concluded "...in neither case [CY or L-PAM] did injection of WR2721 (200 mg/kg) 30 minutes before the alkylating [agent] alter these patterns." This suggests that splenic sequestration of CY does not occur, contrary to the claims of the applicant, but does not address the applicant's speculation regarding altered metabolic activation of CY and metabolite presence in tissues.

Milas et al. used a worst case scenario: an advanced tumor initially implanted as individual cells s.c. in the mouse leg (putative reduced blood flow to the extremities with hypothermia), i.p. injection of chemotherapeutic agent (putative sequestration of drug in peritoneum/spleen), and CY as chemotherapeutic drug with WR2721 (putative decreased metabolism to active

form in liver with hypothermia); yet there was essentially no difference in tumor growth delays with and without WR2721 (DMF with 2 different tumor types) (Milas et al. Cancer Res. 43: 3050, 1983). It should be noted that one tumor type, the FSA fibrosarcoma, showed a 50% decrease in volume between days 10 and 15, then began regrowth, while the other tumor type, the NFSA fibrosarcoma, merely showed a slowing of growth of tumor and DMF's were for FSA and NFSA respectively. Thus, the explanation proposed by the applicant of hypothermia, sequestration or depressed metabolic activation of CY do not appear to be supported by other literature data or experiments. However, in the same paper, micrometastases in the lung (individual cells injected i.v. which lodge in the lung) treated 4 days after initial injection with CY resulted in an increase in number of nodules with WR2721 pretreatment as compared to CY alone (DMF

This protection of tumor micrometastases is of great concern, especially if WR2721 is used with chemotherapy regimens that are curative.

2) The applicant commented that error bars -/- WR2721 overlap at all but the highest concentrations in the Twentyman papers; however, there is a consistent trend where tumors treated with the addition of WR2721 show less growth delay as well as having a statistically significant less delay at the highest concentration of chemotherapeutic agent tested. This could suggest a constant fraction of cells protected (possibly the well-oxygenated set) and with more effective high dose chemotherapy, the surviving fraction protected by WR2721 becomes more evident and has a greater impact on tumor regrowth rate.

3) Researchers in the tumor protection papers acknowledge that there was a large degree of variability in the data. No author of papers favorably to WR2721 cited by the applicant in the NDA makes any mention of this phenomenon, although error bars were frequently at least 10% of the data point. Wasserman et al. (cited in the NDA submission) do not include any statistics in their data. However, the DMF for LD50(30) (dose of chemotherapeutic agent required to kill 50% of the animals at 30 days post-treatment) for i.p. CY in mice as reported by Twentyman (Br. J. Cancer 43: 745, 1981) and Yuhas (Cancer Res. 40: 1519, 1980) was 1.5, showing some degree of reproducibility across laboratory and experiment. However, the applicant is correct in noting that the field as a whole, tends toward large degrees of variability.

4) The uptake of WR2721 into tumors was generally less than levels of drug seen in the blood/plasma. Notable exceptions <u>in vivo</u> included the Morris hepatoma and the RIF-1 tumor (Rasey et al, Int. J. Radiat. Oncol. Biol. Phys. 12: 1487, 1986). The RIF-1 tumor in C3H/Km mice showed a ratio of WR2721 in tumor/blood of approximately 1.3 (range from and a DMF for tumor of with gamma irradiation where cells were exposed to 400 mg/kg WR2721 30 minutes prior to irradiation in situ then excised 10 minutes later for colony formation and cell survival <u>in vitro</u>.

It must be emphasized that in culture (single cell suspensions and 2-3 mm fragments) Lewis lung and normal liver cells accumulated WR721 at the same rate over a 10 minute or 3 hour period at both 4° and 37° C (Millar et al., An. J. Clin. Oncol. CCT 5: 321, 1982). Also of note here. Calabro-Jones (Calabro-Jones et al., Cancer Res. 45: 3634, 1985) demonstrated that WR1065 is

the preferred form for transport and rate is first order up to concentrations of 50 mM in V79 cells. Thus rate of uptake in tumors was most likely related to vascularization of the tumor, availability of alkaline phosphatase, and pH of the tumor instead of an active transport mechanism.

In other words, not all tumors have less uptake of WR2721 than serum, the positive relationship between WR2721 uptake and tumor protection has only been demonstrated by Rasey with the RIF-1 line. As micro-environment (enzymes, pH, vascularization/oxygenation) may affect WR2721 conversion to WR1065 and subsequent uptake into cells, experiments performed in culture with WR2721 are difficult to interpret as truly indicating lack of tumor protection, as one cannot be sure if drug is entering the cells. Unfortunately, none of the in vitro cell lines tested were ones that showed protection as micrometastases in animal models.

5) In the micrometastases models, the applicant suggests that the evidence for protection is a result of membrane disruption during cell disaggregation. (Milas stated that phase contrast microscopy showed >95% viable cells (Milas et al., Cancer Res. 43: 3050, 1983)). However, no rigorous evidence for this explanation was included. The possibility of an environmental difference with anoxic vs. euoxic cells was not discussed.

The available data with Lewis lung carcinoma demonstrated a difference in protection with L-PAM (DMF approximately vs. CY (DMF's approximately which could be due to lack of hepatic activation of CY. No data is available to address this point.

6) There were several cases where there were differences of interpretation/emphasis in the data. First, in the discussion of McNally's data, there is no argument that there was minimal if any tumor protection; however, there was also no normal tissue protection and thus no increase in therapeutic index (p. 12 and 13 of U.S. BioScience analysis, McNally, Br. J. Cancer 46: 670, 1982).

On page 28 of the US Bioscience analysis, figure 18 (Milas et al, Cancer Res. 43: 3050, 1983) shows treatment with CY +/- WR2721 of 9 mm FSa leg tumors resulting in a decrease in lung metastases when leg tumor reached 19 mm with WR2721 vs. CY alone. The question of interpretation/emphasis arises over whether the metastases were extant when WR2721 treatment occu nd thus truly represent an increased effect with WR2721, or only arose subsequent to drug treatment.

Finally, on page 29 in the discussion of the 1983 Milas paper, US Bioscience quotes Milas et al. as stating: "WR2721 might have affected distribution of CY within organs and tissues of animals so that there was a low concentration of CY in the lung and thus less damage of micro metastases. This possibility is indirectly supported by evidence that a significant splenomegaly due to vasodilation develops within 1 hour after injection of WR2721 into mice. This splenomegaly was associated with a reduced peripheral oxygen tension, a change that could alter distribution of drugs throughout tissues and organs." But the author continues to state: "However, if the redistribution of CY did take place, one would expect that large solid tumors would also be protected more profoundly, but this did not occur."

Recommendations

1) The applicant should address the issue raised in the literature of WR2721 uptake in oxic versus anoxic tumor cells and the implications for therapy. The examination could also include tissue culture models in cell lines which have a suggestion of protection in vivo.

2) The applicant should provide information concerning decreased CY activation as an indirect mechanism for WR2721 protection of tumors specific to the mouse.

3) The applicant's discussion of the papers not previously included in the NDA, while helpful, does not dismiss our concern over protection of tumor tissue by WR2721. The animal data suggest that special emphasis should be placed on treatment failure in metastatic disease and possible earlier recurrence with inclusion of WR2721 in the chemotherapeutic regimen in clinical trials.

Suggestions

4) While it is not required that the applicant perform additional animal experiments, if the applicant believes the reported WR2721 tumor protection is peculiar to the mouse and the i.p. route of administration, this should be supported by demonstration that WR2721 tumor protection does not occur in other species when both the chemotherapy and WR2721 are given by the i.v. route of administration.

Wendelyn J. Schmidt

Wendelyn J. Schmidt, Ph.D.

cc:
IND ORIG.
HFD-150
/CSO
/MO
/JDeGeorge
/JJohnson
/LBurke

3/16/93

NDA 20,221

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
SUPPLEMENT 1

NDA 20,221

Reviewer: Wendelyn J. Schmidt, Ph.D.
Received by reviewer: 6/9/92
Completed: 6/24/92

Sponsor: U.S. Bioscience

Drug Name: WR2721, ethyol, amifostine, ethiofos

Indication: chemoprotectant

Related Drugs and IND/NDAs: IND

Proposed Clinical Indication: selective protection against the serious toxicities associated with intensive regimens of platinum and alkylating agent chemotherapy.

Dosage Forms and Route of Administration: 500 mg/vial; i.v.

Studies Received:
I. Pharmacology
1. Effect of WR2721 pretreatment on the cytotoxic activity of 4-HC on Morris hepatoma cell lines FAO and FTO.
2. Effect of WR1065 pretreatment on the cytotoxic activity of cisplatin on four human ovarian cancer cell lines.
3. Effect of WR2721 or WR1065 pretreatment on the cytotoxic activity of antineoplastic agents on human ovarian cancer cells.
4. Effect of WR2721 pretreatment on the cytotoxic activity of anti neoplastic agents in human melanoma (SK-MEL, 28) and ovarian cancer (PA-1) cell lines.
5. Treskes. M., Boven, E., Holwerda, U., Pinedo. H.M., and W.J.F. van der Vijgh. 1992. Time dependence of the selective modulation of cisplatin-induced nephrotoxicity of WR2721 in the mouse. Cancer Res. 52: 2257-2260.

Note - Portions of this review were excerpted directly from the sponsor's submission.

I. Preclinical Pharmacology
1. Effect of WR2721 pretreatment on the cytotoxic activity of 4-HC on Morris hepatoma cell lines FAO and FTO.

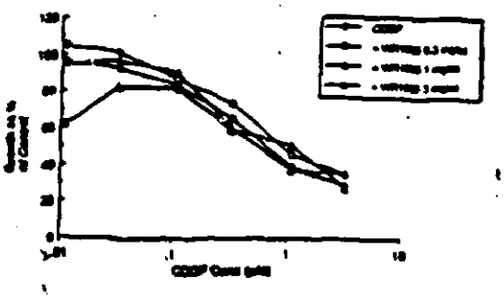
The study was performed by study ETH PH 6. FAO and FTO Morris hepatoma cell lines were pretreated with 3 mg/ml WR2721 for 15 minutes prior to the incubation with 20. 40, 80, 160 or 320 ug/ml 4-HC (controls were no 4-HC). Activity was determined in the MTT assay. Although the OD560 with WR2721 was slightly higher at 4-HC concentrations of ug/ml, indicating a greater number of live cells. the statistical significance of this finding was not determined.

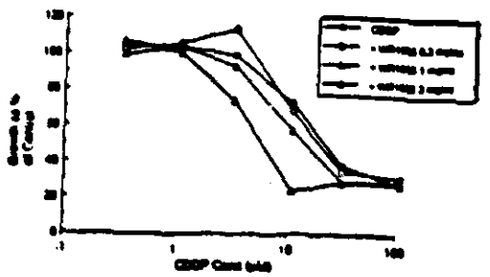
The experiments which should have been performed with these cell lines to answer the question of protection of tumor with WR2721 treatment would be either to use the WR1065 in culture with the chemotherapeutic agents CTX, cis-Pt or HN[1], or, more appropriately, to implant these cell in the mouse and

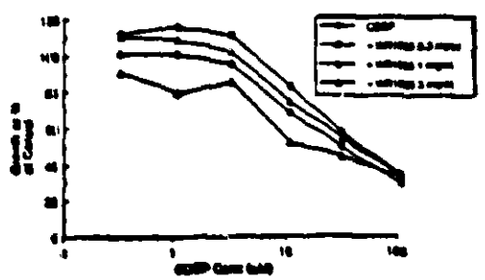
assay the effects of the chemotherapeutic agents previously named. The concentration of WR2721 should have been varied to observed the dose response.

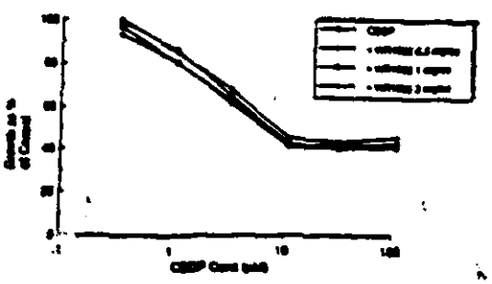
2. Effect of WR1065 pretreatment on the cytotoxic activity of cisplatin on four human ovarian cancer cell lines.

These studies were performed by study ETH PH 5. The effects of WR1065 on cisplatin cytotoxicity in 4 human ovarian cell lines (A2780, A2780-cp70, OVCAR-3 and OVCAR-10, where only A2780 cells were sensitive to cisplatin) were investigated. Cells were incubated with either 0, 0.3, 1.0 or 3.0 mg/ml WR1065 for 15 minutes, washed, then incubated with cisplatin for 2 hours at concentrations ranging from uM. then cloned. No appreciable difference in the growth of A2780 or OVCAR-3 cells with increasing concentrations of WR1065 were noted. With A2780-cp70 and OVCAR-10, the highest concentration of WR1065 increased the cytotoxicity of cisplatin, while the mg/ml concentrations of WR1065 were less toxic in combination with cisplatin. As no error bars were included, (or statistical analysis) the significance of these findings were unevaluable. although the differences appeared minimal. Again. the preferred method of checking the hypothesis that WR2721 is not protecting tumor would have been with implanted tumor lines. and most likely, with non-resistant lines. There is also a question of whether washing the cells. which leads to an unknown quantity of drug taken up by the cells, may not approximate the conditions seen <u>in vivo</u>. Finally, this experiment does not address whether this system accounts for protection of normal tissue.









3. Effect of WR2721 or WR1065 pretreatment on the cytotoxic activity of antineoplastic agents on human ovarian cancer cells.

This study was performed by

study ETH PH 4. BG-1 human ovarian carcinoma cells were preincubated with 3.0 mg/ml WR2721 or WR1065, washed, incubated with several concentrations of nitrogen mustard, cisplatin or ara-C, then assayed for colony formation and growth delay by soft agar cloning. Again, differences between the cells pretreated with the thiols and those untreated were minimal; however, no dose response, whole animal data, or response in normal tissue were included.

4. Effect of WR2721 pretreatment on the cytotoxic activity of anti neoplastic agents in human melanoma (SK-MEL, 28) and ovarian cancer (PA-1) cell lines.

This study was performed by

study ETH PH 3. In SK-MEL or PA-1 cell cultures with or without 15 minute pretreatment with 3.0 or 1.5 mg/ml WR2721 (SK-MEL and PA-1 cells respectively), washed, and the following drugs were incubated with the cells for 2 hours: etoposide, 4-hydroperoxycyclophosphamide, nitrogen mustard, cisplatin, carboplatin, mitomycin C, or doxorubicin. Cytotoxicity after 5 days was determined by counting cells. The concentrations of WR2721 used did not affect the cell growth significantly (98.5% SK-MEL, 96.5 % PA-1). With both SK-MEL and PA-1 cells, pretreatment with WR2721 decreased cell growth, although the significance of the numbers was unestablished. Again, this study did not use WR1065, the active metabolite of WR2721, nor was a dose response to WR2721 established.

5. Treskes, M., Boven, E., Holwerda, U., Pinedo, H.M., and W.J.F. van der Vijgh. 1992. Time dependence of the selective modulation of cisplatin-induced nephrotoxicity of WR2721 in the mouse. Cancer Res. 52: 2257-2260.

In this study, the effect of 200 mg/kg i.p. WR2721 30 minutes prior, 5 minutes prior or 30 minutes post cisplatin in BALB/c male mice was investigated. Nephrotoxicity, as measured by plasma urea levels at 4 days post-injection, diminished in the 30 and 5 minute prior to cisplatin groups, but not the 30 minute post-cisplatin group (DMF Part of this effect may be due to the mannitol in the formulation used. No changes in liver enzymes were noted.

In the nude mice bearing OVCAR-3 ovarian tumor, although tumor volume was slightly increased in the mice given 200 mg/kg WR2721 5 minutes prior to 5 mg/kg cisplatin, and the tumor doubling time and the specific growth delay of tumor were decreased, the size of the tumors at 49 days post-treatment did not differ to a statistically significant extent.

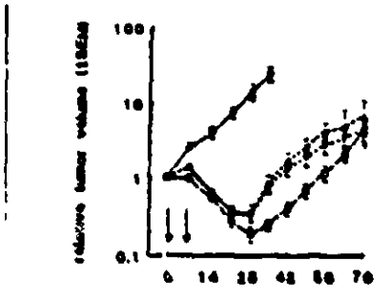


Table 3

Overall Summary and Evaluation

The studies included in this submission do not specifically address protection under conditions where tumor protection would be expected based on previous experiments. However, under the limited conditions employed, these studies confirm a lack of tumor protection with WR2721. There were problems with the design of these investigations in order to answer the specific question of tumor protection. Logically, if the mechanism of differential uptake between tumor and normal tissue is either a change in activation to the active metabolite, WR1065, an altered pH between normal and tumor tissue, or a difference in accessability of the drug to tissue, the choice of model ought to be the whole animal implanted with tumor, preferably a micrometastases model. If the experiment must be done in culture, only the metabolite, WR1065, should be used. In addition, the point to be investigated involves the effects of WR2721 on the activity of antitumor agents; therefore, the dose response effects of WR2721 on tumor growth over time should be the parameters chosen and compared to the effects in normal tissue. Another objection to these studies involves the use of platinum resistant cell lines to investigate WR2721 effects as these cell lines have altered uptake and repair capacity toward platinum compounds. Further, since the sponsor seeks an indication with cyclophosphamide, appropriate tumor and drug treatment should be employed.

In a survey of INDs copies of the 1983 R.E. Durand paper, Br. J. Cancer 47: 387-392 and Yuhas, 1979, Cancer Treat. Rep. 63: 971-976 were found. Papers which were referenced, but not included, were Clement and Johnson, 1981, Int. J. Radiat. Oncol. Biol. Phys. 8: 539-542; Denekamp et al., 1983, Int. J. Radiat. Oncol. Biol. Phys. 9: 1247-1249; Milas et al., 1981, Int. J. Radiat. Oncol. Biol. Phys. 8: 535-538; Milas et al., 1982, Cancer Res. 42: 1888-1895; and Stewart et al., 1982, Int. J. Radiat. Oncol. Biol. Phys. 9:507-513.

It was stated in the Dec. 1982 annual report (IND vol. 3.1, NCI) that:

[redacted]can be seen in Table 1 WR protects mainly the bone marrow, skin, GI tract testes. There is minimal or no protection to the CNS, to skeletal muscle to lung. By contrast, WR-2721 did not protect most of the mouse tumors died from the effect of radiotherapy. Some exceptions, however, have been orted. P388 leukemia (DMF , Ref. 18,20), Lewis lung Carcinoma (DMF 18), oxygenated EMT6 mammary carcinoma (DMF Ref 20), CANTa (DMF calculated, Ref 21).

chanism of the selective protective effect on normal tissues:
Using 35 S marked WR-2721, Yuhas et al (22, 23, 24) have shown that the ug reaches high concentrations in many normal tissues -with the exception the CNS, skeletal muscle and in some studies (23,25) the lung - but is not ncentrated to the same degree in most tumors. One exception to this last tatement is the Morris hepa 7777 which concentrated the drug well (24). his is thought to be due to the high hydrophilicity of the compound (26); st normal cells (RBC are an exception) can absorb higly hydrophylic compounds while most tumor cells cannot. Known inhibitors of active mbrane transport do not affect this concentration so this mechanism es not play a role (22).

It is unfortunate that the Morris hepatoma 7777 has not been tested or radioprotection with WR; no data is available either on the concentration of WR in the other tumors mentioned above which were found to be protected by the compound. This information could help to elucidate the mechanism of protection of the drug. Tumors can be spared the protection either because they do not readily absorb the drug or because they are unable to transform it in active metabolites.

Recommendations

1. The sponsor should reconsider the design of the experiments to reflect the following considerations: the appropriateness of an in vitro versus and in vivo model, the lack of comparison with normal tissue, the use of WR2721 versus WR1065 in the in vitro system, lack of a metastasis model, lack of evidence with cytoxan, and use of platinum resistant cell lines.

2. The sponsor may want to consult with the pharmacologist prior to designing new experiments or review suggestions made during previous meetings.

Wendelyn J. Schmidt, Ph.D.

cc:
IND ORIG.
HFD-150
/KDowns
/JSokol
/JDeGeorge

WJS 7/10/91

JAN 7 1992

PHARMACOLOGY/TOXICOLOGY REVIEW **HFD-150**

NDA# 20221 DRUG NAME: ETHYOL
IND# _____ OTHER NAMES: ETHIOFOS, AMIFOSTINE, WR2721
INDICATIONS: Chemoprotectant _____
SPONSOR: U.S. BIOSCIENCE STEREOISOMER? yes__ no X
DELIVERY SYSTEM? yes__ no X Toxicology Studies

Included in this Review:

	Mouse	Rat	Rabbit	Dog	Other
Single Dose					
intravenous	X	-----	-----	X	-----
identify route	-----	-----	-----	-----	-----
Repeat Dose					
14 day	-----	-----	-----	-----	-----
28 day (1 mo)	-----	X	-----	X	-----
90 day (3 mo)	-----	-----	-----	-----	-----
180 day (6 mo)	-----	-----	-----	-----	-----
1 year (12 mo)	-----	-----	-----	-----	-----
Carcinogenicity	-----	-----	-----	-----	-----
Reproductive Tox					
Segment I	-----	-----	-----	-----	-----
Segment II	-----	X	-----	-----	-----
Segment III	-----	-----	-----	-----	-----
Dermal Toxicity	-----	-----	-----	-----	-----
Ocular Toxicity	-----	-----	-----	-----	-----
Genotoxicity					

1. AMES 2. _____ 3. _____ 4. _____

Pharmacology Studies Included in this Review:

	Mouse	Rat	Rabbit	Dog	Primate	Human
Pharmacokinetics	X	X		X	X	

Pharmacologic Effects	Mouse	Rat	Rabbit	Dog	Primate	In vitro
1. bone marrow protection	X					
2. kidney protection		X				
3.						
4.						

Conclusions
1. IND: No objection ___ Objection _____
NDA: No objection X Objection _____

2. Tumorigen? yes__ no X Neurotoxic? yes__ no__ Immunotoxic? yes__ no__

3. Put an asterisk by the studies that were conducted using the final formulation!

4. Inactive ingredient or metabolite concerns? No

Reviewer [signature] Date 1/7/92

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

JAN 7 1992

NDA 20221

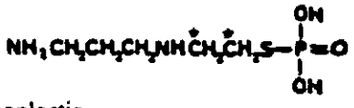
Reviewer: Wendelyn J. Schmidt, Ph.D.
Dated: Oct. 1, 1991
Date Received: Oct. 11, 1991
Date completed: Jan. 6, 1992

Sponsor: U.S. Bioscience, Inc.
One Tower Bridge
100 Front Street
West Conshohocken, PA 19428

Drug Name: Ethyol (WR-2721, NSC 296961, amifostine, ethiofos)

Chemical Name: ethanethiol, 2-[(3-aminopropyl)amino] dihydrogen phosphate (ester)

Structure:



Indication: antineoplastic

Related Drugs and IND/NDAs: IND's

Dosage Forms and Route of Administration:
lyophilized powder for reconstitution, 500 mg/vial, intravenous

Studies Reviewed in Previous Submissions:

1. radiation studies in mice and dogs
2. ADME studies in the rat
3. acute toxicity in mouse (oral and i.p.), rat (oral), guinea pig (oral and i.p.), and monkey (oral).
4. subacute toxicity studies in rat (oral for 28 days), and monkey (i.v. for 5 days, oral for 29 days).
5. Single dose acute i.v. toxicity study in CDF1 mice.
6. Single dose acute i.v. lethality study in CDF1 mice
7. Single dose toxicity study in dogs.

Studies Reviewed in this submission:
Pharmacodynamics:

1. Yuhas, J.M., and F. Culo. Selective inhibition of the nephrotoxicity of cDDP by WR2721 without altering its antitumor properties. Cancer Treat. Rep. 64: 57-64. 1980.
2. Yuhas, J.M., Spellman,J.M., Jordan, S.W., Pardini, M.C., Afzal, S.M.J., and F. Culo. Treatment of tumors with the combination of WR2721 and cDDP or cyclophosphamide. Br. J. Cancer 42: 574-585. 1980.
3. Carfagna P. F., Chaney, S.G., Chang, J. and D.J. Holbrook. Reduction of tetrachloro(d,l, trans) 1,2-diaminocyclohexaneplatinum (IV) (tetraplatin) toxicity by the administration of diethyldithiocarbamate (DDTC), WR2721, or sodium selenite in the Fischer 344 rat. Fundamental and Applied Toxicol. 14: 706-719. 1990.
4. Wasserman, T.H., Phillips, T.L., Ross, G., and L.G. Kane. Differential protection against cytotoxic chemotherapeutic effect on bone marrow CFU's by WR2721. Cancer Clinical Trials 4: 3-6. 1981.
5. Green, D. and P. Schein. Evaluation of chemoprotection by i.p. WR2721 and oral WR151327 in mice. Seventh International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, FLA. 1991
6. Peters, G.J., van der Wilt, C.L., Gyergyay, F., van Laar, J.A.M., Treskes, M., van der Vijgh, W.J.F. and H.M. Pinedo. Protection of WR2721 of the toxicity induced by the combination of cisplatin and 5-FU. Seventh International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, FLA. 1991.

7. Mollman, J.E. Protection against cisplatin neurotoxicity in cultured dorsal root ganglion cells by WR2721. Seventh International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, FLA. 1991.
8. Millar, J.L., McElwain, J.J., Clutterbuck, R.D., and E.A. Wist. The modification of melphalan toxicity in tumor bearing mice by WR2721. Am.J. Clin. Oncol. 5: 321-328. 1982.
9. Valeriote, F. and S. Toten. Protection and potentiation of nitrogen mustard cytotoxicity by WR2721. Cancer Res. 42: 4330-4331. 1982.
10. Phillips, T.L., Yuhas, J.M., and T.M. Wasserman. Differential protection against alkylating agent injury in tumors and normal tissues. In Radioprotectors and Anticarcinogens, Academic Press, Inc., 735-748. 1983.
11. Allalunis-Turner, M.J. and D.W. Siemann. Modification of cyclophosphamide-induced pulmonary toxicity in normal mice. NCI Monographs 6: 51-53. 1988.
12. Nagy, B., Dale, P.J. and D.J. Grdina. Protection against cDDP cytotoxicity and mutagenicity in V79 cells by WR2721. Cancer Res. 46: 1132-1135. 1986.
13. Nagy, B., Dale, P.J. and D.J. Grdina. Protective effects of WR2721 against bleomycin and nitrogen mustard-induced mutagenicity in V79 cells. Int. J. Radiat. Oncol. Biol. Phys. 12: 1475-1478. 1986.
14. Treskes, M., Holwerda, U.,.Nijmans, L., Fichtinger-Schepman, A.M.J., Pinedo, H.M., and W.J.F. van der Vijgh. Modulation of cisplatin and carboplatin with WR2721, molecular aspects. 7th international conference on chemical modifiers of cancer treatment. 1991.

Pharmacokinetics:

1. Plasma concentrations of radioactivity after a single intravenous dose of 14C-WR2721 to male and female rats. Sterling Research. 1990.
2. The excretion and metabolic profiles of 14C-WR2721 following a single intravenous dose to male and female rats. Sterling Research. 1990.
3. Measurement of WR2721 in plasma: preliminary pharmacokinetics in the beagle. Southwest Research. 1985.
4. The disposition of 14C-WR2721 following a single oral or i.v. dose to male dogs. Sterling Research. 1990.
5. Swynnerton, N.F., Huelle, B.K., Mangold, D., and T.M. Ludden. A method for the combined measurement of WR2721 and WR1065 in plasma: application to pharmacokinetic experiments with WR2721 and its metabolites. Int. H. Radiation Oncology Biol. Phys. 12: 1495-1499. 1986.
6. Washburn, L.C., Rafter, J.J., Hayes, R.L. and J.M. Yuhas. Prediction of the effective radioprotective dose of WR2721 in humans through an interspecies tissue distribution study. Radiation Research 66: 100-105. 1976.
7. Washburn, L.C., Carlton, J.E., Hayes, R.L., and J.M. Yuhas. Distribution of WR2721 in normal and malignant tissues of mice and rats bearing solid tumors: dependence on tumor types, drug dose, and species. Radiation Research 59: 475-483. 1974.
8. Utley, J.F., Seaver, N., Newton, G.L., and R.C. Fahey. Pharmacokinetics of WR1065 in mouse tissue following treatment with WR2721. Int. J. Radiation Oncol. Biol. Phys. 10: 1525-1528. 1984.
9. Mangold, D.J., Miller, M.A., Huell, B.K., Sanchez-Barona, D.O.T., Synnerton, N.F., Fleckenstein, L., and T.M. Ludden. Disposition of the radioprotector WR2721 in the rhesus monkey: influence of route of administration. Drug Metabolism and Disposition 17: 304-310. 1989.

Toxicology:

1. A dose ranging study to assess the toxicity of WR2721 following daily intravenous administration to rats for 7 days. Sterling Research Group. 1989.
2. An assessment of the toxicity of WR2721 in rats following daily intravenous administration for 28 days. Sterling Research Group. 1989.
3. Exploratory study of WR2721 administered intravenously to Beagle dogs for two weeks. Sterling Research Group. 1989.
4. One month subchronic safety evaluation and plasma concentration analysis study of WR2721 administered intravenously to Beagle dogs. Sterling Research Group. 1989.
5. Sodicoff et al. Effect of WR2721 on fetal development in the rat. Radiation Research 107: 49-57. 1986.
6. Effect of WR2721 in the Ames test. Walter Reed Army Institute of Research. 1983.

Note - Portions of this review were excerpted directly from the sponsor's submission.

I. Preclinical Pharmacology

1. Yuhas, J.M. and F. Culo. Selective inhibition of the nephrotoxicity of cDDP by WR2721 without altering its antitumor properties. Cancer Treatment Reports 64: 57-64. 1980.

Seventy day old female Fischer 344 rats were given 200 mg/kg i.p. WR2721 30 minutes prior to cDDP. Parameters measured included BUN, hematocrit, and body weight at days 0, 3 ,5, 7, and 10. In a separate experiment. mammary tumor was implanted in the hind leg and tumor regrowth was monitored following cDDP and WR2721 administration.

Treatment with WR2721 prior to administration of 7.5 mg/kg cDDP resulted in a reduction of mortality from 60% to 0%. At 10 mg/kg cDDP, WR2721 pretreatment reduced mortality at 30 days from 100% to 80%. Body weight decreases at Day 3 were unchanged by WR2721 administration; however, at day 7, body weight in WR2721 rats was increased by 5 g at 5.0 mg/kg cDDP and 15 g at 7.5 mg/kg cDDP as compared to rats treated with cDDP alone. Hematocrit values were unchanged. BUN levels at day 3 in WR2721 were similar to control values at 2.5, 5.0 and 7.5 mg/kg cDDP, and were 3-4 times higher at 10.0 mg/kg. while rats treated with cDDP alone BUN levels doubled over controls at 5.0 mg/kg, rose 4 times at 7.5 mg/kg and rose 6 times at 10 mg/kg. At day 5, 10 mg/kg cDDP alone rats were dead. BUN levels in rats treated with WR2721 were comparable to controls up to doses of 7.5 mg/kg , while at 10 mg/kg, BUN levels rose approximately 10 fold. cDDP only animals at 5.0 mg/kg had 3 fold elevations in BUN, 12 fold elevations at 7.5 mg/kg.

In aninals with transplanted mammary tumors, the time for tumor to grow an additional 4 mm following 5.0 mg/kg cDDP treatment did not change significantly with the addition of WR2721 to therapy. Due to diminished lethality to the tumor-bearing rats, 7.5 mg/kg cDDP could be administered with WR2721 which allowed a 1.6 fold increase in the time to progression compared to a 5.0 mg/kg cDDP dose.

2. Yuhas, J.M., Spellman, J.M., Jordan, S.W., Pardini, M.C. Afzal, S.M.J. and F. Culo. Treatment of tumors with the combination of WR2721 and cDDP or cyclophosphamide. British Journal of Cancer 42: 574 (1980).

Limited dose response curves of varying WR2721 concentrations 30 minutes prior to 7.5 mg/kg of cDDP showed a protective effect on BUN levels which plateaued at 200 mg/kg WR2721, while protection to kidney as evidenced by histopathology grade for tubule degeneration and enlarged tubular epithelial nuclei increased linearly between 50 mg/kg and 300 mg/kg WR2721, the maximum dose used.

With multiple dose WR2721, 100 mg/kg WR2721 administered 30 minutes prior to cDDP allowed 1.7 times more cDDP to be given to rats before an equivalent increase in BUN was seen (DMF). A DMF of 1.5 was seen with cDDP administration in the mouse with WR2721. Neither MCA-11 carcinoma in the mouse nor DMBA-14 mammary carcinoma in the rat was protected from cDDP toxicity with WR2721 as evidenced by lack of change in the growth delay of the tumor with either weekly or daily dosing with the drug combination.

Addition of WR2721 to weekly cyclophosphamide treatment (125 mg/kg twice weekly or 200 mg/kg once weekly) in mice allowed the mice to survive 1.6 times longer than when treated with cyclophosphamide alone. Regrowth time of MCa-11 tumor in mice did not differ when WR2721 was added to the treatment.

3. Carfagna, P.F., Chaney, S.G., Charg, J. and D.J. Holbrook. Reduction of tetrachloro (d,l-trans) 1,2-diaminocyclohexaneplatinum (IV) (TP) toxicity by the administration of DDTC, WR2721, or sodium selenite in the Fischer 344 rat. Fundamental and Applied Toxicology 14: 706-719.

Effects on TP toxicity to kidney and circulating blood cells by 200 mg/kg WR2721 were monitored at 3 and 5 days following drug treatment by peripheral blood counts, BUN levels, and on day 5, histopathologic examination of the kidney. On day 5, 67% of the rats treated with 16 mg/kg TP alone survived, while 100% of the TP/WR2721 treated rats lived to day 5. No WR2721 protected rat showed diarrhea following treatment, while 95% of the TP alone rats did.

THE EFFECT OF DDTC AND WR-2721 ON HISTOLOGICAL CHANGES IN THE FISCHER 344 RAT

Organ	Histologic changes	Experimental groups*			
		Control	TP	TP + DDTC	TP + WR-2721
Stomach	Mucosal ulceration and atrophy	0	+	0	+
Small intestine	Villous atrophy and abscission	0	+++	0	0
Large intestine	Villous atrophy and abscission, reduction of goblet cells	0	+++	0	0
Kidney	Coagulative necrosis of tubules, proteinuric fluids and deformation of nucleoli	0	++	0	0
		0	++	0	0
		0	++	0	0
Thymus	Atrophy and reduction of lymphocyte number	0	+++	+	+++
Spleen	Atrophy and reduction in size of lymphoid follicles, reduction of T-cell cuffers, reductions of hematopoietic cells	0	++	0	++
		0	+++	0	++
		0	+++	0	++

Note. All tissues were taken on Day 5 after injection. The doses used were cisplatin, 16.5 mg/kg; DDTC, 750 mg/kg; WR-2721, 200 mg/kg.

* 0, normal; +, mild changes; ++, moderate changes; +++, severe changes.

[illegible] LYMPHOCYTE AND NEUTROPHIL + MONOCYTE CELL NUMBER FOR TP-TREATED RATS WITH AND WITHOUT PROTECTIVE AGENT*

Treatment	N	Platelets* (cells/ml) × 10^{-5}	Lymphocytes* (cells/ml) × 10^{-6}	Neutrophils + Monocytes* (cells/ml) × 10^{-6}
Vehicle	4	704 ± 30	3.10 ± 0.16	1.01 ± 0.18
16.5 mg/kg TP	3	196 ± 36*	0.61 ± 0.06*	0.66 ± 0.19*
16.5 mg/kg TP + 750 mg/kg DDTC	3	324 ± 10*	1.23 ± 0.05*	0.54 ± 0.07
16.5 mg/kg TP + 200 mg/kg WR-2721	3	185 ± 25	0.70 ± 0.01	0.80 ± 0.07

* The parameters were measured on Day 3 only. Statistical comparisons were made by Student's *t* test. The groups were TP vs vehicle, TP + DDTC vs TP, and TP + WR-2721 vs TP.

* Mean ± standard error.

* Significantly different from vehicle group ($p \leq 0.05$).

* Significantly different from tetrachloro-treated group ($p \leq 0.05$).

BUN and creatinine levels at both days [illegible] n controls with WR2721 treatment, while with TP alone, rat BUN and creatinine rose 48% and [illegible] ay 3 and 142 and 127% at day 5. White blood cell parameters, including neutrophil + monocyte [illegible] t protect 1 from TP toxicity (see table). Similarly, while WR2721 completely abrogated TP toxicity to [illegible] small intestine and kidney, thymic atrophy and reduction in lymphocyte number, stomach mucosal ulceration and splenic atrophy and reduction in size of lymphoid follicles were not protected by WR2721.

4. Wasserman, T.H., Phillips, T.L., Ross, G., and L.J. Kane. Differential protection against cytotoxic chemotherapeutic effects on bone marrow CFU's by WR2721. Cancer Clinical Trials 4: 3-6. 1981.

Marrow donor mice (male LAF1) were injected with 600 mg/kg WR2721 30 minutes prior to receiving doses of cyclophosphamide, cDDP, 5-FU, nitrogen mustard, or BCNU. From the number of splenic colonies in the irradiated LAF1 female mice, a Do value and dose modification factor (DMF) were calculated. Lowest protection was seen with BCNU (DMF and greatest protection was seen with nitrogen mustard (DMF Time to regrowth of EMT6/SK tumor implanted in BALB/c mice when pretreated with 525 mg/kg WR2721 then administered any one of the compounds did not significantly differ from tumor treatment with cytotoxin alone, indicating a lack of protection for tumor versus marrow.

TABLE 1

Maximally Tolerated Doses

Drug	MTD (mg/kg) LAF1, Male	BALB/c
Cyclophosphamide	250	200
Carboplatin	13	7
5-Fluorouracil	125	125
Nitrogen mustard	3	1.75
BCNU	25	20

TABLE 2

Bone Marrow D_0 Values and Dose-Modifying Factors

Agent	Alone	+ WR-2721	DMF
X-ray	80 rads	191 rads	2.4
Nitrogen mustard	0.53 mg/kg	2.4 mg/kg	4.6
Carboplatin	2.8 mg/kg	12 mg/kg	3.2
Cyclophosphamide	64 mg/kg	155 mg/kg	2.4
BCNU	19 mg/kg	35 mg/kg	1.8
5-Fluorouracil	34 mg/kg	89* mg/kg	2.7

* Peak dose

5. Green, D. and P. Schein. Evaluation of chemoprotection by i.p. WR2721 and oral 151327 in mice. 7th international conference on chemical modifiers of cancer treatment. 1991.

Toxicity of carboplatin and mitomycin C i.p. with 400 mg/kg WR2721 pretreatment was determined by % ILS and peripheral blood counts. Percent ILS in mice with P388 leukemia treated with mitomycin C or carboplatin alone or in combination with WR2721 did not differ significantly. Peripheral murine WBC count at 4 days was 49% of control with 100 mg/kg carboplatin but 74% of control in combination with WR2721. Mitomycin C alone had WBC counts at 57% of control, while in combination WBC levels were 87% of control.

6. Peters, C.L., van der Wilt, C.L., Gyergyagy, F., van Laar, J.A.M., Treskes, M. van der Vijgh, W.J.F., and H.M. Pinedo. Protection by WR2721 of the toxicity induced by the combination of cisplatin and 5-FU. 7th International conference on chemical modifiers of cancer treatment. 1991.

In vitro experiments on the growth inhibition of the combination of WR2721 and 5-FU or cDDP were performed with concentrations of WR2721 ranging from [illegible] uM and scheduling ranging from 24 hours pre-cytotoxin to 24 hours post-cytotoxin. No change in growth inhibition was observed; however, the better experiment would be to use WR1065, the active metabolite instead.

In Balb/c and C57B1/6 mice, 200 mg/kg WR2721 did not change the MTD 100 mg/kg, of 5-FU. In combination, the MTD dose of cDDP used was 3 mg/kg without WR2721, 7 mg/kg cDDP with the addition of WR2721. WR2721 addition returned thrombocyte level to control (thrombocyte level was reduced by 55% without WR2721 as compared to control). Leucocyte level was also increased (15% of control without WR2721, 40% of control with WR2721). Tumor bearing mice did not tolerate 7 mg/kg cDDP in combination with WR2721, but dose escalation of platinum to 5.5 mg/kg cDDP was tolerated and increased the efficacy (data not given).

7. Mollman, J. Protection against cisplatin neurotoxicity in cultured dorsal root ganglion cells by WR2721. 7th International conference on chemical modifiers of cancer treatment. 1991.

In cultured ganglion cells, LDH level in the media was used as an index of cDDP damage. WR2721 (100 ug/ml) was exposed to cells for 1 hour before, during, or after a 45 minute exposure to 27 ug/ml cDDP. No provision for acid phosphatase reduction of WR2721 to WR1065 was made. Peak levels of LDH in the media were reached at approximately 7 days in the cDDP alone cultures. At 4 days, LDH levels in the media of cells treated

with cDDP were elevated 117% over controls while cells treated with cDDP and WR2721 were only elevated by 71% over controls. As the control levels of LDH also rose with time, a question arises as to the viability of the cells in this culture system for a 14 day period.

8. Millar, J.L., McElwain, T.J. Clutterbuck, R.D. and E.A. Wist. The modification of melphalan toxicity in tumor-bearing mice by WR2721. American Journal of Clinical Oncology. 5. 321-328. 1982.

A 20 mg/kg dose of melphalan in mice killed 10/10 mice in 5-6 days; however, when 400 mg/kg WR2721 is administered 30 minutes prior to melphalan, all the animals survived. The plasma clearance of 14C-melphalan was unaffected by WR2721 administration. The DMF at 400 mg/kg WR2721 for marrow protection against melphalan toxicity by CFU assay was 1.6. Survival of intestinal crypt cells with i.v. or i.p. melphalan alone was 10%, administration of 400 mg/kg i.p. WR2721 increased survival to 60%, with i.v. WR2721 survival was 32%. Human melanoma growth delay in immune-deprived (thymectomized) mice was 22 days with 12 mg/kg melphalan, 8 days with 400 mg/kg WR2721 alone, and 27 days with the combination of melphalan and WR2721.

Tissue levels of 14C-WR2721 relative to blood varied for each organ depending on the tumor type and route of administration of the WR2721. The ratio of i.p. or i.v. WR2721 in tumor relative to blood for B16 melanoma, adenocarcinoma, Lewis lung and human melanoma xenograft was consistently below 0.9 (minimum ratio = 0.4). (Organs such as liver, marrow, and kidney all showed label ratios in tissue versus blood of greater than 1.) When 2-3 mm fragments of liver and Lewis lung carcinoma were incubated with 14C-WR2721 for up to 3 hours at 4 or 37 C, no appreciable difference in tissue levels of 14C label were seen. Single cell suspensions of bone marrow and Lewis lung cells also showed minimal differences in 0 and 10 minute uptake of 14C-WR2721. The authors concluded that differential uptake of WR2721 was related to exposure. i.e. poorer vascularity in tumor and short half life of compound resulting in a minimal concentration at the tumor.

9. Valeriote, F. and S. Toten. Protection and potentiation of nitrogen mustard cytotoxicity by WR2721. Cancer Research 42: 4330-4331. 1982.

Normal versus leukemic marrow protection from nitrogen mustard damage was assessed by CFU assay and survival DMF in tumor bearing AKR mice. Mice receiving nitrogen mustard alone had LD50/30 (dose to kill 50% of the animals at 30 days) values of 0.14 mg/mouse while pretreatment with 15 mg/mouse (625 to 750 mg/kg) WR2721 gave LD 50/30 values of 0.28, a DMF of 2.0. With nonleukemic marrow, the dose of nitrogen mustard required to reduce survival by 50% (D0.5) was 0.047 mg/mouse, while with WR2721 pretreatment, D0.5 was 0.13 mg/mouse, a DMF In contrast, leukemic marrow D0.5 with nitrogen mustard alone was 0.050 mg/mouse and with the combination of drugs, 0.025 mg/mouse, a potentiation factor of 2.

10. Phillips, T.L., Yuhas, J.M., and T.H. Wasserman. Differential protection against alkylating agent injury in tumors and normal tissue. IN Radioprotectors and Anticarcinogens, Academic Press, INc., p. 735-748. 1983.

Protective effects of 600 mg/kg WR2721 in mice was investigated by CFU assay, intestinal crypt cell regeneration following irradiation and drug treatment, and normal versus tumor protection. Bone marrow protection levels were highest for cyclophosphamide and nitrogen mustard at 50% and 10% survival levels, intermediate for adriamycin, BCNU, cisplatin, and X-ray, and low for melphalan and 5-FU. There was no consistent pattern for protection at high versus low survival with drug class. Gaps in the table were not explained: i.e. was the data ignored or technically impossible to obtain.

TABLE IV. Bone Marrow CFU-S Chemoprotection

Agent	D.M.F. at survival level 50%	10%	1%	Terminal slope D_o ratio
Adriamycin	2.5	—	—	1.6
BCNU	2.6		—	1.5
Cis-platinum	2	3	—	3.2
Cyclophosphamide	10	3.4	—	2.4
5-fluorouracil	1.0	1.0	1.8	2.7
Melphalan	1.4	1.7	1.8	1.9
Nitrogen mustard	6	4.6	—	4.6
X rays	3.0	2.8	2.8	2.4

TABLE V. Intestinal Crypt Cell Chemoprotection

Agent	D.M.F. at survival of crypt cell number 30	10	1
Adriamycin	1.2	1.3	—
Bleomycin	10.0	—	—
BCNU	2.0	1.3	—
Cis-platinum	—	1.8	—
5-fluorouracil	1.0	0.8	—
Melphalan	2.0	—	—
Methotrexate	—	1.0	1.1
Nitrogen mustard	4.0	2.0	—
X ray	1.6	1.7	1.8

TABLE VI. Growth Delay[a] Caused by Drugs and Radiation + WR-2721 in EMT6/S.F. Tumors

Agent	Dose	Days delay to 3x	Days delay to 3x with WR-2721	Days difference
X ray	15 Gy	8.0	8.5	+ 0.5
BCNU	30 mg/kg	3.0	4.0	+ 1.0
	60 mg/kg	—	> 17	—
Cyclophosphamide	200 mg/kg	10.5	8.5	- 2
5-fluorouracil	120 mg/kg	2.0	1.5	- 0.5
Melphalan	10 mg/kg	9.0	7.5	- 1.5
Nitrogen mustard	2 mg/kg	6.5	3.0	- 3.5
Cis-platinum	7 mg/kg	3.5	7.0, 0.5	+3.5, -3

11. Allalunis-Turner, M.J., and D.W. Siemann. Modification of cyclophosphamide-induced pulmonary toxicity in normal mice. NCI monographs 6: 51-53. 1988.

Cyclophosphamide damage to lung, pulmonary pneumonitis and fibrosis, correlated with increased breathing rate and an increase in total lung protein at day 4 following administration of the drug. WR2721 (400 mg/kg) was administered to mice 30 minutes prior to 300 mg/kg cyclophosphamide and lung lavage samples taken on day 4. While total lung protein in cyclophosphamide only animals was 2.08 mg, WR2721 treated animals had 0.95 mg protein, which was not significantly different from the 0.97 mg protein in control animals. Breathing rate was also unchanged from controls in WR2721 combination treated animals.

12. Nagy, B., Dale, P.J., and D.J. Grdina. Protection against cDDP cytotoxicity and mutagenicity in V79 cells by WR2721. Cancer Research 46: 1132-1135. 1986.

The active metabolite of WR2721, WR1065, was assayed for reduction of cytotoxicity and protection from mutation at the HGPRT locus in chinese hamster cells. The concentration of cDDP necessary to reduce cell survival to 1% of control was 52 ug/ml for cDDP alone, 134 ug/ml with 4 mM WR1065 added for 30 minutes before washout and cDDP administration, 84 ug/ml when added with cDDP and 92 ug/ml when WR2721 was added after cDDP. (DMF's of 2.6, 1.6, and 1.7 respectively.) WR1065 alone was not toxic to the cells in either of these experiments.

Mutation frequency increased linearly with increasing cDDP concentrations. WR1065 addition reduced the frequency from 25 X 10^{-7} for cDDP alone to 1 X 10^{-7} when WR1065 is administered before, 5 X 10^{-7} during, and 11 X 10^{-7} after, with mutation protection factor (MPF) by slope comparison of 10, 4, and 2 respectively.

13. Nagy, B., Dale, P.J., and D.J. Grdina. Protective effects of WR2721 against bleomycin and nitrogen mustard-induced mutagenicity in V79 cells. International Journal of Radiation Oncology, Biology and Physics 12: 1475-1478.

Mutation frequency and single strand breaks in chinese hamster V79 cells were assessed with bleomycin and nitrogen mustard. The MPF with WR1065 added 30 minutes prior to cytotoxin was 2.8 for bleomycin and 3.4 for nitrogen mustard. The fraction of label remaining filter bound after alkaline elution was increased by WR1065 from 0.2 to 0.3, which is related to the amount of unbroken DNA.

14. Treskes, M., Holwerda, U., Nijtmans, L., Fichtinger-Schepman, A.M.J., Pinedo, H.M., and W.J.F. van der Vijgh. Modulation of cisplatin and carboplatin with WR2721, molecular aspects. 7th international conference on chemical modifiers of cancer treatment. 1991.

Rate constants for the interaction of platinum compounds cDDP, CBDCA, and dien Pt conjugated to S-methyl glutathione with WR compounds were calculated. WR1065 reacted at a greater rate than WR2721 or the thiol bridged compound, WR33278. Ability to prevent platinum adduct formation or remove platinum bound to DNA was also measured, with WR1065 as the most active of the 3 compounds at both prevention and removal of Pt adducts. The caveat in this experiment was the type of adducts which were removed from DNA: it was not stated whether the "cDDP-treated DNA" was aged to form predominantly diadducts or was still in the monoadduct form.

Table I. Second order reaction rate constants for the interaction of CDDP, CBDCA and Pt(dien)SMeG with various modulating agents at pH7.4 and 37°C as well as prevention of formation and the reversal of Pt-DNA adducts.

	k_{cpsr} ($10^{-3}M^{-1}s^{-1}$)	k_{cbdca} ($10^{-3}M^{-1}s^{-1}$)	$k_{monoadduct}$ ($10^{-3}M^{-1}s^{-1}$)	CDDP-DNA adducts % prevention	% reversal
DDTC	614*	78.2*	10100	84	32
TS	570*	88.1*	3980	89	13
WR1065	42.1	12.4	142.5	73	22
WR2721	25.3	6.07	5.29	51	14
WR33278	8.60	0.39	0.0	63	13

* P.C. Dedon and R.F. Borch, Biochem. Pharmacol. 36(12), 1965 (1987).

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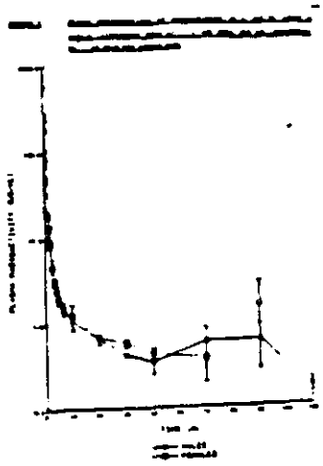
II. Pharmacokinetics

1. Plasma concentrations of radioactivity after a single intravenous dose of 14C-WR2721 to male and female rats. Sterling Research. 1990.

Four male and 4 female Sprague Dawley rats aged 6-10 weeks were deprived of food for 16 hours prior to and 2 hours post-dosing. 50 mg/kg 14C WR2721 was administered by i.v. bolus via the tail vein (mean dose 20.2 uCi).

After the first 10 minutes there was little inter-animal variation or variation between males and females. Plasma levels of radioactivity decreased ≈100 fold over the first 6 hours, from ug equiv./g to ug equiv./g. The overall AUC for males and females was ug equiv.hr/g. Detectable amounts of radioactivity were still present at 120 hours post-dosing ug equiv./g).



2. The excretion and metabolic profiles of 14C-WR2721 following a single intravenous dose to male and female rats. Sterling Research. 1990.

Four male and female Sprague Dawley rats were administered 50 mg/kg 14C-WR 2721 i.v. Urine, feces, carcasses and bedding were analyzed for radioactivity. Urine metabolites were separated by TLC. No significant difference between excretion profiles in males and females was seen. The majority of the drug was excreted in the urine (≈75%) with ≈10% in feces and 8% remaining in the carcass after 144 hours. Total recovery of the dose was 95-97%. Minimal differences in the urinary metabolites between males and females were seen: the major metabolites were analogous to WR33278, the disulfide of WR1065 (20% of the total dose), WR 254380 (20%) and RU3 (12%). Minimal amounts of WR2721 were found in the urine (2-3%).

Sample	Collection period (h)	PER CENT RADIOACTIVE DOSE					
		[illegible]	[illegible]	[illegible]	[illegible]	mean (± SD)	Cumulative mean (± SD)
Urine	0-12	80.8	48.9	57.6	68.3	58.9 ± 6.4	58.9 ± 6.4
	12-24	7.8	6.3	7.5	7.0	7.4 ± 1.1	66.3 ± 7.2
	24-48	3.7	5.8	4.4	3.8	3.9 ± 1.0	68.7 ± 6.7
	48-72	2.1	1.8	1.5	2.0	1.9 ± 0.3	70.7 ± 6.8
	72-96	0.6	0.6	0.6	1.2	0.8 ± 0.3	71.5 ± 7.0
	96-120	0.6	0.6	0.6	0.7	0.6 ± 0.1	72.1 ± 7.0
	120-144	0.6	0.4	0.2	0.7	0.5 ± 0.2	72.6 ± 7.1
Total urine	0-144	77.4	68.8	72.4	76.0	72.6 ± 7.1	
Faeces	0-24	6.3	7.6	3.8	3.8	4.1 ± 2.1	4.1 ± 2.1
	24-48	2.2	3.7	2.8	2.4	2.8 ± 0.7	7.1 ± 2.8
	48-72	1.0	2.2	1.2	0.8	1.3 ± 0.6	8.4 ± 3.2
	72-96	0.7	0.8	0.8	0.6	0.8 ± 0.2	9.2 ± 3.3
	96-120	0.5	0.6	0.4	0.4	0.5 ± 0.1	9.6 ± 3.4
	120-144	0.2	0.6	0.6	0.3	0.4 ± 0.2	10.1 ± 3.6
Total faeces	0-144	9.9	15.3	9.7	8.6	10.1 ± 3.6	
Cage wash	0-144	0.7	2.3	1.9	0.6	1.4 ± 1.0	
Cage debris	0-144	1.3	2.1	2.1	nd	1.8 ± 1.0	
Expired CO_2	0-144	1.1	1.2	1.1	1.0	1.1 ± 0.1	
Carcass	0-144	7.3	8.3	8.7	8.3	8.2 ± 0.8	
Overall recovery		96.7	98.3	93.8	94.4	94.8 ± 1.9	

f = female
m = [illegible]

Sample	Dose (0-144h)	PER CENT OF SAMPLE RADIOACTIVITY					
		Urinary Metabolite					Non Recovered
		1[a]	2[b]	3[c]	4	5[d]	
Urine (Male, 0-24h)	77.6 ± 3.8[e]	4 (9.1)	10 (21.7)	7 (15.2)	4 (9.1)	17 (37.0)	14 (30.4)
Urine (Female, 0-24h)	73.6 ± 7.1[e]	3 (3.8)	27 (19.6)	16 (11.6)	4 (2.9)	28 (20.3)	18 (11.6)

a [illegible] similar RI to WR 2721
b [illegible] similar RI to WR 33278
c [illegible] similar RI to WR 254380
d [illegible]
e Figures in parentheses are expressed as percentages of the total dose excreted over the study period

3. Measurement of WR2721 in plasma: preliminary pharmacokinetics in the beagle. Southwest Research. 1985.

Two 1 year-old male beagles had indwelling catheters placed in the cephalic vein (for drug infusion) and jugular vein (for sampling). 150 mg/kg WR2721 was administered following an overnight fast. Drug kinetics were measured in the same dogs after 35 days. An HPLC method with an internal standard was used for detection of the parent WR2721 only.

A 2 compartment model best fit the plasma kinetics. Several parameters varied greatly between the first and second dosing, including volume of peripheral compartment, distributional clearance, beta elimination phase macro rate constant, and beta half-life. Alpha half-life was approximately minutes, beta half-life was approximately minutes. With a 2 hour infusion of 150 mg/kg WR2721, a 2 compartment model remained the best fit for the data.

4. The disposition of 14C-WR2721 following a single oral or i.v. dose to male dogs. Sterling Research. 1990.

Four male beagle dogs were administered 8 mg/kg 14C-WR2721 i.v. and the blood, plasma, urine, and feces concentrations of 14C were followed. Three weeks later, 16 mg/kg 14C-WR2721 was administered orally and the same parameters followed. Oral levels of 14 C in blood, plasma and urine exceeded those in i.v. animals after 45 minutes. The percentage of radioactivity excreted in the urine was essentially the same for oral (65% of the total administered) and i.v. (63%) administration, indicating similar bioavailability; however, orally dosed dogs also excreted an additional 15% of the dose in the feces. Small amounts of radioactivity were still seen in the urine 19 days after dosing. AUC values were not calculated.

The major metabolite following oral or i.v. dosing was the sulphinic acid derivative of WR 1065 (32%). The other metabolites had similar rf's to WR254677 (the cystine conjugate of WR1065, 6% of the i.v. dose), WR254380 (the methyl compound, 11% of the dose) and cysteamine (6% of the i.v. dose).

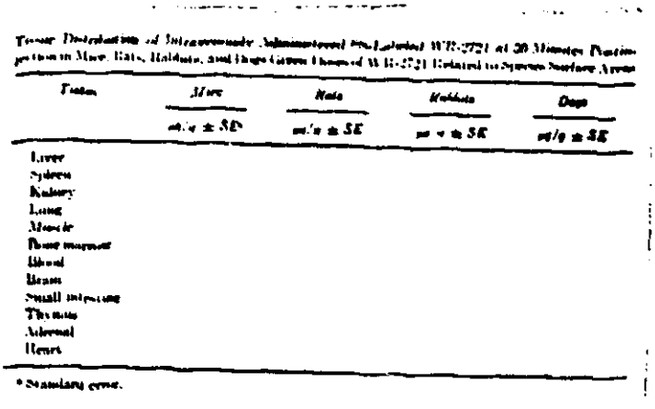
5. Swynnerton, N.F., Huelle, B.K., Mangold, D., and T.M. Ludden. A method for the combined measurement of WR2721 and WR1065 in plasma: application to pharmacokinetic experiments with WR2721 and its metabolites. Int. H. Radiation Oncology Biol. PHys. 12: 1495-1499. 1986.

Five monkeys were dosed with 120-150 mg/kg WR2721 i.v. and plasma samples were analyzed for parent drug (WR2721) and all compounds convertible to WR1065, which included the sulfide linked metabolites of the drug. Total WR1065 ranged from ug/ml mM) immediately after infusion to ug/ml mM) at hours. Parent drug ranged from ug/ml mM) post-infusion to ug/ml, the limit of detection, at minutes. The clearance was ml/min/kg and half-life was minutes.

6. Washburn, L.C., Rafter, J.J., Hayes, R.L. and J.M. Yuhas. Prediction of the effective radioprotective dose of WR2721 in humans through an interspecies tissue distribution study. Radiation research 66: 100-105. 1976.

Male C57BL/6 mice, buffalo rats, female New Zealand white rabbits, and both sexes of beagle dogs were administered i.v. WR2721 at an equivalent dose on a mg/sq.m. basis.

Both the concentration of WR2721 (ug drug/g tissue) and the tissue distribution varied with species at 15 and 30 minutes post-injection. While mice had the highest level of drug in the liver at 15 or 30 minutes, rats, rabbits, and dogs had the highest concentration of drug in the kidney. In all species tested, concentrations in the brain were minimal. Plasma levels at minutes post-injection varied from ug/g in dog to ug/g in rabbit. At minutes, blood values for mouse, rat, and rabbit were all near ug/g. The changes in tissue concentration between minutes were minimal. The concentration in most tissues was intermediate between correlating with absolute dose and dose on a mg/sq.m. basis.



[illegible]

Tissue	Mice	Rats	Rabbits	Dogs
	[illegible] ± SE*	[illegible] ± SE	[illegible] ± SE	[illegible] ± SE
Liver				
Spleen				
Kidney				
Lung				
Muscle				
Bone marrow				
Blood				
Brain				
Small intestine				
Thymus				
Adrenal				
Heart				

* Standard error.

7. Washburn, L.C., Carlton, J.E., Hayes, R.L., and J.M. Yuhas. Distribution of WR2721 in normal and malignant tissues of mice and rats bearing solid tumors: dependence on tumor types, drug dose, and species. Radiation Research 59: 475-483. 1974.

Male mice or rats were implanted with either P-1787 lymphosarcoma (mouse), CA755 adenocarcinoma (mouse), RFT tumor (rat), or Morris hepatoma (rat) and treated with 4 different doses of WR2721: 1, 50, 100, or 300 mg/kg i.v. Tissue samples were taken at 30 minutes.

There was no significant difference between the concentration of label in blood and tumor in the mouse lymphosarcoma and rat RFT tumor. Tumor level of label was 45% lower in tumor than blood in mouse adenocarcinoma and 242% higher in Morris hepatoma in rat. Mouse had a higher concentration of drug in liver while in rat, drug concentrated in kidneys. Lowest concentrations of drug were seen in the brain. A linear dose response curve was seen in all tissues.

8. Utley, J.F., Seaver, N., Newton, G.L., and R.C. Fahey. Pharmacokinetics of WR1065 in mouse tissue following treatment with WR2721. Int. J. Radiation Oncol. Biol. Phys. 10: 1525-1528. 1984.

Mice with EMT6 tumors were injected i.v. with 500 mg/kg WR2721 and sacrificed between 5 minutes and 48 hours post-injection. Samples were analyzed by HPLC for WR1065 concentration.

Maximal concentrations for tissues was reached between 5 and 15 minutes. Highest concentrations of WR1065 were seen in the kidney, lowest concentration in brain and tumor.

Table 1. WR-1065 levels in mouse after treatment with WR-2721 (WR-1065, nmoles per gm fresh tissue*)

	Time after WR-2721 injection (IV)							
Tissue	5 min. (3)	15 min. (3)	30 min. (4)	1 hr. (4)	3 hr. (4)	8 hr. (4)	24 hr. (4)	48 hr. (4)
Liver								
Kidney								
Lung								
Heart								
Muscle								
Brain								
Tumor								
Spleen								
Salivary gland								

9. Mangold, D.J., Miller, M.A., Huell, B.K., Sanchez-Barona, D.O.T., Synnerton, N.F., Fleckenstein, L., and T.M. Ludden. Disposition of the radioprotector WR2721 in the rhesus monkey: influence of route of administration. Drug Metabolism and Disposition 17: 304-310. 1989.

150 mg/kg WR 2721 was administered i.v. bolus, i.v. infusion, i.p., intraduodonally (i.d.) or via hepatic portal vein to rhesus monkeys. Radioactive compound was also administered i.p., i.v. and i.d. Plasma concentrations of drug were monitored over 72 hours by HPLC or by scintillation counting.

Concentrations of WR2721 in plasma decreased rapidly, dropping by a factor of > 100 within the first hour and becoming undetectable by 6-8 hours post infusion regardless of route of administration. By the i.d. route, no parent compound was detectable at any time.

In i.v., i.p. or i.d. 14C-WR2721 administration, concentration of radioactive label in plasma (AUC) was much greater than total WR1065 (WR1065 and WR1065 conjugates) and WR2721 together (1.7X by i.v, 2.6X i.p., and 28X i.d.), indicating that metabolites other than WR1065 derivatives are formed extensively. When WR2721 is given as a 2 hour i.v. infusion, concentration of free WR1065 remained similar to that seen with i.v. bolus, while AUC of WR2721 and total WR1065 decreased by 67 and 48% respectively. A similar result was seen in portal vein i.v. bolus and infusion: WR 2721 AUC decreased by 86%, free WR1065 decreased by 83% and total WR1065 decreased by 61%. The authors suggested that bolus infusion saturated metabolic pathways for WR2721.

AUC values after administration of 150 mg/kg aminothiol to rhesus monkeys

Route of Administration	Monkey Weight	AUC (nM-hr)			
	kg	Aminothiol	Free WR-1065	Total WR-1065	Total ^{14}C
i.v. (10 min)	3.35 (0.46, 4)	239 (119, 4)	201 (62.5, 4)	1360 (185, 4)	-
i.v. (10 min, ^{14}C)	4.83 (0.51, 3)				2702* (370, 3)
i.v. (120 min)	3.65 (0.25, 3)	78.8 (15.9, 3)	197 (7.5, 3)	844 (164, 3)	-
i.p.	4.47 (0.79, 3)	256 (224, 3)	NA†	811 (761, 3)	-
i.p. (^{14}C)	3.78 (0.22, 4)				2767* (364, 4)
Portal (10 min)	4.76 (1.30, 4)	239 (172, 4)	344 (152, 4)	1269 (336, 4)	-
Portal (120 min)	3.36 (0.12, 3)	48.7 (8.8, 3)	53.4 (32.9, 3)	308 (227, 3)	-
Intraduodenal‡	3.88 (0.64, 2)	d (N = 2)	8.6 (N = 1)	69.4 (2.75, 2)	-
Intraduodenal‡ (^{14}C)	3.49 (0.37, 3)				1733* (1144, 3)

* Radioactivity expressed as aminothiol equivalents (μM-equiv-hr).
‡ Single bolus administration.
† NA, not assayed.
d Plasma levels were below detectable limits.

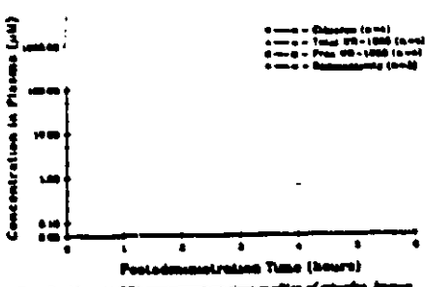


Fig. 2. Mean (n=3) concentration-time profiles of aminothiol, bound metabolites, and ^{14}C (as aminothiol equivalents) following 10-min IV infusions of 150 mg/kg labeled and unlabeled aminothiol.

III. Toxicology

Acute Toxicity Summary

(This summary was comprised of previous reviews by A.W. Coulter.)

Mouse (CD1) i.v. toxicity of WR2721 (combined male and female)

LD10	508 mg/kg	1524 mg/sq.m.
LD50	589 mg/kg	1767 mg/sq.m.
LD90	682 mg/kg	2046 mg/sq.m.

In the dog study, all dogs (beagle) dosed below 214 mg/kg (male) or 186 mg/kg (female) survived the study, while all 4 dogs dosed between 279 and 428 mg/kg (5580 and 8560 mg/sq.m.) were sacrificed moribund at 4 hours post-dosing.

Clinical signs observed in both the mouse and dog included hypoactivity, hypothermia and ataxia. Mice also showed decreased limb tone, bradypnea, decreased righting reflex and tremors, while dogs were emetic, and had ptyalism/ectropion of the lower eyelid. The moribund dogs had tonic convulsions. Upon autopsy, lesions described as moist/gelatinous/red/dark red were found on the liver, lung, spleen, gall bladder, kidney, urinary bladder, stomach, lymph node, bile duct and mammary gland. Minor changes were seen in the ophthalmic examination in some dogs; opacity was seen only on the first day of dosing in mice.

In the mouse, there were no changes in hematological parameters, while dogs showed lymphocytopenia, eosinopenia and neutrophilia. Isolated dogs had decreases in hemacrit and RBC number. Liver enzymes AST and ALT were slightly elevated in mice and one dog while several dogs had elevated acid phosphatase.

Significant histopathologic findings included lymphoid necrosis in the thymus, spleen, mesenteric and mediastinal lymph nodes, and renal tubular epithelial hyaline degeneration in the mouse. Toxicities in the dog, seen mostly in HD/moribund animals, were liver congestion, gall bladder edema and hemorrhage, kidney congestion and protein casts, and moderate to mild g.i. congestion.

Subchronic toxicity

A. A dose ranging study to assess the toxicity of WR2721 following daily intravenous administration to rats for 7 days. (vol 10:80-197)

A GLP/QA statement accompanied the experiment which was performed at

Male and female rats, 3/sex/dose, were administered 0, 25, 50, 100 or 200 mg/kg WR2721 in 0.9% saline (2 ml/kg) via the lateral tail vein for 7 days.

Measurements and Observations:
Hourly for 6 hours post-injection: clinical signs
Daily: body weight, mortality and water consumption
Day 2 and 6: urinalysis
Day 8 (termination): hematology, serum chemistry, organ weights, gross and histopathology

Clinical Observations:

In the 200 mg/kg group, 1 male and 1 female were found dead on days 5-6, the remaining HD animals were sacrificed moribund on day 6. All other animals survived the study.

No male or female in the control or 25 mg/kg group had any observable behavioral changes during the study. In the 50 mg/kg group, unsteady gait and lethargy was seen in 1 male on the first day of treatment, excessive salivation was seen in 1 male on the 6th and 7th days of treatment. Two females in the 50 mg/kg group were irritable on the 7th day of treatment. The following changes were seen beginning on day 3 in 100 mg/kg males, on day 1 for all 200 mg/kg males and females: hunched posture, unsteady gait, lethargy, partial eye closure, hypothermia and piloerection with the addition of abnormal respiration for the females.

The change in body weight in males decreased in a dose dependent fashion: even at 25 mg/kg a 12%

decrease in weight gain versus controls was seen; however, a decrease representing a 5 g difference in total weight gain over 1 week may not be statistically significant. There was no significant change or dose response in weight gain in females. Both males and females treated with 200 mg/kg WR2721 lost weight throughout the dosing while males in the 100 mg/kg group did not begin to gain weight until day 5.

Note: blood samples for the 200 mg/kg group were taken on Day 6 prior to sacrifice.

Hematology:

WBC # decreased in a dose dependent manner in both males and females with a maximum decrease of 78%. This was reflected in the decrease in lymphocyte number by 96% at the 200 mg/kg dose. Neutrophil count approximately doubled at the 200 mg/kg dose.

Clinical Chemistry:

In the 200 mg/kg group, AST and ALT (1 male) and glucose (1 male and 1 female) were doubled. There was a trend toward increased glucose levels in the 100 mg/kg group but single animals had glucose levels below that of control. Urea levels in both males and females were dramatically elevated at 200 mg/kg: 5 fold higher than levels seen in controls. Sodium, chloride and calcium levels were all decreased in HD males and females (see table).

	MALES	FEMALES
SODIUM	8% DECR.	9% DECR.
CHLORIDE	14% DECR.	21% DECR.
CALCIUM	32% DECR.	40% DECR.
CREATININE	26% INCR.	25% INCR.
PHOSPHATE	24% INCR.	49% INCR.

Urinalysis:

There was a trend with increasing dose of WR2721 for increased output of urine and concomitant decrease in osmolality of urine, especially in the 6-24 hour period following dosing on Day 6. Protein, glucose, and blood levels in the urine did not change significantly with treatment.

Gross pathology:

Drug treated animals did not differ from controls until the 200 mg/kg treatment level. The findings in the HD group included enlarged and flaccid hearts and absence of adipose tissue. Organs were not weighed when the HD animals were sacrificed at day 6. In males, there was a trend to decreasing organ weight (both absolute and relative weight) with increasing WR2721 dose for heart, liver, and spleen which was not necessarily statistically significant. Maximal decrease in the 100 mg/kg males compared to control was 13% for heart, 18% for liver, and 31% for spleen (males and females).

Upon microscopic evaluation the following changes were seen only at 200 mg/kg: 1 male and 1 female had focal centrilobular necrosis, all animals had diffuse cortical sinusoidal dilation, all animals had minimal to mild necrosis/atrophy of the white pulp of the spleen, the thymus, and the cervical lymph nodes; and minimal proximal and distal tubular degeneration of the kidney. Findings also seen at 100 mg/kg were bone marrow hypoplasia increasing in severity with dose, and cortical tubular basophilia in the kidney.

B. An assessment of the toxicity of WR2721 in rats following daily intravenous administration for 28 days. (vol. 11:1-12:213)

A GLP/QA statement accompanied the experimental data which was collected by

The main study consisted of 10 males and 10 females/dose treated for 28 days, with two additional groups of 5 rats/sex/dose for assessing toxicity at 15 days of treatment and after 28 days of recovery. Doses of WR 2721

studied were 0, 25, 50 and 100 mg/kg administered by lateral tail vein daily.

Measurements and observations:

Clinical observations: hourly following dosing, daily during recovery.
Mortality: daily
Body weight: pre-study, days 1, 2, 5, 8, and twice weekly thereafter
Food and water consumption: weekly
Ophthalmoscopy: pre-study, between days 25-28
Blood samples: for 15 day sacrifice group: day 8 and 15, for 28 day group day 29, for recovery group: day 29 and 57.
Urinalysis: day 8, 14, 28, and 56.
Terminal data: gross pathology, organ weights, histopathology

Clinical observations:

Three rats died in the course of the study: 1 male 100 mg/kg rat was sacrificed moribund on day 32 with respiratory distress, severe lethargy, and immobility; 1 male and 1 female control rat died during blood collection on day 29.

Earlier onset and greater frequency and multiplicity of abnormal signs were observed with increasing dose. Signs seen at the lowest dose included piloerection, increased salivation, staining of fur, and irritability with an onset of greater than 15 days. As the dose increased other additional observations included hunched posture, unsteady gait, lethargy, hypothermia, and partial eye closure. The onset in the 50 mg/kg group was 7-9 days, while at 100 mg/kg, signs became apparent by 3-4 days. No abnormal signs were observed after day 30 of the study.

Body weight was decreased significantly in HD males from week 1 (7% decrease in weight gain) to a maximum at 28 days (21% decrease in gain) until the end of the study (8% deficit in gain compared to controls). Weight in HD females was significantly diminished at day 28 (10%) but was comparable to controls for the rest of the study. Water consumption increased with increasing dose, while food consumption decreased.

Hematology:

The severity of depression of white blood cell number, particularly lymphocytes, but also platelets, increased with increasing dose of WR2721. Neutrophil numbers increased with increasing dose. Males were affected earlier and to a greater initial extent than females, although the decrease in WBC and lymphocyte # at 28 days of recovery from treatment was greater in females than in males (40% decrease in WBC # in females versus 22% in males). Platelet number in both males and females remained depressed 28 days after cessation of treatment while neutrophil number returned to normal. (See table).

Clinical chemistry:

Changes in clinical chemistry values were only seen at the 100 mg/kg dose level with the exception of AST (day 15), cholesterol (day 15), and ALT (day 29) which were elevated at 50 mg/kg in males (14%, 36%, 42% respectively). All values except cholesterol in the male, returned to normal by the end of the recovery period. Liver enzymes ALT and AST, total bilirubin and glucose levels were elevated at day 29 in both males and females. Cholesterol levels were elevated in males only, while creatinine levels fluctuated in females. Decreases of less than 7% were seen in plasma sodium, chloride, and calcium levels in both males and females.

Urinalysis:

At day 7 of the study, there was at least a doubling in the volume of urine excreted over 16 hours at HD, an accompanying decrease in urine osmolality (<50% compared to controls at HD), a decrease in excretion of creatinine (males only 20-30% at HD during the 4 weeks of dosing), and an increase in total urine electrolytes excreted over the 16 hour period (Ca, Cl, Mg, PO4, Na increasing by 30-170% over controls during the 4 week dosing period). At day 14, the electrolyte increases were also seen at the 50 mg/kg dose level. By day 56, all parameters had returned to levels comparable to controls.

	PERCENT CHANGE VERSUS CONTROL					
	DAY	DOSE (mg/kg)	WBC #	PLT #	LYMPH #	NEUT #
MALE	8	25	—	—	—	—
		50	30% ↓	—	31% ↓	—
		100	44% ↓	11% ↓	48% ↓	32% ↑
	15	25	—	—	30% ↓	—
		50	32% ↓	—	40% ↓	75% ↑
		100	57% ↓	19% ↓	77% ↓	232% ↑
	29	25	37% ↓	—	30% ↓	—
		50	50% ↓	—	55% ↓	15% ↑
		100	54% ↓	7% ↓	83% ↓	363% ↑
	57	25	—	—	—	—
		50	—	—	—	98% ↑
		100	22% ↓	18% ↓	26% ↓	—
FEMALE	8	25	—	—	—	—
		50	—	—	—	—
		100	28% ↓	10% ↓	27% ↓	—
	15	25	12% ↓	—	11% ↓	—
		50	24% ↓	—	27% ↓	—
		100	65% ↓	23% ↓	75% ↓	52% ↑
	29	25	38% ↓	—	37% ↓	—
		50	54% ↓	—	57% ↓	—
		100	64% ↓	23% ↓	86% ↓	325% ↑
	57	25	—	—	—	—
		50	—	—	—	—
		100	40% ↓	22% ↓	40% ↓	—

— INDICATES EITHER LESS THAN A STATISTICALLY SIGNIFICANT CHANGE OR A CHANGE OF LESS THAN 10%.

	% CHANGE VERSUS CONTROL IN 100 MG/KG TREATED RATS					
	MALES			FEMALES		
	DAY 15	DAY 29	DAY 57	DAY 15	DAY 29	DAY 57
AST	28% ↑	42% ↑	—	32% ↓	40% ↑	—
ALT	—	124% ↑	—	43% ↑	96% ↑	—
LDH	—	45% ↓	—	—	—	30% ↓
T Bilir	—	8% ↑	—	16% ↑	—	—
Albumin	8% ↓	7% ↓	—	—	9% ↓	—
Chol.	49% ↑	71% ↑	52% ↑	—	—	—
Glucose	86% ↑	36% ↑	—	42% ↑	19% ↑	—
Creat.	—	—	—	35% ↑	36% ↓	—
Urea	—	—	—	—	16% ↑	—

Gross Pathology:

Macroscopic finding at necrospy included reductions in size of spleen in males and thymus in females, dark/firm contents throughout the digestive tract, and pale kidneys in 1/5 HD males at 15 days. At 29 days, 2/10 male and 1/10 female HD rats had pale liver, 4/10 MD males had pale spleens, and 6/10 male and 1 MD and HD female had distended/edematous pancreas. Reduction of the size of the testes/seminal vessicles in the HD males increased in frequency with time (6/10 at 28 days) and did not recover. At 57 days, all other parameters had returned to normal.

By day 15 and continuing through day 29, the weight of the thymus was decreased in both males and females by approximately 50% as compared to controls. The relative weight of the adrenal gland in males increased in a time and dose dependent manner reaching a maximum increase at 29 days of 118%; females had a 36% increase over control at HD. Epididymis, testes, prostate, and seminal vesicle weight were all decreased by 35% to 60% in HD males at 29 days. Ovarian weights were reduced in a dose dependent manner to a maximum of 26% at HD, while uterus weight was decreased by 31% at HD. At day 57 (28 day recovery period), testes and epididymis weight remained diminished by approximately 50% in males. Spleen and liver weight were increased in males by 43% and 13% at HD respectively. No differences in organ weight between treated and control animals was seen in females at 57 days.

Changes seen at 29 days were increases in frequency and severity of the changes seen at 15 days. A high background of liver inflammation and necrosis was seen in control animals made interpretation of drug effects on liver problematical. Changes remaining at 57 days included pancreatic atrophy, spleen fibrosis, gland atrophy, or damage to reproductive organs.

Histopathologic changes at 29 days.
6/10 ♂, 1/10 ♀ HD, pancreas: edema
1/10 ♂ HD, pancreas: focal inflammation
2/10 ♀ HD, adrenal: cortical enlargement
2/10 LD, 9/10 MD, 10/10 HD ♂; 2/10 LD, 5/10 MD, 10/10 HD ♀ bone marrow: dose dependent increase in severity of atrophy
10/10 ♂, 10/10 ♀ HD spleen: dose dependent increase in severity of peritonitis, atrophy
10/10 ♂, 8/10 ♀ HD thymus: minimum to marked atrophy
10/10 ♂, 8/10 ♀ HD mesenteric lympha/salivary gland/lacrimal gland: minimal to marked atrophy

1/10 ♂, 2/10 ♀ HD kidney: pyelitis
10/10 ♂ HD reproductive organs: decreased spermatozoa in epididymis, necrotic debris from seminiferous tubules in epididymis, decreased secretion from prostate, decreased secretion from seminiferous vesicles, minimum to moderate atrophy of the testes
2/10 HD ♀ ovary: decreased # corpora lutea

C. Exploratory study of WR2721 administered intravenously to Beagle dogs for 2 weeks. (vol. 13:1-318)

The study was performed at Male and female beagle dogs 2/sex/dose were treated with 0, 16, 48, or 144 mg/kg WR2721 i.v. bolus or 48 and 144 mg/kg daily by 15 minute infusion for 2 weeks.

Measurements and Observations:
Daily: clinical signs, mortality, food consumption
Twice weekly: body weight
Pretreatment, Day 2, 8, and 14: blood pressure, respiratory rate, body temp., ECG
Pretreatment, Day 7 and termination: hematology and serum chem.
Pretreatment and day 14: urinalysis
Termination: organ weights, pathology

Clinical Observations:

All of the dogs in both the 144 mg/kg i.v. bolus and infusion groups died or were sacrificed moribund prior to the end of the study. Two males were sacrificed on day 8, the remaining males were sacrificed on day 13. All HD females were sacrificed on day 12.

Clinical signs observed included emesis, increased watery salivation, ptosis of the superior eye lid, prolapse of the nictitating membrane, mydriasis and loss of pupillary reflex, decreased motor activity, staggering gait and tremors. Emesis was dose dependent in relation to onset, frequency, and duration. Fewer episodes were seen with repeated administration. In contrast, incidence of watery salivation, although dose dependent in onset and duration, increased with repeated administration. Incidence was greater in the infused group compared to the bolus group. Response was variable even within individual dogs. Incidence and duration of ptosis of the superior eyelid, prolapse of the nictitating membrane and mydriasis were also dose dependent. Episodes of these signs began shortly after administration and lasted a maximum of 7 hours in the HD group. Tremors, staggering gait, and/or decreased motor activity were seen in all treated groups. In the HD group, deficits were seen following the first treatment; recovery occurred overnight.

A clear dose response in loss of body weight was not seen, although the HD group did have a body weight decrement of 13% in males and 22% in females prior to sacrifice. A consistent decrease in food consumption (partial to no consumption) was seen at HD beginning at day 2. With MD animals, half had a slight decrease in food consumption while the other half had little or no consumption of food.

There was a significant drop in both diastolic and systolic blood pressure only in the HD group dogs 6 hours after WR2721 administration at the midpoint of the experiment. Blood pressure at 2 hours post-treatment was unchanged.

Body temperature dropped appreciably in males and females in the HD group only. At day 2, rectal body temperature dropped an average of 1.2° C in males and 1.0° C in females 2 hours after dosing. At the midpoint of the study, body temperature in males dropped by 1.6° C at 2 hours and had recovered by 24 hours; in females the drop was greater (2.2° C) but recovery was complete by 24 hours.

Changes in the ECG were seen in the 144 mg/kg dogs only.

Hematology and Clinical Chemistry:

Changes in hematological parameters in the dog included decreases in WBC #, and percentages of lymphocytes and eosinophils; while percentage of neutrophils increased. Decreases in WBC # were more severe in the infused animals compared to the bolus dogs (insignificant changes in bolus MD and HD animals, 13% MD and 25% HD reduction in infused dogs at 1 week, 12% MD reduction at 2 weeks). Lymphocyte percentage was reduced in a dose dependent fashion to a maximum of 72% with HD bolus; reduction at MD was similar at 2 weeks.

Percentage of eosinophils was reduced only at HD at week 1 (93% in bolus animals) while at 2 weeks, MD bolus animals had reductions of 32% compared to controls. The percentage of neutrophils increased over the 2 weeks period in a dose dependent manner with a 36% increase in i.v. bolus animals at 1 week and a 26% increase vs. controls at 2 weeks.

Changes in serum chemistry seen in HD animals only at 1 week included increases in total bilirubin, alanine transferase, creatinine, and total protein. Decreases were seen in calcium and potassium levels. At 2 weeks changes seen in serum components in MD animals included decreases in potassium and phosphorus levels, and increases in nitrogen, and creatinine.

Urinalysis: No significant changes were seen between control and LD, MD animals at 2 weeks.

Pathology:

Organ weight at termination was not recorded for the HD animals. Thymus weight alone was decreased at MD for both infused and bolus dogs. Ovary and uterine weight in females was also decreased. The major gross changes at autopsy were pale discoloration of the kidney and black discoloration of the intestinal mucosa in the HD animals. Histopathologic observations included inflammatory and degenerative lesions of the renal cortex and subcortex in HD dogs, degenerative/inflammatory lesions of the mucosa/submucosa in MD and HD dogs, lymphoid depletion in mesenteric and submandibular lymph nodes and spleen at MD and HD, and diffuse atrophy of the thymus at all doses.

D. One month subchronic safety evaluation and plasma concentration analysis study of WR2721 administered intravenously to Beagle dogs. (Vol 14:1-359)

The study was performed at and a GLP/QA statement accompanied the data. Male and female beagle dogs (4/sex/dose) were administered 0, 8, 20, or 50 mg/kg WR2721 daily by i.v. bolus injection for 28 days.

Measurements and Observations:
Daily: clinical signs, mortality, food consumption
Twice weekly: body weight
Pretreatment, Day 2, 7, 8, 15, 22, and 28: B.P.,body temp., respiratory rate, and heart rate.
Pretreatment, Day 15, 28: ECG
Pretreatment, Day 16, day 30: hematology, clinical chemistry
Pretreatment, Day 30: urinalysis
Termination: ophthalmologic exam, necropsy, histopathology

Clinical Observations etc.:

No dogs died during the study.

Repeated emesis was observed with all dogs in the MD and HD groups with episodes decreasing after the first week of dosing. Watery salivation was dose-related and increased with the length of treatment, also occuring prior to treatment after repeated dosing. Ptosis of the superior eyelid, and prolapse of the nictitating membrane occurred in HD dogs. Mydriasis was seen in all treated animals, with the duration increasing with dose. Tremors and decreased motor activity were seen in the last 2 weeks of the study in HD dogs. One case of multifocal alopecia was also seen at HD in the last week. Percentage body weight decrement was dose and time related in MD and HD dogs. Maximal decreases were 13 to 20% in males and females.

Blood pressure in either males or females did not change significantly over the course of treatment. Rectal temperature in males sporadically decreased by 0.5°C during treatment. Respiratory rates did not change. Heart rate appeared to increase sporadically (usually between 4-6 hours after treatment) in HD males and females by ≈ 20 beats/minute. Minimal sporadic increases in QRS complex durations and complex intervals were seen in MD and HD animals.

Hematology:

RBC parameters were not affected by drug treatment. Platelet number was decreased in both males (max

36% at 2 weeks, 91% at 4 weeks) and females (max. 77% at 4 weeks); however, the decreases did not correlate well with dose. Total WBC number was either essentially unchanged at 2 weeks and increased at 4 weeks by as much as 49% over controls. Absolute number of lymphocytes and eosinophils were decreased at high doses in both males and females, while total number of neutrophils (both band and segmented) were increased. (*Please see following page for table of percent change in each parameter.*)

Changes in hematologic parameters with 1 month treatment with WR 2721

% Change Versus Control at 4 Weeks						
	Males			Females		
	8 mg/kg	20 mg/kg	50 mg/kg	8 mg/kg	20 mg/kg	50 mg/kg
plate #	73% ↓	91% ↓	—	51% ↓	77% ↓	71% ↓
#WBC's	15% ↑	49% ↑	42% ↑	—	30% ↑	38% ↑
%lymphs	—	34% ↓	41% ↓	—	—	56% ↓
#lymphs	—	—	—	—	—	38% ↓
% bands	—	71% ↑	75% ↑	—	—	—
# bands	—	142% ↑	141% ↑	—	—	48% ↑
% seg.	—	—	—	18% ↑	19% ↑	44% ↑
# seg.	—	—	—	22% ↑	58% ↑	99% ↑
%eosins	—	—	—	59% ↓	30% ↓	80% ↓
#eosins	—	—	—	56% ↓	—	74% ↓

Clinical Chemistry:

In male dogs, chloride levels were decreased by 10% at week 2 but did not differ from controls at week 4. Serum bilirubin levels were sporadically increased over time and dosage level with a maximum increase over control of 132%. ALP levels at HD decreased by 25% over controls at both 2 and 4 weeks. One male dog had BUN and creatinine levels increased over controls by 201% and 163% at 2 weeks, 142% and 74% at 4 weeks.

In female dogs, only ALT levels were consistently affected throughout the group, increasing by 41% at MD and 70% at HD as compared to controls. A single HD female dog had diminished sodium levels at 2 and 4 weeks (decrease 9.7% and 20% compared to control). Another HD female had elevated ALP levels at 2 and 4 weeks (160% and 173%) as compared to controls. Finally, a third HD female had elevated lactic dehydrogenase and creatinine kinase levels at 4 weeks (490% and 86% respectively).

Gross Pathology:

The major macroscopic findings at necropsy were thymic atrophy (3/4 males and females at MD, 3/4 males and all females at HD), 1 HD male with cortical pale discoloration of the kidney, and 1 MD male dog with yellow discoloration of the organs, increased spleen size and decreased mesenteric lymph node size. The icterus was accompanied by anemia, and was evaluated by the sponsor as intravascular hemolysis. Focal/diffuse red discoloration was observed sporadically at all dose levels in the digestive tract, pancreas, urinary bladder, and thyroid. Other findings included 1 HD ♀ with diffuse pale liver discoloration, pituitary cysts in 1 LD, 2 MD and 2 HD ♂, diffuse ovarian enlargement in 2 MD ♀, focal ovarian cysts in 1 MD and 1 HD ♀, and lung focal/multifocal white subpleural discolorations in 3 HD ♂.

Relative organ weights did not differ to a statistically significant level with the exception of prostate in the male (50% increase in LD and MD) and uterus and thymus in the female (maximum increase of 142%, 44%

decrease at HD respectively).

Histopathology:

The most striking alterations in histology were the multifocal follicular lymphoid depletion in the mesenteric lymph nodes (1 HD ♂, 3 HD ♀), the multifocal follicular lymphocytolysis in the spleen (3 HD ♂, 2 HD ♀), and the interstitial lymphohistiocytic infiltration (3 LD ♂, 2 LD ♀, 1 MD ♂ and ♀, 3 HD ♂, 2 HD ♀), tubular basophilia (1 MD ♂ and ♀, 3 HD ♂ and 4 HD ♀), tubular casts (1 MD ♂ and 1 HD ♂ and ♀), and glomerulo-sclerosis (2 HD ♂ and 1 HD ♀) in the kidney.

Summary of Subchronic Toxicity

The similar responses to WR2721 treatment occurred across species and sex with comparable dosage. Clinical signs were similar in the rat and dog (with the obvious exception of emesis in the dog) with prominent watery salivation, ptosis, unsteady gait and lethargy, all manifestations of neurologic toxicity.

In both dog and rat, white blood cell parameters changed: lymphocyte and platelet numbers were decreased while neutrophil numbers increased. Oddly enough, marrow atrophy was seen at necropsy in the rat, while dog marrow looked normal; however, in the dog, lymphocytolysis was seen in the spleen and lymphoid depletion in the mesenteric lymph nodes and thymus, suggesting peripheral destruction of blood units. One connection between drug and lymphoid depletion may be the observation that concentrations of WR1065 remained high in the salivary gland for several hours after dosing. Thymic weights in rats decreased, while thymic atrophy was seen in both species. Observation of the animals throughout a 28 day recovery period was only performed in the rats. Platelet levels did not return to pretreatment levels after 28 days drug-free, while lymphocyte and overall WBC levels partially recovered. Neutrophil numbers at 28 days post-treatment were indistinguishable from controls.

Liver enzymes elevated consistently in the rat were AST and ALT while in dogs, ALT was elevated in females only, ALP in males only. Enzymes were sporadically elevated in dogs, e.g. ALP was increased by ≈ 300% in 1 HD female, CK and BUN were elevated in 1 male only. However, histopathologic examination of the liver showed few changes in either rat or dog. Kidney parameters were changed in female rats (increased urea and fluctuating creatinine) as well as decreases in serum electrolytes and urine osmolality. Changes were also seen in the histopathologic examination of these tissues: tubular basophilia, glomerulo-sclerosis, and pyelitis in the kidney. These results are not surprising considering the high localization of radiolabelled drug in liver and kidney.

A difference in the response of rat and dog to WR2721 seen was in the 4 week dog experiment, which was the only time weight increases were seen in prostate, ovary, and uterus as opposed to decreases.

Neither of these studies established a no effect level. The recommended dosage in humans is 910 mg/sq.m (25 mg/kg) with the possibility of administration of a second dose of 910 mg/sq.m. within 2 hours. The maximum dose used in the rat study was 590 mg/sq.m./day while in the dog study, the highest dose used was 1000 mg/sq.m./day.

Reproductive Toxicity

(Single study published in Radiation Research 107: 49-57,(1986).)

Female rats were mated with untreated males until there was evidence of sperm in daily vaginal lavage. Two rats were injected i.p. at 14 days of pregnancy with 14C WR2721 to determine placental transfer of drug. The remaining females were injected i.p. with 2.5-600 mg/kg at either day 9, 11 or 14. Females were sacrificed on day 20 post-coital and the fetuses examined.

Measurements and Observations:

Daily: maternal mortality

Day 20: # implantation sites, # live, dead, resorbed fetuses,

weight of fetuses, head measurements on fetuses, external defects in fetuses.

Isotope study of placental transfer:

Two dams were injected i.p. with 100 mg WR 2721 on day 14. Three fetuses were removed at 15 minute intervals up to 1 hour and digested for scintillation counting. The radioactivity did continue to accumulate in the fetus over the 1 hour period studied (1 hour accumulation 2000 cpm/fetus).

Maternal Toxicity:

Only maternal mortality was measured. No females died in the 0-300 mg/kg range. 3/60 died at 400 mg/kg, 10/60 at 500 mg/kg, and 17/30 at 600 mg/kg to give an LD50 in pregnant females of 580 mg/kg. LD 50 in nonpregnant females was 640 mg/kg.

Fetal toxicity:

The number of live fetuses/litter decreased with increasing dose at all administration times, becoming statistically significant above 300 mg/kg (>15% loss for day 9, >5% loss for day 11 and > 10% loss for day 14 at 300 mg/kg). Small but significant losses in fetal number were also observed at 10 to 50 mg/kg doses. Fetal weight also decreased significantly compared to control at 500 mg/kg with injection at day 9 and day 11, 300 mg/kg with injection at day 14. No significant changes in head size were seen. No external malformations were observed at any dose. Internal examinations were not performed.

Summary of reproductive toxicity:

WR2721 in rats is embryotoxic; however, whether this only occurs at maternally toxic doses is not clear from this study.

The segment II reproductive study performed in support of the NDA is not adequate for the following reasons. The route of administration was i.p. while the dosage route used in humans will be i.v. The maternal toxicity of the drug was not monitored: no record of maternal weights or clinical observations were included. The administration schedule of drug was not correct: drug was administered on either day 9, 11, or 14, not throughout the organogenesis period of days 6-15. Internal examinations of the fetuses were not performed.

Further deficiencies in the reproductive toxicology included in this NDA include the lack of a segment II toxicity study in a second species, and the absence of a segment I toxicity study.

Genotoxicity

A. The mutagenic potential of ethyol (vol. 15: 159-171).

A stock 5% solution of WR2721 was used in these experiments. Test strains of salmonella used were TA98, TA100, TA1535, TA1537, and TA1538. S-9 microsomal fractions were used with the compound. Positive controls were benzo(a)pyrene, 2-aminofluorene, aminoanthracene and N-methyl-N'-nitrosoguanidine. Maximum dose was 5 mg/plate. The number of revertants/plate in all strains of salmonella with increasing concentrations of WR2721 with and without microsomal S-9 fraction did not differ statistically from negative controls.

Summary of Mutagenicity/Carcinogenicity:

WR2721 was not mutagenic in the Ames test. No carcinogenicity tests were performed on the compound; however, data from the efficacy studies suggest that WR2721 can protect somewhat against the mutagenicity of chemotherapeutic compounds. For example, administration of WR2721 prior to cisplatin, bleomycin, or nitrogen mustard decreased the mutation rate at the HGPRT locus in V79 cells, but not to control levels. Mice treated with WR2721 prior to radiation therapy developed fewer secondary tumors within the following 2 years.

Summary of Preclinical Pharmacodynamics

WR2721 is metabolized by alkaline phosphatase to WR1065 and taken up by normal cells to exert a protective action against radiation and some chemotherapeutic agents. Preferential uptake by normal versus tumor cells appears to be governed by increased vascularity of normal tissue, pH differential between normal and tumor tissue and increased concentration of alkaline phosphatase in capillary beds near normal tissue. There is the possibility that active transport of WR2721 occurs in normal tissue, however, the experiment was performed in an inappropriate system, thereby confusing the interpretation. Tissue distribution studies in tumor bearing animals show a ratio of WR compound (14C label) in tumor to blood of less than 0.9. Only the brain and spinal cord have lower concentrations of drug, implying that protection from CNS toxicity is unlikely.

Once in the cell, the thiol compound protects cellular integrity and/or function through scavenging of free radicals and binding to alkylating agents/platinum moieties. WR2721 did not, within experimental error, affect the anti-tumor effects of cDDP, cyclophosphamide, 5-FU, nitrogen mustard, BCNU, or melphalan in several types of tumor including melanoma, mammary carcinoma, leukemia, and adenocarcinoma as evidenced in tumor regrowth assays in animal models.

The data on hematopoietic protection was mostly derived from murine bone marrow colony-forming unit assays, although the platinum and mitomycin C data was obtained from peripheral white blood cell counts at 4 days post-treatment. The data on cDDP and TP was the only study done in the rat with peripheral white blood cell counts. Minimal protection from anti-metabolite hematologic toxicity was seen with WR2721. Marrow protection from alkylating agent damage ranged from a DMF for BCNU to a DMF for cyclophosphamide. Marrow protection was seen for cDDP by CFU assay and peripheral counts. Peripheral WBC counts showed WBC protection for CBDCA, but not for TP. Adriamycin and mitomycin C toxicity to hematopoietic cells was also diminished with WR2721 treatment.

CLASS	DRUG	MARROW DMF	INTEST DMF	OTHER
ALKYLATING AGENTS	NITROGEN MUSTARD			—
	CYCLOPHOS-PHAMIDE		—	PULMONARY
	BCNU			—
	MELPHALAN			—
PLATINUM	cDDP			KIDNEY
	ORMAPLATIN	NO PROTECT (WBC CT)	PROTECT (MICROSC)	KIDNEY
	CBDCA	PROTECT (WBC CT)	—	—
ANTI-METABOLITES	5-FU			—
	MTX	—		—
ANTIBIOTICS	ADRIAMYCIN			—
	BLEOMYCIN	—		—
	MITOMYCIN C	PROTECT (WBC CT)	—	—

Protection from damage to intestinal crypt cells by WR2721 was similar for all the alkylating agents (DMF=2) and cisplatin. Protection from antimetabolite damage in the intestinal crypt was minimal, while protection with antibiotics was variable.

Special organ toxicities where WR2721 showed protection against drug induced damage included kidney, lung in in vivo studies, and peripheral nerve cells in in vitro studies. Both tetraplatin and cisplatin damage as manifested in increased BUN/creatinine levels and histopathologic damage were dramatically decreased with WR2721 treatment. Cyclophosphamide damage to lung was also decreased with WR2721 treatment.

In vitro tests used to further characterize the protective effects of WR2721 included a peripheral ganglion nerve assay, where WR2721 contributed to the decreased release of LDH indicative of damage by cisplatin, and mutation assays at the HGPRT locus of chinese hamster cells. WR2721 decreased the mutation frequency by as large a factor as 10 when administered prior to cisplatin.

The evidence in animals suggests that scavenging free radicals and detoxifying agents by binding to the thiol moiety may be the mechanism by which WR2721 exerts its protective action once in the cell as the compound was essentially inactive against antimetabolites.

Summary of PK/ADME Studies

WR2721, and its dephosphorylated metabolite WR1065 were rapidly eliminated from the plasma of both dog, rat, and monkey. The half-life of WR2721 is less than 10 minutes for all species observed. However, small amounts of radiolabel were detectable in blood even after 19 days. Similar kinetics were seen in the human (half-life under 10 minutes, minimal elimination of the parent drug by the kidney).

The majority of the drug, as metabolites, is eliminated from the kidney, 75% of the dose in the rat, 65% of the dose in the dog. Minimal amounts of parent compound were seen in the rat; none in the dog. The major metabolites in the rat were the disulfide bridged form of WR1065 (20%) and the methylated sulfide (20%). The dog excreted mostly the sulfinic acid form (33%) with the next major metabolite as the methylated sulfide (11%). The majority of the dose is excreted within the first 12 hours in both rat and dog, although small amounts are still detectable after 144 hours.

The tissue distribution was similar in dogs, rabbits and rats, with the kidney as the target organ. In one mouse study, the target organ was the liver (kidney was the major target in the other study). Uniformly low concentrations of WR2721 and metabolites were seen in the brain. Tumor concentration ranged from less than half the concentration in blood to 242% of blood levels with the Morris hepatoma. The high concentration of label in that tumor may be related to its origin, liver, and the higher concentration of alkaline phosphatase found there.

Tissue and blood levels were not equivalent across species on either a mg/kg or mg/sq.m. basis. There did not appear to be any sex-related alterations in plasma kinetics or metabolism of WR2721. The major metabolite in the dog was the sulfinic acid conjugate of WR1065, while in the rat it was the disulfide bridged form of WR1065 and the methyl form of WR1065, which was also seen in the dog at a lower extent. Cysteamine was seen in the urine of the dog, but not in the rat.

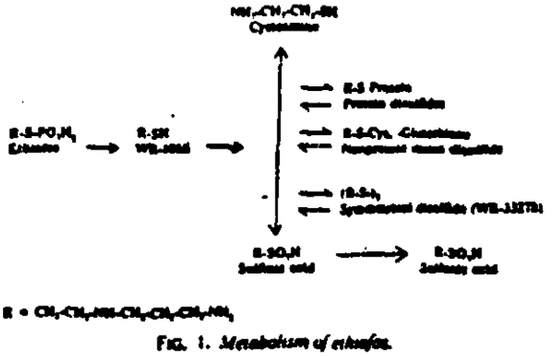


FIG. 1. *Metabolism of ethiofos.*

Evaluation

WR2721 (ethyol) protects against some of the toxicities of some of the platinum, alkylating agent, and antibiotic cancer chemotherapeutic compounds by preferential uptake into normal tissue, where cytotoxins can be bound to the sulfhydryl group of WR1065 (the active dephosphorylated form of the drug) and free radicals scavenged. Levels of drug in the CNS and tumor is generally low, with the exception of the Morris hepatoma, indicating the possibility that some tumors may be protected from cytotoxicity by this compound. Kidney damage with the platinum compounds cisplatin and ormaplatin, measured both by serum levels of BUN and creatinine and histopathologically was decreased with WR2721 administration. Depletion of peripheral WBC's by cisplatin, CBDCA, and mitomycin C was decreased with WR2721. Toxicity to colony forming units of the bone marrow with nitrogen mustard, cyclophosphamide, BCNU, melphalan, cisplatin, and adriamycin were all decreased with WR2721 administration. Finally, pulmonary toxicity seen with cyclophosphamide was decreased with WR2721 administration.

WR2721 is rapidly cleared from the blood, as is its dephosphorylated metabolite, WR1065 (the half-life of both compounds is less than 10 minutes for all species observed). Excretion is via the kidneys, with over half of the dose removed within the first 12 hours (smallest time increment studied).

Toxicities seen with the drug include neurologic-related clinical signs (mydriasis, watery salivation, emesis from an i.v. administered drug, ptosis of the eyelid and gait abnormalities) elevations of some liver enzymes (less than 2 fold), marrow toxicity in the rat but not the dog, and kidney damage. Paradoxically, protection of hematopoietic units when WR2721 is combined with other marrow-toxic agents is well documented. Hence, liver and kidney function, and hematological parameters should be monitored closely during therapy. Reproductive toxicity was not well characterized (studies are currently in progress), but, the compound will be used with known teratogens, so pregnancy issues are somewhat irrelevant. A similar argument can be used for carcinogenicity testing, as the compound will be administered with known carcinogens; however, animal studies have shown some protection against delayed tumor formation with radiotherapy and tissue culture studies have shown a protective effect on HGPRT locus mutation.

Note: previous pharmacologic deficiencies, lack of multiple dose and reproductive toxicologic studies, have been corrected.

Labelling Issues

1. Eliminate the sentence in the second paragraph of the clinical pharmacology section reading "Other studies suggest a facilitated..."
as this experiment was not performed in an appropriate system.

2. The final sentence in the second paragraph of the clinical pharmacology section should begin with "In a cell-free system..."

3. In the precautions section under "Carcinogenesis, mutagenesis, and impairment of fertility" the third sentence should read: "Data from in vitro studies demonstrate that ethyol decreases mutations at the HGPRT locus in Chinese hamster cells by 60 to 95% compared to mutations with the cytotoxic agent alone using cisplatin, bleomycin, or nitrogen mustard (Nagy et al., 1986). In vivo rodent experiments show a 60% reduction in tumor incidence following X-ray irradiation of the leg with ethyol therapy (Hunter et al., 1991)."

4. In the pregnancy section under precautions, this study the sponsors quote is woefully inadequate and the conclusion of nonteratogenic unfounded. The reproductive toxicity experiments are being repeated, so this may be acceptable once that data is submitted. As currently submitted, the labelling should read "Adequate teratogenicity testing has not been performed with WR2721".

NDA 20221

Notes to the Medical Officer on Labelling Issues

The largest question in the labelling is whether to allow the vague description of uses and agents: i.e. should their indications and usage read "as a chemoprotective agent against the serious toxicities associated with intensive regimens of platinum and alkylating agent chemotherapy" or should it be more specific as to which toxicities and which drugs, especially as some of these agents have not actually been tested with WR2721 in well-controlled human trials. If specificity is desired, the first sentence of the clinical pharmacology section and the indications and usage section could read as follows.

> From the evidence in rodents, ethyol is indicated as a chemoprotective agent against some of the toxicities (bone marrow and nephrotoxicity) of platinum compounds (cisplatin, CBDCA, and ormaplatin), and the marrow toxicities of cyclophosphamide and alkylating agents (nitrogen mustard, cyclophosphamide, BCNU, and melphalan).

Wendelyn J. Schmidt, Ph.D.

cc:

/orig. NDA 20221
/HFD-150/Division File
/KDowns
/JSokol
/JDeGeorge
/ACoulter
/HFD-340
/HFD-502/JWeissinger

1/7/92

Pharm and Bio

NOV 30 1994

PHARMACOKINETIC SECTION

NDA 20-221
Amifostine
Lyophilized powder - IV use
500 mg/vial
Ethyol™
US Bioscience

Submission Date: July 12, 1994
September 21, 1994.

Reviewer: Lydia C. Kaus, M.S., Ph.D.

Type of Submission: Amendments 17 and 18 to NDA 20-221

Background:
This is Amendment #17 and 18 to NDA 20-221.

Dates:	Notes:
9/30/91 1/10/92 1/23/92	An NDA review of the original submission. Completed by Dr. Mallikaarjun 8/12 /92. Many omissions of data resulting in incomplete conclusions being able to be made. Information requested from the firm.
11/25/92	Review of amendment #13 for meeting. Completed 3/23/93 (see below).
3/19/93	Meeting held with firm . Biopharm comments submitted to firm. Biopharm. still awaiting dose proportionality study, Phase 3 clinical PK studies, drug interaction studies.
11/1/93	Discussion with firm of Amendment #14 submitted 8/30/93. No PK per se mentioned in minutes of meeting.
11/23/93	Memo to file from Biopnarm referring to pre-NDA meeting. Promise of PK of cyclophosphamide, cisplatin or carboplatin after pretreatment with drug. Three clinical studies with PK promised still not submitted.

The three PK studies that are ongoing are:

"Pharmacokinetics of single dose amifostine (ethyol)" Investigator: Leslie S. Shaw, PhD, Philadelphia, PA

"Pharmacokinetics of double dose amifostine with corresponding measurements of WR-1065 in plasma and bone marrow cells." Investigator: Leslie Shaw, PhD, Philadelphia, PA.

"Pharmacokinetics of cisplatin following a 15-minute infusion of amifostine". Investigator: Joan

Schiller, MD, Madison, WI.

Summary of NDA and Amendment:
Review of Submissions 9/30/91, 1/10/92, 1/23/92 by Dr. S. Mallikaarjun, Div. Biopharm.
"Synopsis: The sponsor has submitted incomplete information on the pharmacokinetics of amifostine at doses of 710 mg/m^2, with or without dexamethasone and metoclopramide. No information has been provided at 910 mg/m^2, the proposed dose.

Recommendation: The Human Pharmacokinetics Section of NDA 20-221, Amifostine cannot be evaluated, since the information requested by the Division of Biopharmaceutics has not been provided by the sponsor."

Comments :
Assay validation to date has been provided in the form of 2 published articles on the HPLC-EC assay method by the same author, who is the Investigator for two of the three ongoing pharmacokinetic studies in Amendment #17 (Dr. Shaw).

To date information on the 910 mg/m^2 PK study has not been provided. In Amendment #17 there is mention of 4 patients being treated previously at 910 mg/m^2 apart from the ongoing study ETH-PK3 . The information on these 4 patients cannot be located.

The sponsor stated that no dose proportionality information is available in humans.

The sponsor gave an article on the hydrolysis of amifostine as additional assay techniques to the submitted published articles on HPLC-EC method.

Information requested:
1. Provide information on the 4 patients on 910 mg/m^2 pharmacokinetic study by Dr. Shaw, including:
a. subject demography
b. formulation and dosing schedule
c. blood/urine sampling scheme
d. complete assay validation/methodology, not published reference.
e. methodology for pharmacokinetic analysis.

2. Provide the following information for the drug interaction study with dexamethasone and metoclopramide:
a.. individual and mean plasma concentration-time data and profiles
b. assay validation to include method validation, individual study validation

3. Provide the following information for the 740 mg/m^2 study by Dr. Shaw:
a. subject demography (data was provided only for patients with urinary excretion measurements)

b. study design
c. individual and mean plasma concentration-time data and profiles
d. assay validation
e. pharmacokinetic parameters both individual and mean.

4. Provide information on binding of the drug in human plasma/blood (some information provided in the form of 1994 ASCO abstract - full data/information needs to be provided).

The above information was requested on January 17, 1992 meeting with the sponsor.

5. Provide the following information for the study on single dose amifostine 910 mg/m^2 - ongoing study by Dr. Shaw:
a. study demography
b. individual and mean plasma concentration-time data and profiles
c. assay validation to include method validation, individual study validation
d. clinical study dates - start and end
e. analytical assay dates - start and finish
f. batch(es) of amifostine used in the study
h. define AUC as to whether it represents the AUC to last sampling time point or extrapolated to infinity

6. Provide the following information for the study in patients receiving 200-340 mg/m^2 given daily as 5-8 minute infusion (Dr. Shaw as investigator):
a. study demography
b. study design
c. individual and mean plasma concentration-time data and profiles
d. any determined individual and mean pharmacokinetic parameters
e. assay validation to include method validation, individual study validation
f. clinical study dates - start and end
g. analytical assay dates - start and finish

7. Provide the following information for the study on double dose (as two 15 minute infusions) amifostine with measurements of the free thiol in plasma and bone marrow cells:
a. study demography
b. individual and mean plasma concentration-time data and profiles
c. statistical methodology and analysis
d. individual pharmacokinetic parameters
e. assay validation to include method validation, individual study validation
f. clinical study dates - start and end
g. analytical assay dates - start and finish
h. batch(es) of amifostine used in the study
i. levels in bone marrow for all individuals
j. tables with p-values and a description of the statistical test used for comparison of dose 1 to dose 2

k. with the free thiol , define the average concentration and the test used for statistical comparison

l. define AUC as to whether it represents the AUC to last sampling time point or extrapolated to infinity

8. Provide the following information for the study on cisplatin and amifostine - investigator Dr. Schiller:

a. study demography
b. individual and mean plasma concentration-time data and profiles
c. individual pharmacokinetic parameters
d. assay validation to include method validation, individual study validation
e. clinical study dates - start and end
f. analytical assay dates - start and finish
g. batch(es) of amifostine used in the study

9. Provide information such as mass balance and activity of metabolites of amifostine.

10. Provide information on the stability of amifostine in the biological samples eg. how long these can be stored and at what temperature prior to analysis.

11. Provide complete information on the data used for the basis of the 1994 ASCO abstract (Shaw, Bonner, Nakashima et al.) with the method of analysis and the proposed PK/PD model and method of analysis.

Recommendation:

Amendments #17 and 18 of NDA 20-221 are unacceptable for adequate review by the Division of Biopharmaceutics. Since incomplete information has been provided for the Pharmacokinetic section, the firm cannot support the labeling claims described under Pharmacokinetics in the Clinical Pharmacology section of the proposed labeling. The information requests 1-11 should be forwarded to the sponsor. The firm should be sent updated guidelines from the Division of Biopharmaceutics.

11/22/94

Lydia C. Kaus, M.S., Ph.D.
Pharmacokinetics Evaluation Branch

FT 11/30/94
Mehul Mehta, Ph.D., Section Head.

cc
HFD-150 NDA 20-221 Div. files
HFD-150:McCollum

HFD-150:Delap
HFD-150:Schmidt
HFD-150:Tolgyesi
HFD-426:Biopharm/Drug File
HFD-426: Biopharm/Mehta
HFD-426: Biopharm/Fleischer
HFD-426: Biopharm/Chen L
HFD-340: Viswanathan

1. SINGLE DOSE STUDY

TITLE: Pharmacokinetics of single dose amifostine (WR-2721; Ethyol)

OBJECTIVES:
To evaluate the single dose pharmacokinetics of amifostine given as 910 mg/m^2 IV as a 15-minute infusion.

Clinical Investigator and Site: Leslie M. Shaw, Ph.D., Depart. Pathology and Lab. Med., Pennsylvania, PA.

Clinical Study Dates: Ongoing
Subject Demographics: Not provided
Drug Supplies: Not stated

STUDY DESIGN AND DOSAGE ADMINISTRATION:
13 patients were given amifostine 910 mg/m^2 IV as a 15-minute infusion. This was followed fifteen minutes later by an infusion of high-dose cisplatin.

BIOLOGICAL SAMPLING:
Blood samples were taken at 0, 3, 6, 9, 12, 15, 116, 16.5, 17, 17.5, 18, 20, 30, 45 and 60 minutes after the start of infusion of amifostine.

ANALYTICAL METHODOLOGIES:
HPLC-EC method. Validation data not provided.
Analytical Site: Same as investigator site.
Analytical Dates: Not provided.

PHARMACOKINETIC RESULTS:
No individual plasma concentration-time data was provided. A table summarizing the pharmacokinetic parameters, Cmax, Vdss, AUC, $t_{1/2}$, CL and MRT was provided for individual patients. The AUC was not defined as to whether it represented the AUC to last sampling time point or extrapolated to infinity. The investigator's intent is to combine the pharmacokinetics from a previous study of four patients on the same dose. Additional future studies were also very briefly described: assessment of the pharmacokinetics of amifostine after multiple dosing and the pharmacokinetics in patients receiving 200-340 mg/m^2 as a 5-8 minute infusion prior to radiation.

COMMENTS:
The study description, results and conclusions are incomplete for adequate review.

2. MULTI-DOSE STUDY

TITLE: Pharmacokinetics of double dose amifostine with corresponding measurements of WR-1065 in plasma and bone marrow cells.

OBJECTIVES: To evaluate the PK behavior of the parent drug, WR-2721, in cancer patients receiving 500 mg/m^2 carboplatin therapy.

Clinical Investigator and Site: Leslie M. Shaw, Ph.D., Depart. Pathology and Lab. Med., Pennsylvania, PA.

Clinical Study Dates: Ongoing
Subject Demographics: Not provided
Drug Supplies: Not stated

STUDY DESIGN AND DOSAGE ADMINISTRATION:
Seven patients have been enrolled to date. Each patient was administered a 15 minute infusion of 910 mg/m^2 amifostine followed by a 30 minute infusion of carboplatinum beginning 15 minutes post-infusion of amifostine. The second dose of 910 mg/m^2 of amifostine was administered 1 hour 45 minutes post-infusion of the first.

BIOLOGICAL SAMPLING: Blood samples were collected pre-dose, 3, 6, 9, 12, 15, 16, 17, 18, 19, 20, 21, 22, 25, 30, 45, 60 minutes post-dose.

ANALYTICAL METHODOLOGIES:
HPLC-EC method. Validation data not provided.
Analytical Site: Same as investigator site.
Analytical Dates: Not provided.

PHARMACOKINETIC RESULTS:
No individual plasma concentration-time data was provided. A table summarizing the pharmacokinetic parameters of Cmax Vdss, AUC, $t_{1/2}$, CL and MRT was provided for individual patients. The AUC was not defined as to whether it represented the AUC to last sampling time point or extrapolated to infinity. Individual plasma concentration data was provided for the free thiol metabolite WR-1065. Levels in bone marrow were not provided.

STATISTICAL ANALYSIS:
The firm presented basic statistical information on mean, kurtosis etc. of the PK data on amifostine from the two doses. No tables were produced with p-values and the statistical test used for comparison of dose 1 to dose 2 was not described. With the free thiol, the firm concluded that the average plasma concentration was no different between the two doses; the average concentration was not defined and the test for statistical comparison was not described.

COMMENTS:
The study description, results and conclusions are incomplete for adequate review.

3. DRUG INTERACTION STUDY

TITLE: Pharmacokinetics of cisplatin (CDDP) following a 15-minute infusion of amifostine.

OBJECTIVES: To determine the effect, if any, of a 15-minute infusion of amifostine on the pharmacokinetics of cisplatin.

Clinical Investigator and Site: Joan Schiller, M.D., Univ. Wisconsin Cancer Center, Madison, WI.

Clinical Study Dates: Ongoing
Subject Demographics: Not provided
Drug Supplies: Not stated

STUDY DESIGN AND DOSAGE ADMINISTRATION:
Cisplatin was administered as a 30-minute infusion, about 25 minutes post-infusion of amifostine to six patients. Amifostine was given as a 15-minute infusion at a dose of 740 mg/m^2. There was no analysis of amifostine in plasma.

BIOLOGICAL SAMPLING: Blood samples were collected pre-dose (cisplatin), 0, 15, 30, 60 and 90 minutes, and also 2, 3, 4, 6, 24, 30, 48 and 54 hours post-infusion of cisplatin. Urine was collected form the start of cisplatin infusion up to 48 hours post-infusion.

ANALYTICAL METHODOLOGIES:
Platinum was measured in plasma, ultrafiltered plasma and urine using Atomic Absorption method (the firm refer to a published method).
Analytical Site: Same as investigator site.
Analytical Dates: Not provided.

PHARMACOKINETIC RESULTS:
Mean total plasma Pt and ultrafilterable Pt pharmacokinetic parameters were summarized in a table. No individual plasma concentration-time data was provided. The AUC was not defined as to whether it represented the AUC to last sampling time point or extrapolated to infinity.

STATISTICAL ANALYSIS:
The firm presented the mean pharmacokinetic parameters obtained in the study and that from six published reports. No statistical (meta-analysis for instance) was carried out for comparison across studies.

COMMENTS:
The study description, results and conclusions are incomplete for adequate review.

Amifostine was given at a reduced dose, not the initial 910 mg/m^2 dose recommended in the labeling.

The study population for the PK study was patients with non-small cell lung cancer. The proposed labeled population are patients with ovarian cancer.

CLINICAL PHARMACOLOGY & BIOPHARMACEUTICS REVIEW

Submission :	NDA Original Amendment (AL): **NDA 20,221**
Submission Date:	October 27, 1995
Established name:	**Amifostine (WR-2721, Ethyol^{R})**
Sponsor:	U.S. Bioscience, Inc. One Tower Bridge, 100 Front Street West Conshohocken, PA 19428
Formulation:	Lyophilized powder for reconstitution (intravenous administration)
Reviewer:	Peter Zannikos, Ph. D.

BACKGROUND:

In this submission, the sponsor has responded to the approvable letter for Ethyol (WR-2721, amifostine) dated October 6, 1995. A point-by-point response to the following pharmacokinetics/ biopharmaceutics issues has been submitted for review:

ATTACHMENTS

4.1) the need for evaluation of gender differences in the clearance of amifostine

4.2) the need for additional drug-drug interaction studies with amifostine (and its active metabolite WR-1065) and representative therapeutic agents

4.3) characterization of amifostine and WR-1065 protein binding over the therapeutic range

4.4) submission of the ongoing clinical trial entitled "Phase I Study of Bone Marrow Protection by WR-2721 (amifostine) in Patients with Solid Tumors Treated with Carboplatin" at its completion

ATTACHMENT 4.1

There is some evidence for gender differences in the clearance of Ethyol. This is based on data combined from the two pharmacokinetic studies (ETH-PK 1 & 3). On average, there appeared to be a 30 % lower clearance of amifostine in women than in men. These results can only be considered preliminary, however, in view of the small sample size, and the inclusion of data from subjects who received either 740 or 910 mg/m² dose. As you hope to expand claims for amifostine beyond ovarian cancer, it will be important to examine the potential for differences in pharmacokinetics between men and women. You should design a study to address this issue.

Sponsor's Response

When data from various studies evaluating WR-2721 pharmacokinetics in patients dosed with 740 mg/m² are combined, the clearance of WR-2721 is found to be approximately 40 % lower in females as compared to males (2.5 versus 4.3 L/min). The sponsor reports that this does not reach statistical significance. In contrast, the mean clearance at the 910 mg/m² dose level for both genders are within 5 % of each other. An analysis of variance, which included both dose and gender effects, indicates that there is no evidence for a difference in the clearance between genders.

Comments

1. In comparison to the sponsor's reported p value of 0.148, a re-analysis of the WR-2721 data at the 740 mg/m² dose yielded a similar mean clearance (and standard deviation) but a p value of 0.09 (two-sample, two-sided t-test, homoscedastic variance assumed). When the corrected value of 1.79 L/min for patient [redacted] in the metoclopramide/WR-2721 study is included, the differences in mean clearance between genders reaches statistical significance ($p = 0.046$). In agreement with the sponsor, there is virtually no difference in the clearance of WR-2721 in male and females at 910 mg/m², the dose most frequently used in the clinical trials (two-sample t-test).

2. The ANOVA provided by the sponsor indicates a 31 % lower clearance at the 910 mg/m² dose as compared to the 740 mg/m² dose (2.4 versus 3.5 L/min; $p < 0.02$). In Amendment #023, the sponsor included an abstract which provided evidence for dose-dependent WR-2721 clearance. The authors hypothesized that capacity-limited metabolism of alkaline phosphatase, the enzyme which converts amifostine to its active metabolite occurs during drug infusion.

3. The difficulty in evaluating these data is that the assessment of dose proportionality and potential differences in WR-2721 pharmacokinetics between genders were not studied prospectively. Further, the sponsor has combined results from four separate studies performed over a number of years resulting in variability which is difficult to take into account. At present, the clinical relevance of the apparent non-linearity in WR-2721 clearance cannot be determined.

ATTACHMENT 4.2

The possibility that Ethyol or its active metabolite might affect the pharmacokinetics of co-administered chemotherapeutic agents has not been adequately addressed. You should describe the effects of Ethyol administration on the pharmacokinetics of representative chemotherapeutic agents that will be given while WR-1065 is still present.

Sponsor's Response

WR-2721/Cisplatin

Data on the effect of WR-2721 on the pharmacokinetics of cisplatin was submitted to the NDA in Amendment #18.1 (September, 1994). The pharmacokinetics of cisplatin in these patients pre-treated with WR-2721 were reported to be comparable to published pharmacokinetic data evaluating patients treated with cisplatin alone.

WR-2721/Carboplatin

A copy of an abstract discussing this information has been provided in this amendment. Pharmacokinetic data from eleven patients treated with carboplatin (400 or 500 mg/m^2) and three doses of WR-2721 (740 - 910 mg/m^2) were compared with that from six patients treated with carboplatin alone (400 mg/m^2). The results suggest that WR-2721 increased the area under the curve and half-life of carboplatin, total platinum and ultrafilterable platinum. This is believed to be associated with transient hypotension leading to a transient decrease in renal function.

These results contrast those published by Budd et al. (also provided in the amendment). The pharmacokinetics of carboplatin were assessed in patients treated with WR-2721 (740 mg/m^2) prior to and two hours following the carboplatin infusion (525 and 625 mg/m^2). These data show that the pharmacokinetics for carboplatin in patients treated with WR-2721 are similar to those reported for carboplatin alone.

Recently, pharmacokinetic data has been obtained from Vermorken et al. on four patients who were treated with carboplatin alone and four weeks later with carboplatin and three doses of WR-2721 (740 mg/m^2). WR-2721 was administered prior to and at 2 and 4 hours after the carboplatin. Pharmacokinetic parameters for total platinum, ultrafilterable platinum and intact carboplatin were calculated. Differences in area under the curve, initial half-life, terminal half-life, volume of distribution, clearance and percent of dose excreted in the urine were compared using a paired t-test. None of the comparisons were statistically significant.

In support of these findings, the sponsor points out that in randomized clinical trials where patients pre-treated with WR-2721 prior to receiving cisplatin and cyclophosphamide, no new adverse events were reported. Known serious toxicities with these drugs were decreased with WR-2721 without decreasing efficacy. Similarly, WR-2721 reduced the toxicities associated with carboplatin in two randomized clinical trials.

The sponsor proposes a new study to evaluate the effect of WR-2721 on the pharmacokinetics of paclitaxel, since this drug is commonly used in combination with cisplatin for the treatment of advanced solid tumors.

Comments

1. As indicated in the Biopharmaceutics Review dated Sept. 18, 1995, deficiencies in the WR-2721/cisplatin study including the lack of a control group, differences in assay methodology and pharmacokinetic analysis between studies etc.. make it difficult to draw firm conclusions regarding the effect of WR-2721 on cisplatin pharmacokinetics. The study by Budd et al. (WR-2721/carboplatin) suffers from similar deficiencies. Results of these studies should not be included in the labeling.

2. The final report by Vermorken et al. should provide supportive information regarding the potential for interaction between carboplatin and WR-2721. Unfortunately, the starting dose recommended in the labeling (910 mg/m^2) was not used limiting the relevance of this study. Future drug interaction studies should be performed at the dose recommended in the labeling.

3. The sponsor is encouraged to submit the proposed protocol evaluating the effect of WR-2721 on paclitaxel disposition for review. Preliminary recommendations include:

i) the use of doses recommended in the labeling (ie.910 mg/m^2 WR-2721)
ii) a crossover design where each subject receives paclitaxel with and without WR-2721
iii) justification of sample size based on a power analysis
iv) sampling for over 3 half-lives to adequately describe the pharmacokinetics of the compounds of interest
v) assay validation data for all species measured should be provided

ATTACHMENT 4.3

Plasma protein binding of Ethyol and its metabolites over the therapeutic range has not been characterized.

Sponsor's Response

The protein binding of amifostine and the free thiol (WR-1065) has recently been characterized by Dr. Leslie Shaw, Director of Clinical Pharmacology at the Hospital of the University of Pennsylvania. The studies demonstrate that little, if any, amifostine is bound to plasma protein. In contrast, 45 - 65 % of the free thiol, WR-1065, is bound to albumin (see APPENDIX).

Comments

1. Unfortunately, the experiments were carried out at room temperature. Since plasma protein binding is, in general, influenced by temperature, it is difficult to determine if these results represent binding *in vivo*. Results from these studies should not be included in the labeling.

ATTACHMENT 4.4

The clinical trial entitled "Phase I Study of Bone Marrow Protection by WR-2721 (amifostine) in Patients with Solid Tumors Treated with Carboplatin" is apparently ongoing and will provide information on the pharmacokinetics of the active metabolite. We would like to know when the results of that study are anticipated. A report of the study should be provided to the agency as soon as possible. The report should include subject demography, including any medications taken during the study periods, individual and mean plasma concentration-time data for amifostine and WR-1065 after single versus multiple dosing, and assay validation for all species measured.

Sponsor's Response

Accrual has been completed. The sponsor intends to submit this data by the end of the year.

TEAM LEADER'S COMMENTS ON PACKAGE INSERT

Draft Labeling (October 26, 1995)	Proposed Revision
Pharmacokinetics: Clinical Pharmacokinetic studies show that ETHYOL is rapidly cleared from the plasma with an α t½ of < 1 minute and β t½ of approximately of 8 minutes.	**Pharmacokinetics:** Clinical Pharmacokinetic studies show that ETHYOL is rapidly cleared from the plasma with a distribution half-life of < 1 minute and an elimination half-life of approximately of 8 minutes.

RECOMMENDATION:

The amendment to pending application NDA 20,221 (WR-2721, Ethyol) has been reviewed. The general comments should be conveyed to the sponsor.

Atiqur Rahman, Ph.D. 12/6/95
Group Leader
Pharmaceutical Evaluation I

Peter N. Zannikos, Ph.D. 12/6/95
Pharmaceutical Evaluation I

cc. HFD-150: NDA 20-221 Div. Files
HFD-150: Delap
HFD-426: Biopharm/Drug File
HFD-860: Biopharm/Malinowski
HFD-860: Biopharm/Mehta
HFD-860: Biopharm/Rahman
HFD-860: Biopha..n/Zannikos
HFD-150: McCollum

APPENDIX:

Ethyol Clearance: 910mg/m²

Study	Patient ID	Gender	Clearance (L/min)
Various (ETH-3)		M	
		F	
		F	
		F	
		M	
		F	
		F	
		M	
		M	
		F	
		M	
		M	
		M	
Ethyol± metoclopromide (ETH-1)		M	
		F	
		M	
		F	
		F	
Ethyol+ carboplatin (2 doses of Ethyol 2 hours apart) (Amendment 18.1)		M	
		F	
		M	
		F	
		F	
		M	
		M	
Mean	(n = 25 patients)		2.5 ± 1.0 (n = 32 clearance values)

Ethyol Clearance, 740 mg/m²

Study	Patient ID	Gender	Clearance (L/min)
Ethyol and calcium metabolism (Amendment 18.1)		M	
		M	
		M	
		F	
		?	
Ethyol ± dexamethasone (ETH-1)		F	
		M	
		M	
		M	
		M	
		M	
Ethyol ± metoclopromide (ETH-1)		F	
		F	
		M	
Mean	(n = 14)		3.5 ± 1.6

Amifostine Binding to Plasma Proteins Using Ultrafiltration

Methods:

Incubations were carried out at room temperature for a minimum of one hour (personal communication with Dr. Leslie Shaw, 11-04-95). Samples were pipetted into Amicon centrifree micropartition system devices. The free fraction of the microsolute was seperated from the protein bind fraction by centrifugation. All samples were prepared in duplicate.

Amifostine binding to human albumin, globulin-free
albumin - 40 mg/ml
[^{14}C]amifostine - 10, 100 and 1000 µmole/L

Amifostine binding to human albumin, fatty acid-free
albumin - 40 mg/ml
[^{14}C]amifostine - 10, 20, 50, 100, 200, 500, 1000 and 2000 µmole/L

Amifostine binding human alpha-1-acid glycoprotein
alpha-1-acid glycoprotein - 1.875 mg/ml
[^{14}C]amifostine - 10, 20, 50, 100 and 200 µmole/L

Amifostine binding to cancer study patient plasma
[^{14}C]amifostine - 100 µmole/L

WR-1065 binding to human albumin, fatty acid-free
albumin - 40 mg/ml
[^{14}C]WR-1065 - 10, 20, 50, 100 and 200 µmole/L

WR-1065 binding to human alpha-1-acid glycoprotein
alpha-1-acid glycoprotein - 1.875 mg/ml
[^{14}C]WR-1065 - 10, 20, 50, 100 and 200 µmole/L

WR-1065 binding to cancer study patient plasma
[^{14}C]WR-1065 - 100 µmole/L

BIOPHARMACEUTICS/PHARMACOKINETICS REVEIW

Submission :	Amendments to pending **NDA 20,221**
Submission Dates:	July 10, 1995 Sept. 21, 1994
Established name:	**Amifostine (WR-2721, Ethyol®)**
Sponsor:	**U.S. Bioscience**, Inc. One Tower Bridge, 100 Front Street West Conshohocken, PA 19428
Formulation:	Lyophilized powder for reconstitution (intravenous administration)

SYNOPSIS

In this submission the sponsor has provided descriptive pharmacokinetics of WR-2721 in cancer patients. Average WR-2721 peak concentrations of 164 µmole/L and 280 µmole/L are achieved after a 740 mg/m^2 and 910 mg/m^2 dose, respectively, when administered as a single fifteen minute intravenous infusion. WR-2721 exhibits short distribution (≈1 minute) and elimination half-lives (≈ 8 minutes). More than 90 % of WR-2721 is cleared from the plasma within 6 minutes of completion of the infusion. The short half-life of WR-2721 is attributable to the relatively small distribution volume (Vdss: 7 - 30 L) and high systemic clearance (2.0 - 4.3 L/min). An analysis of combined data from two studies is suggestive of a 30 % lower clearance of WR-2721 in females as compared to males. Limited information has been provided in regards to pharmacokinetics of WR-2721 after multiple dosing. WR-2721 does not appear to accumulate when dosed every 2 hours (740 mg/m^2). In contrast, a two-fold increase in the levels of the active metabolite, WR-1065, were observed following this treatment regimen. Binding of WR-2721 to plasma proteins has not been evaluated. Although a range of doses has been proposed by the sponsor (740 vs 910 mg/m^2), no formal dose proportionality studies have been performed. The sponsor has not provided any information regarding the influence of age or gender on the pharmacokinetics of WR-2721 or possible changes in drug disposition in patients with impaired renal and/or hepatic function. Premedication with either metoclopramide or dexamethasone was

found to have no noticeable effect on the levels of WR-2721. The disposition of the active metabolite, WR-1065, has not been characterized to any significant extent.

Recommendation: The NDA 20,221 (Ethyol, WR-2127) has been reviewed and has been found to partially meets the requirements of tne Division of Biopharmaceutics. The list of deficiencies and comments should be conveyed to the sponsor.

9/18/95
Mehul Mehta, Ph.D.
Section Head
Pharmacokinetics Evaluation Branch I

9/18/95
Peter N. Zannikos, Ph.D.
Pharmacokinetics Evaluation Branch I

cc. HFD-150: NDA 20-221 Div. Files
HFD-426: Biopharm/Drug File
HFD-426: Biopharm/ChenL
HFD-150: Biopharm/Mehta
HFD-426: Biopharm/Fleischer
HFD-150: Delap
HFD-150: McCollum
HFD-340: Viswanathan
HFD-150: Tolgyesi

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ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the plasma-concentration time curve
Cl	Clearance
Cmax	Peak plasma concentration
CV	Coefficient of variation
DV	Observed Concentration (NOMEM output)
HPLC	High performance liquid chromatography
i.v.	Intravenous
L	Liter
min	Minute
MTD	Maximum tolerated dose
Pred	Predicted concentration (NONMEM output)
std	Standard deviation
Vd	Volume of distribution
WR-1065	Amifostine active metabolite, free thiol $NH_2(CH_2)_3NH(CH_2)_2SH$
WR-2721	Amifostine, ethyol $NH_2(CH_2)_3NH(CH_2)_2S\text{-}PO_3H_2$
WR-33278	Amifostine metabolite, disulfide $[NH_2(CH_2)_3NH(CH_2)_2S\text{-}]_2$

BACKGROUND

Ethyol (WR-2721) is a prodrug that is dephosphorylated to an active metabolite (WR-1065) at the tissue site by a membrane bound alkaline phosphatase. Once it has entered the cell, WR-1065 has the ability to detoxify the active species of alkylating and platinum agents. Differential metabolism and uptake of WR-1065 into normal tissue versus tumor tissue is the basis for the apparent selective protection of normal tissue by WR-2721. Both animal and cell culture studies indicate that differences in capillary alkaline phosphatase and pH differences favor the conversion of the prodrug to the active form in normal tissue. In addition, cell culture studies have shown that normal tissue incubated with [^{35}S]WR-2721 demonstrate facilitated uptake of radiolabel (either WR-2721 and/or WR-1065) against a concentration gradient, whereas tumor tissue relies on passive absorption. Preferential uptake of WR-1065 into normal tissue as compared to tumor *in vivo* following WR-2721 administration in humans has not been demonstrated.

PHYSICOCHEMICAL PROPERTIES

Amifostine (WR-2721, Ethyol) is an organic phosphorothioate cytoprotective agent. It is chemically known as ethanethiol, 2-[(3-aminopropyl)amino]-,dihydrogen phosphate (ester) and has the following structural formula:

$$H_2N(CH_2)_3NH(CH_2)_2S\text{-}PO_3H_2 \quad \text{(molecular weight: 214.22)}$$

It is a white crystalline powder which is freely soluble in water.

FORMULATION

Ethyol is manufactured by Ben Venue Labs. and supplied as a sterile lyophilized powder mixture with mannitol. It requires reconstitution for intravenous infusion. Each vial contains 500 mg of WR-2721 and 500 mg of mannitol.

INDICATIONS & USAGE

Ethyol is indicated as a cytoprotective agent against the acute and cumulative hematologic and renal toxicities associated with alkylating agents such as cyclophosphamide and platinum agents such as cisplatin in patients with ovarian cancer.

RECOMMENDED DOSAGE & ADMINISTRATION

The starting dose of Ethyol is 910 mg/m^2 administered once as a minute i.v. infusion starting within minutes prior to chemotherapy.

The infusion should be interrupted if the systolic blood pressure significantly decreases. -If the blood pressure returns to normal with 5 minutes, the infusion may be restarted so that the full dose is administered. If the full dose of Ethyol cannot be administered, the dose of Ethyol for subsequent cycles should be 740 mg/m^2.

It is recommended that antiemetic medication be administered prior to and in conjunction with Ethyol.

SUMMARY OF PHARMACOKINETICS STUDIES

I. Bioavailability/Bioequivalence

No bioavailability/bioequivalence studies have been performed since Ethyol will be administered intravenously.

II. Pharmacokinetics (from Reports ETH-PK-1 and ETH-PK-3)

The pharmacokinetics of WR-2721 were investigated in a number of clinical trials. In all cases the participants were cancer patients. WR-2721 was administered as a min intravenous infusion. Following a dose of 740 mg/m^2 an average maximum concentration of µmole/L is achieved as compared to µmole/L after a 910 mg/m^2 dose. Typically, the concentration time profile for WR-2721 is characterized by a two-compartment model. A rapid

"distribution phase" (half life ≈ 1 min) is evident during which a majority of the drug is cleared from the systemic circulation followed by a "terminal elimination phase" (half life ≈ 8 min). The short half-life of WR-2721 can be attributed to the rapid clearance (L/min) and small distribution volume (Vdss, 15.9.± 17.0 L). No information has been provided in regards to the binding of WR-2721 to plasma proteins.

Limited information is available in regards to the pharmacokinetics of WR-2721 after multiple dosing. WR-2721 does not appear to accumulate when dosed every 2 hours (740 mg/m^2). In contrast, a two-fold increase in the peak levels of the active metabolite, WR-1065, was observed following this treatment regimen (see Phase 1 Study of Bone Marrow Protection by Ethyol in Patients with Solid Tumors Treated with Carboplatin). Plasma levels of WR-1065 have not been further characterized.

Levels of the free thiol were measured in bone marrow cells of three patients after i.v. infusion of WR-2721. Several problems with "infusion technique and consistency with amifostine administration" makes it difficult to determine the amount of the targeted 910 mg/m^2 dose that was actually administered. Concentrations between μmol/kg were reported in these bone marrow aspirates minutes following completion of the infusion.

III. Metabolism & Excretion

Ethyol (WR-2721) is a prodrug that is dephosphorylated to a pharmacologically active metabolite, the free thiol (WR-1065), at the tissue level site by alkaline phosphatase. A disulfide metabolite (WR-33278; $[NH_2(CH_2)_3\text{-}NHCH_2CH_2S\text{-}]_2$) is subsequently produced and is less active than WR-1065. After administration of 150 mg/m^2 as a 10 second bolus, the urinary excretion of WR-2721, WR-1065 and WR-33278 was 0.69±0.42, 2.64±3.6 and 2.22±2.9 % of the WR-2721 dose, respectively, within 45 minutes (n=6, data published article). Similar results were seen following a 15 minute infusion of 740 mg/m^2 where the mean percent excretion of WR-2721 was 1.1±1.0 % within 45 minutes after the infusion. No mass balance studies have been done with WR-2721.

IV. Dose Proportionality

The recommended starting dose of Ethyol is 910 mg/m^2. If the full dose cannot not be tolerated, the recommended dose of Ethyol for subsequent cycles is 740 mg/m^2.

No studies have been performed specifically aimed at comparing the disposition of WR-2721 and/or the active metabolite in the recommended dose range. A published abstract (Appendix 1) which included data from the NDA submission suggests a greater systemic clearance of WR-2721 at the lower dose (L/min for 910 mg/m^2 vs L/min for 740 mg/m^2). However, problems with study design (ie. two different groups of patients evaluated over a number of years) make it difficult to draw firm conclusions from this study

V. Special Populations

A. Gender

As part of the review, pharmacokinetics parameters were compared with respect to differences due to gender. For this analysis, data from the dexamethasone/metoclopramide plus WR-2721 interaction study (ETH-PK-1; baseline data) and trials performed at the (ETH-PK-3) were combined. Females appear to have a 30 % lower clearance as compared to males (2.88±1.2 vs. 2.04±0.68 L/min; $p<0.05$, 2-sample t-test). No other differences in WR-2721 disposition could be detected.

B. Renal Impairment: Not evaluated

C. Hepatic Impairment: Not evaluated

D. Pediatrics: Not evaluated

VI. Drug Interactions

Pretreatment with dexamethasone or metoclopramide prior to WR-2721 administration does not appear to effect the pharmacokinetics profile of WR-2721 (see ETH-PK-1).

VII Pharmacokinetics/Pharmacodynamic Relationships

The sponsor did not provide any information on pharmacokinetics/pharmacodynamic relationships in the submission.

VIII. Formulation

Five batches of WR-2721 were manufactured by Ben Venue Labs. to supply investigators participating in the pivotal clinical study. Drug product from these lots was available for use to evaluate the pharmacokinetics of WR-2721 in Study ETH-PK-3. The source of WR-2721 used in the other pharmacokinetics studies was not specified.

IX. Assay Methodology

Two publications regarding analysis of WR-2721 and WR-1065 have been submitted as part of the NDA (J Liq Chrom, 7, 1984; J Liq Chrom, 9, 1986). The first author to both papers is Dr. Leslie Shaw from the University of Pennsylvania. He was also the principal investigator to most of the pharmacokinetic studies submitted in the NDA package (Report ETH-PK 1, ETH-PK 3 and the two Phase I studies). During a telecon with Dr. Shaw (8/20/95), he indicated that the assay methodology used to quantitate WR-2721 throughout the clinical trials was consistent with methods described in the publications. At the Division's request, Dr. Shaw provided additional assay validation data (Appendix II).

The concentration of WR-2721, WR-1065 and the symmetrical disulfide WR-33278 in plasma and urine samples were measured by a high performance liquid chromatography system equipped with an Hg/Au electrochemical detector. Prior to measuring WR-2721, internal standard and acetonitrile was added (the latter to precipitate proteins). The samples were centrifuged and an aliquot of supernatant was injected. The mobile phase for parent WR-2721 was an aqueous solution of monochloroacetic acid and sodium octylsulfate, pH = 3.0. Samples to be analyzed for WR-2721 metabolites were prepared similarly. However, a solution of perchloric acid and EDTA was used to precipitate proteins and stabilize the free thiol. Elution of WR-1065 was achieved using a 40 % methanol/water mobile phase containing monochloroacetic acid and sodium octylsulfate, pH = 3.0. WR33278 was eluted with an aqueous mobile phase that

contained ethylamine, 1 % methanol and monochloroacetic acid, pH=3.0 (Shaw & Bonner, J Liq Chrom, 10, 1987).

	WR-2721*	WR-1065 *	WR33278**
Limit of quantitation	?	0.1 µmol/L	0.2 µmol/L
Linearity	1-1000 µmol/L r^2=0.998	2.5 - 250 µmol/L r^2=0.998	1-1000 µmol/L r^2=0.999
Accuracy$	-13.0 - 6.0 %	0.2 - 7.2 %	-20 - 12.4 %
Precision (CV)			
within run	1.2 - 6.5 %	1.7 - 2.7 %	1.2 - 1.8 %
between run	8.1 - 13.2 %	?	?
Absolute Recovery	76.5 %	?	86.7 - 108 %
Specificity	satisfactory	satisfactory	satisfactory
Internal Std.	WR80855	WR251833	WR183159

* data obtained from NDA submission and published reports; the matrix was plasma
**data obtained solely from published reports; the matrix was whole blood and liver tissue
$ percent deviation of the measured concentration from the spike-in concentration

No assay validation data was provided in regards to measurement of WR-2721 and metabolites in urine.

DEFICIENCIES:

1) There appears to be some evidence of nonlinearity in the disposition of WR-2721 at the doses recommended. This preliminary data is based on a published abstract submitted in the NDA which shows a decrease in clearance of WR-2721 when 910 mg/m^2 is administered L/min) as compared to the 740 mg/m^2 dose L/min). The sponsor should perform studies specifically designed to compare the pharmacokinetics of WR-2721 in range of doses found in the labeling.

2) There is some evidence for gender differences in the clearance of WR-2721. This is based on data combined from two pharmacokinetics studies (ETH-PK 1 & 3). On average, there appeared to be a 30 % lower clearance of WR-2721 in women as compared to men. However, these results can only be considered preliminary given: i) the small sample size, ii) the analysis includes data from subjects who received either the 740 or 910 mg/m^2 dose which may confound the results and iii) evaluating the effect of gender on WR-2721 pharmacokinetics was not a stated objective of these clinical trials *a priori*. The sponsor should design a prospective study(ies) to specifically address this issue.

3) The protective effects of WR-2721 are attributed to its active metabolite, WR-1065. Lack of proper evaluation of the WR-1065 disposition in humans represents a major deficiency in this submission.

4) Considering that WR-2721 is to be administered immediately prior to the administration of chemotherapeutics agents, the sponsor has not adequately addressed the possibility of drug interactions. This is somewhat justifiable given the short half-life of WR-2721. In contrast, levels of active metabolite (WR-1065) appear to accumulate when dosed approximately every 2 hours suggesting a longer half-life for this species. It would seem that there is the possibility for WR-1065 to interact with other co-administered drugs. The sponsor should describe the effect of WR-2721 administration on the

pharmacokinetics of representative chemotherapeutic agents.

5) Plasma protein binding of WR-2721 and its metabolites over the therapeutic range has not been characterized.

COMMENTS (general)

1) No information has been provided in the submission in regards to the vehicle used to dilute WR-2721 prior to administration. Dr. Shaw has indicated (personal communication 8-20-95) that the diluent used could have been one of the following:
   i) lactated solution buffered with to pH
   ii) sodium chloride, %
   iii) D5W
   The sponsor recommends reconstituting Ethyol for injection with sodium chloride, USP 0.9 % in the proposed labeling.

2) The clinical trial entitled "Phase I Study of Bone Marrow Protection by Ethyol (Amifostine) in Patients with Solid Tumors Treated with Carboplatin" is apparently ongoing. A final report should be provided to the agency when the study has been completed particularly if no other human data will be submitted in regards to the disposition of the active metabolite (WR-1065). The following information should be submitted for review:
   i) Clinical start and end dates
   ii) Subject demography including concurrent medications used during the study periods
   iii) Individual and mean plasma concentration-time data
   iv) Statistical methodology for analyzing changes in the disposition of WR-2721 and its metabolites after single versus multiple dosing
   v) Assay validation for all species measured

COMMENTS (Package insert):

Pharmacokinetics: Clinical pharmacokinetic studies show that ETHYOL is rapidly cleared from the plasma with an α t1/2 of < 1 minute and a β t1/2 of approximately 8 minutes. [a]Less than 10 % of ETHYOL remains in the plasma 6 minutes after drug administration. ETHYOL is rapidly metabolized, especially in normal tissues to the active free thiol metabolite WR-1065. The disulfide metabolite, WR-33278 which is subsequently produced, is less active than WR-1065. [b]After a 15-minute infusion of a dose of 740-910 mg/m^2, renal excretion of ETHYOL and its two metabolites, WR-1065 (free thiol) and WR-33278 (disulfide), during the hour following drug administration was low, averaging 0.69%, 2.64% and 2.22% of the administered dose, respectively. [c]Concentrations of 82-227 lmol/L of the free thiol metabolite, WR-1065, are detectable in aspirated bone marrow cells at 5-8 minutes after intravenous infusion of 910 mg/m^2 of amifostine. [d]This observation, taken together with its rapid clearance from plasma, its very small volume of distribution and the low percentage of ETHYOL and metabolites excreted in the urine are consistent with the rapid dephosphorylation and entry into normal tissues that is suggested by animal studies. Pretreatment with dexamethasone or metoclopramide has no effect on ETHYOL pharmacokinetics. [e]Likewise, studies in six patients show no apparent effect of ETHYOL on the pharmacokinetics of cisplatin administered 15 minutes after the completion of the amifostine infusion.

[a] Based on AUC data. Correct as written.

[b]An incorrect statement and should be changed to: **After a 10-second bolus dose of 150 mg/m^2**, renal excretion of ETHYOL and its two metabolite, WR-1065 (free thiol) and WR-33278 (disulfide) during the hour following drug administration was low averaging 0.69 %, 2.64 % and 2.22 % of the administered dose, respectively.

[c]Details of the studies involving repeat WR-2721 dose administration and resulting concentrations in plasma and aspirated bone marrow were requested by the Division. The sponsor has subsequently (July 10, 1995) requested to withdraw this data from consideration due to "problems with infusion technique and consistency with amifostine administration." **The sponsor should therefore remove this statement from the labeling.**

[d]This statement should be changed to: Measurable levels of the free thiol metabolite, WR-1065, have been found in bone marrow cells 5 - 8 minutes after intravenous infusion of amifostine. This observation, taken together with its rapid clearance from plasma, its very small volume of distribution and the low percentage of ETHYOL and metabolites excreted in the urine are consistent with the

rapid dephosphorylation and entry into normal tissues that is suggested by animal studies.

'The information in the proposed labeling regarding cisplatin should be deleted. There are several problems with the design of the study supporting this statement, some of which were cited by the investigators:

i) "First, none of these patients were given cisplatin alone, so no control group is available for direct comparison"

ii) "Second, an extensive literature search reveals no studies using the 30 minute infusion time at a dose (of cisplatin) close to one used here."

iii) Other problems cited by the investigators include the use of assays in earlier studies with poor sensitivity for ultrafilterable platinum measurement, differences in the way pharmacokinetic data was analyzed and the limited availability of some pharmacokinetic data.

iv) In addition, the sample size is relatively small (n=6) and validation data regarding the cisplatin assay methodology were not provided.

These deficiencies in the study make it difficult to draw firm conclusions regarding the effect of WR-2721 on cisplatin pharmacokinetics.

TITLE: **PHARMACOKINETICS OF WR-2721 WITH AND WITHOUT METOCLOPRAMIDE OR DEXAMETHASONE**

REPORT: ETH-PK-1

INVESTIGATOR: Leslie M. Shaw, Ph.D.
Dept. of Pathology and Laboratory Medicine
Hospital of the University of Pennsylvania

STUDY DATES: 1987 - 1989

REPORT DATE: May 22, 1992 (Revised September, 1994)

OBJECTIVE: To evaluate the pharmacokinetics of WR-2721 administered as an intravenous (i.v.) infusion with and without dexamethasone or metoclopramide pretreatment.

METHODS:

Study Drug

WR-2721 (dihydrogen phosphorothioate, amifostine, Ethyol). No information regarding WR-2721, batch or lot information was submitted. Throughout the clinical trials various diluents were used to reconstitute WR-2721 prior to administration. These included: i) lactated solution buffered with sodium bicarbonate to pH 7.4, ii) sodium chloride, % and iii) D5W (personal communication with Dr. Shaw on 8-20-95).

Parenteral formulations of dexamethasone and metoclopramide are commercially available, however, no information has been submitted with regards the source of these drugs.

Subjects

No subject exclusion/inclusion criteria was incorporated in the study. Six patients (1 female, 5 males) aged 34 to 62 years were administered WR-2721 with and without dexamethasone. Nine patients (6 females, 3 males) aged 28 to 70 years were included in the metoclopramide WR-2721 interaction trial. Most patients were diagnosed with Stage IV melanoma. In two subjects the primary tumor site was the breast (see Table 1).

Study Protocol

WR-2721 +/- dexamethasone
Six patients were randomized to receive a single dose of i.v. WR-2721 (740 mg/m^2 over 15 min) with and without dexamethasone pretreatment (10 mg i.v. over 15 min) in a cross-over study

design. The dexamethasone infusion was started 30 minutes prior to administration of WR-2721 (personal communication with Dr. Shaw, 8/20/95). The washout period between treatments was 3-4 weeks.

WR-2721 +/- metoclopramide

Nine patients were randomized to receive WR-2721 with and without metoclopramide pretreatment (1-2 mg/kg i.v. over 15 minutes) in a cross-over design. Four patients were intravenously administered 740 mg/m^2 WR-2721 and five received 910 mg/m^2 WR-272 over a 15 minute period. The metoclopramide infusion was started 30 minutes prior to administration of WR-2721 (personal communication with Dr. Shaw, 8/20/95). The washout period between treatments was 3-4 weeks.

Sample Collection

Blood samples were collected at 0, 3, 6, 9, 12, 15, 16, 16.5, 17, 17.5, 18, 20, 30, 45 and 60 minutes after the start of the WR-2721 infusion.

Assay

A high performance liquid chromatographic system equipped was a Hg/Au electrochemical detector was utilized to measure WR-2721 concentrations in blood samples. Based on published literature, an internal standard (WR-80855) was added to each sample.

Pharmacokinetics/Statistical Analysis

Pharmacokinetic parameters were estimated using the Lagran7p model-independent non-linear regression package. The effects of pretreatment with the two antiemetics on WR-2721 disposition were analyzed further by analysis-of variance (ANOVA). With respect to systemic exposure, logarithmically transformed AUC were used. The data for both doses of WR-2721 were pooled when examining (ANOVA) the effect of concurrent metoclopramide treatment. Effects due to sequence of drug administration, subject (within sequence), period of drug administration and pretreatment were also considered. Type III Sums of Squares were performed testing the significance of each effect after adjusting for all other effects.

As a part of the review, a population pharmacokinetics approach was used to describe the disposition of WR-2721. The baseline data (ie. without antiemetic pretreatment) were combined with data from one other clinical trial (Appendix I; Population Pharmacokinetics Analysis of WR-2721 (NONMEM)).

RESULTS:

The pharmacokinetics of WR-2721 are presented in Table 2-5. Mean baseline values for clearance, Vdss, half-life and observed Cmax were L/min, L, min and

μmole/L after the 740 mg/m^2 dose (n=10) and L/min, min
and μmole/L after the 910 mg/m^2 dose (n=5), respectively.

The disposition of WR-2721 (including systemic clearance, volume of distribution, half-life and peak blood level) did not appear to be significantly altered by pretreatment with either dexamethasone or metoclopramide (Tables 2-5).

CONCLUSION:

This trial may be considered to be a pivotal pharmacokinetics study. It appears to be well controlled and included a moderate sample size. Besides estimation of basic pharmacokinetic parameters, this study provided evidence for a lack of interaction between WR-2721 and two commonly used antiemetics.

Table 1.

Patient Demographics: WR-2721 ± Dexamethasone

Pt. #	Sex	Age	Weight (kg)	Height (cm)	Body surface (m^2)	Primary Site	Stage	Performance Status
	F	34	62.0	165.0	1.66	Melanoma	IV	0
	M	59	70.0	172.0	1.82	Melanoma	IV	1
	M	60	90.0	180.0	1.86	Melanoma	IV	0
	M	62	81.1	177.5	1.95	Melanoma	IV	1
	M	56	77.0	178.0	1.95	Melanoma	IV	1
	M	48	104.5	182.5	2.00	Melanoma	IV	1

Patient Demographics: WR-2721 ± Metoclopramide

Pt. #	Sex	Age	Weight (kg)	Height (cm)	Body surface (m^2)	Primary Site	Stage	Performance Status
	F	66	57.0	160.0	1.58	Melanoma	IV	0
	F	42	60.5	172.7	1.70	Melanoma	IV	1
	F	28	54.0	173.0	1.60	Melanoma	IV	1
	M	43	64.0	170.0	1.75	Melanoma	IV	1
	M	76	78.0	182.0	2.00	Melanoma	IV	0
	F	46	57.0	168.0	1.78	Breast	IV	0
	M	37	96.0	174.0	2.10	Melanoma	IV	1
	F	41	76.0	167.0	1.75	Melanoma	IV	0
	F	43	68.0	156.0	1.55	Breast	IV	2

Table 2. Effect of Dexamethasone (10 mg) on the Pharmacokinetics of WR-2721 (740 mg/m^2) in Six Patients

Parameter	WR-2721	Dexamethasone + WR-2721
AUC	2563.2	2350.7
Vdss (L)	27.3	25.6
CL (L/min)	2.82	3.0
T1/2 (β, min)	11.6	6.48
MRT (min)	8.58	8.77
Cmax (μmol/L)	173.4	166.0

Table 3. Effect of Metoclopramide (1 - 2 mg/kg) on the Pharmacokinetics of WR-2721 (740 or 910 mg/m^2) in Nine Patients

Parameter	WR-2721	Metoclopramide + WR-2721
AUC	3137.3	2995.0
Vdss (L)	20.9	25.3
CL (L/min)	2.51	2.45
T1/2 (β, min)	8.94	9.27
MRT (min)	9.59	10.3
Cmax (μmol/L)	233.0	241.4

Table 4. Pharmacokinetics of WR-2721 With and Without Dexamethasone - Raw Data

	Period	AUC	Cls (L/min)	T1/2β (min)	Vdss (L)	MRT (min)	Cmax (μmol/L)	Tmax (min)
Patient								
(-)Dex	**1**	**3820**						
(+)Dex	**2**	**3190**						
Patient								
(-)Dex	**1**	**1544**						
(+)Dex	**2**	**1275**						
Patient								
(-)Dex	**2**	**2139**						
(+)Dex	**1**	**2186**						
Patient								
(-)Dex	**2**	**3038**						
(+)Dex	**1**	**3009**						
Patient								
(-)Dex	**1**	**1781**						
(+)Dex	**2**	**2021**						
Patient								
(-)Dex	**2**	**3057**						
(+)Dex	**1**	**2423**						

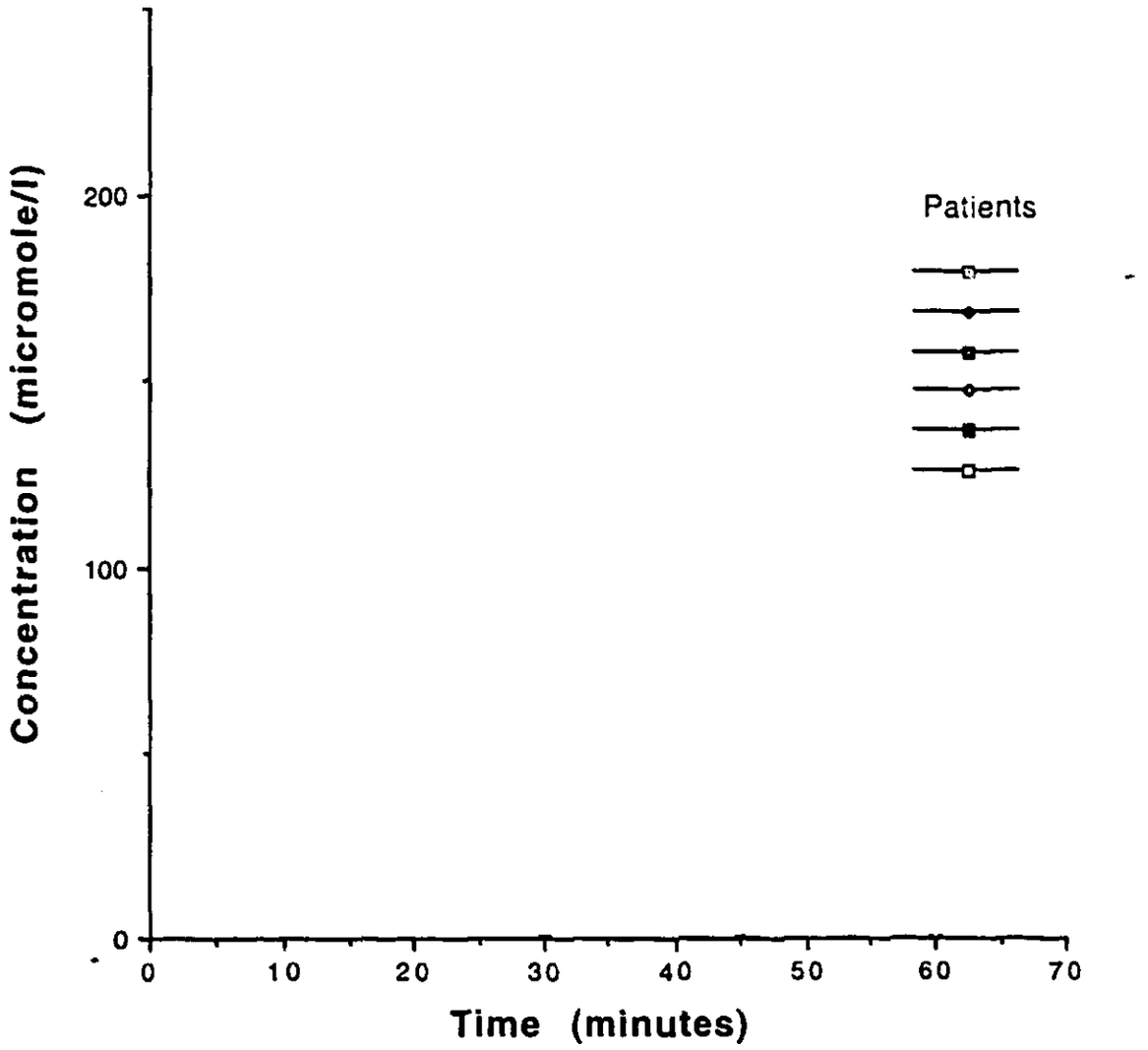
AUC = Area under concentration-time curve; μmol*min/L

Table 5. Pharmacokinetics of WR-2721 With and Without Metoclopramide - Raw Data

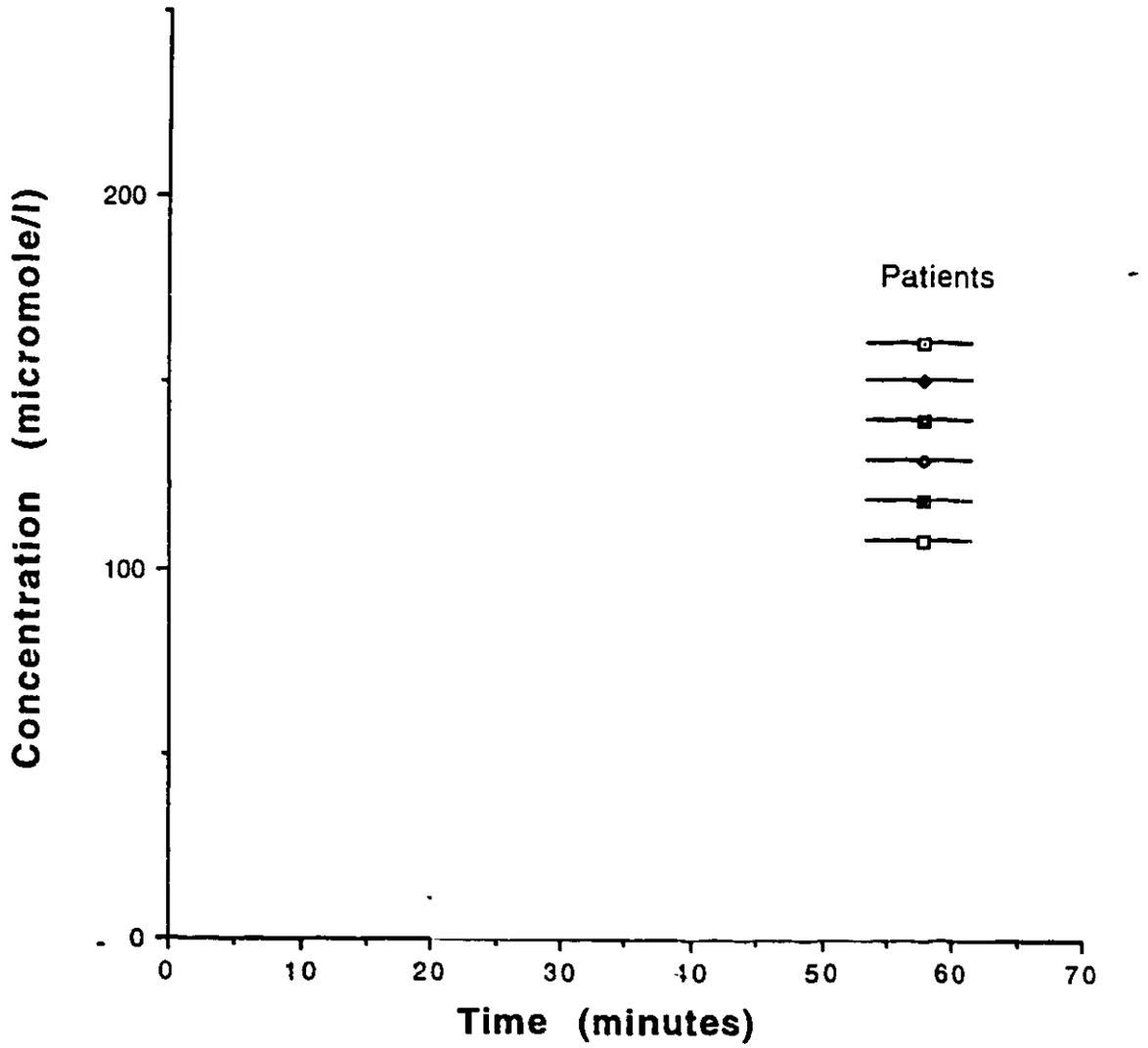
	Period	AUC	Cls (L/min)	T1/2β (min)	Vdss (L)	MRT (min)	Cmax (μmol/L)
				* 740 MG/M² WR-2721 *			
Patient							
(-)Met	**2**	**3036**					
(+)Met	**1**	**2951**					
Patient#							
(-)Met	**2**	**3461**					
(+)Met	**1**	**2539**					
Patient#							
(-)Met	**1**	**2175**					
(+)Met	**2**	**3490**					
Patient#							
(-)Met	**1**	**1001**					
(+)Met	**2**	**1856**					
				* 910 MG/M² WR-2721 *			
Patient#							
(-)Met	**2**	**3030**					
(+)Met	**1**	**2120**					
Patient#							
(-)Met	**1**	**3684**					
(+)Met	**2**	**3199**					
Patient#							
(-)Met	**2**	**3849**					
(+)Met	**1**	**2839**					
Patient#							
(-)Met	**1**	**4349**					
(+)Met	**2**	**4883**					
Patient#							
(-)Met	**1**	**3651**					
(+)Met	**2**	**3078**					

AUC = Area under concentration-time curve; μmol*min/L

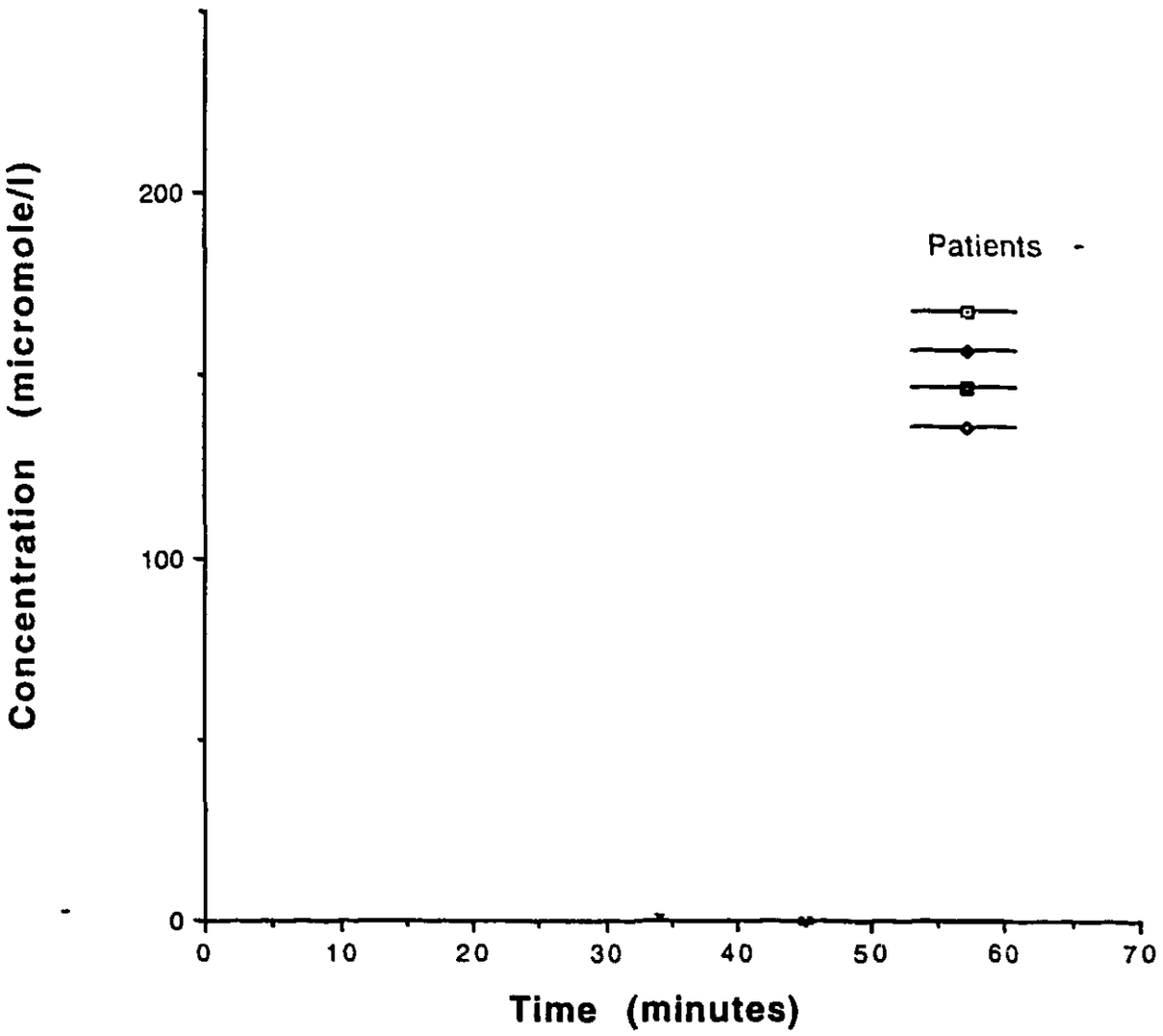
ETH-PK-1
Plasma Amifostine
IV infusion (15 min): 740 mg/m2**
(without dexamethasone pretreatment)



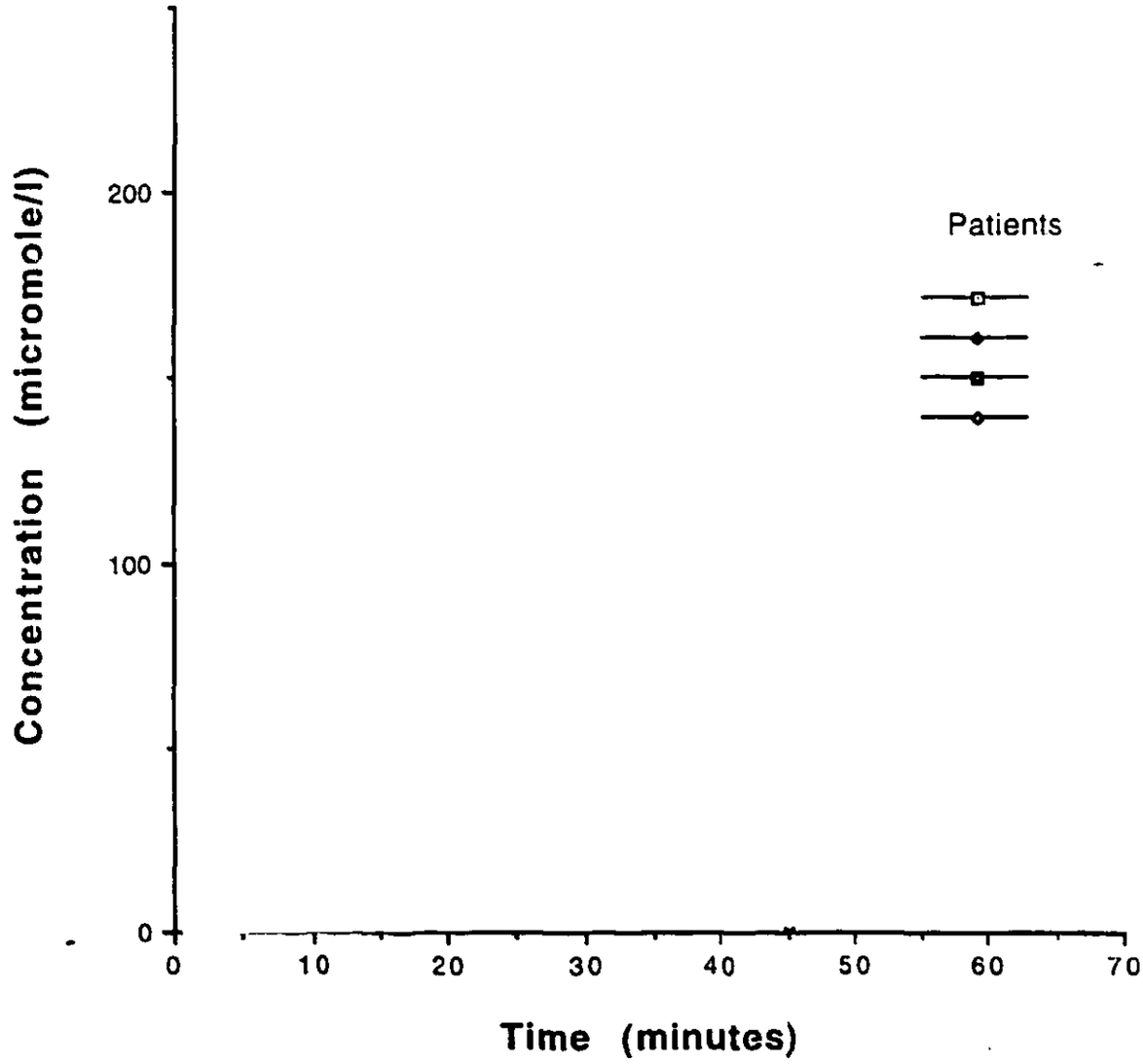
ETH-PK
Plasma Amifostine
IV infusion (15 min): 740 mg/m*2
(with dexamethasone pretreatment)



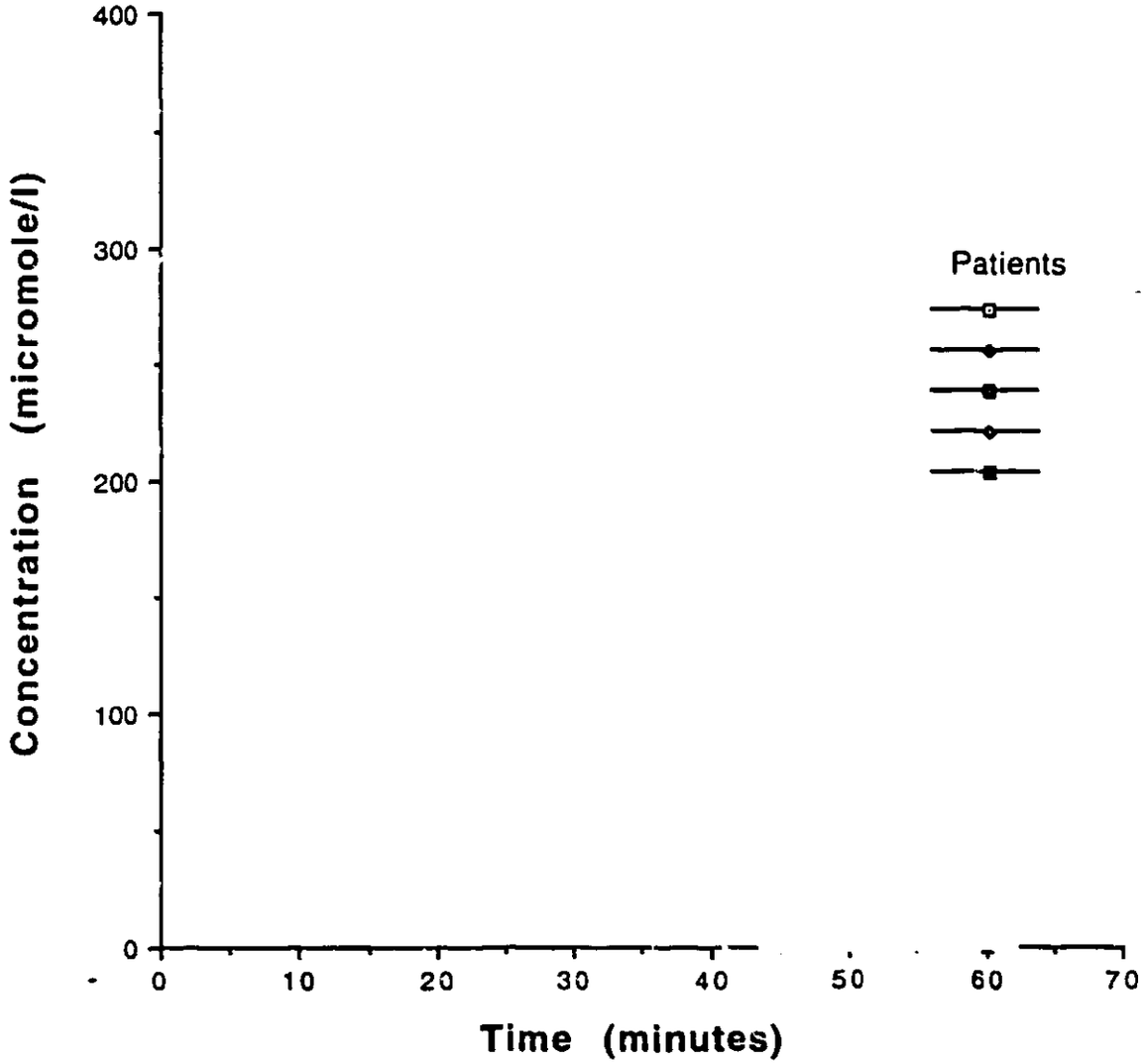
ETH-PK-1
Plasma Amifostine
IV infusion (15 min): 740 mg/m2**
(without metoclopramide)



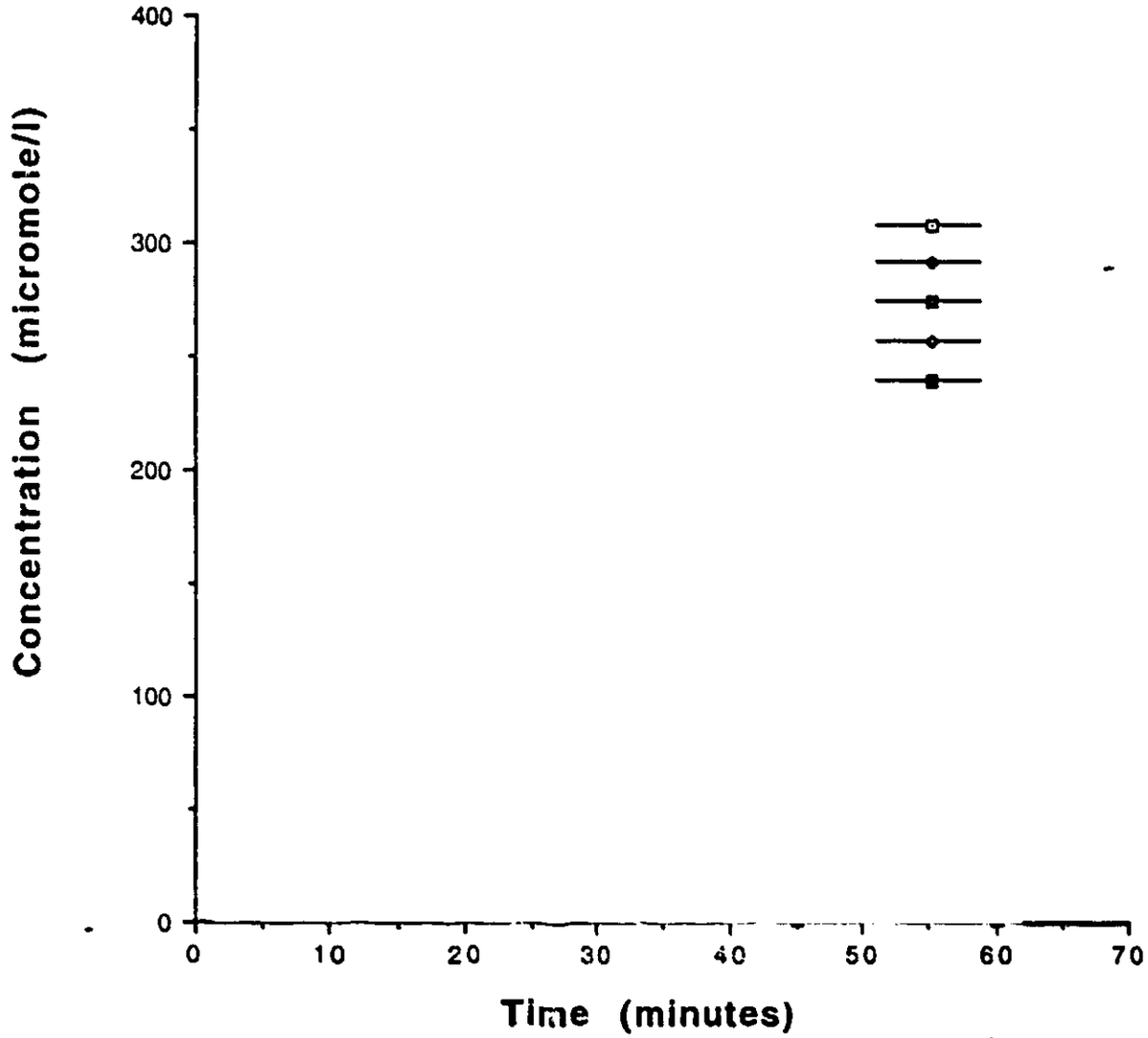
ETH-PK-1
Plasma Amifostine
IV infusion (15 min): 740 mg/m2**
(with *metoclopromide*)



ETH-PK-1
Plasma Amifostine
IV infusion (15 min): 910 mg/m2**
(without metoclopramide)



ETH-PK-1
Plasma Amifostine
IV infusion (15 min): 910 mg/m2**
(with metoclopramide)



TITLES: **PHASE 1 TRIALS OF THE CHEMOPROTECTOR WR-2721 (740 mg/m^2) IN CONJUNCTION WITH THE ALKYLATING AGENTS CYCLOPHSOPHAMIDE (1200-4000 mg/m^2) AND CISPLATINUM (50-300 mg/m^2) (UPCC Protocol)**

PHASE 1 PROTOCOL FOR THE INITIAL CLINICAL STUDY OF MULTIPLE DOSE WR-2721 (RTOG Protocol)

INVESTIGATOR: Leslie M. Shaw, Ph.D.
Dept. of Pathology and Laboratory Medicine
Hospital of the University of Pennsylvania

STUDY DATES: 1985 - 1986

REPORT DATE: September, 1994

OBJECTIVES: This was not explicitly stated by the sponsor. Apparently the aim of these studies was to describe the pharmacokinetic characteristics of WR-2721 after a single intravenous dose of 740 mg/m^2 (infused over 15 minutes).

METHODS:

Study Drug

WR-2721 (dihydrogen phosphorothioate, amifostine, Ethyol). No specific information regarding WR-2721, batch or lot information was submitted. Throughout the clinical trials various diluents were used to reconstitute WR-2721 prior to administration. These included: i) lactated Ringer's solution buffered with sodium bicarbonate to pH 7.4, ii) sodium chloride, 0.9 % and iii) D5W (personal communication with Dr. Shaw on 8-20-95).

Subjects

No subject exclusion/inclusion criteria was incorporated in the study. Demography for four of five subjects were provided (see Table 6). The studies were conducted in patients with a wide range of tumor types, performance status and concurrent therapy.

Study Protocol

Patients included in this report are from two separate early phase I trials. WR-2721 was administered as a single-dose 15 minute intravenous infusion. Apparently, no one study design can be used to describe the protocol.

Assay

Based on published literature, a high performance liquid chromatographic system equipped with a Hg/Au electrochemical detector was utilized to measure WR-2721 concentrations in blood and urine samples. An internal standard (WR-80855) is added to each sample.

Pharmacokinetic Analysis

Model-independent parameters of WR-2721 disposition were provided (area under the plasma concentration time curve, clearance, half-life and Cmax) The percent of dose administered found in urine during the study period (45 minutes) was reported.

RESULTS:

The pharmacokinetics of WR-2721 are presented in Table 7 and the attached graph. After a minute intravenous infusion of 740 mg/m^2, the average observed peak concentration achieved was μmole/L μg/ml). The amount of WR-2721 (as a percent of the dose administered) excreted unchanged in the urine was low (2.6% or less).

CONCLUSION:

This study does provide some information regarding the pharmacokinetics of WR-2721 It is the only study that evaluated the renal excretion of WR-2721 The amount of parent drug found to be excreted unchanged in the urine is small and agrees with published reports. However, this study does not appear to have been well controlled. The data have been combined from four separate early Phase 1 trials. In addition, the patients were diagnosed with a wide range of tumor types and performance status and were apparently receiving chemotherapy and probably other medications, radiation therapy etc. during the study period. Therefore, this study is not considered to be a pivotal pharmacokinetic study.

Table 6. Patient demographics

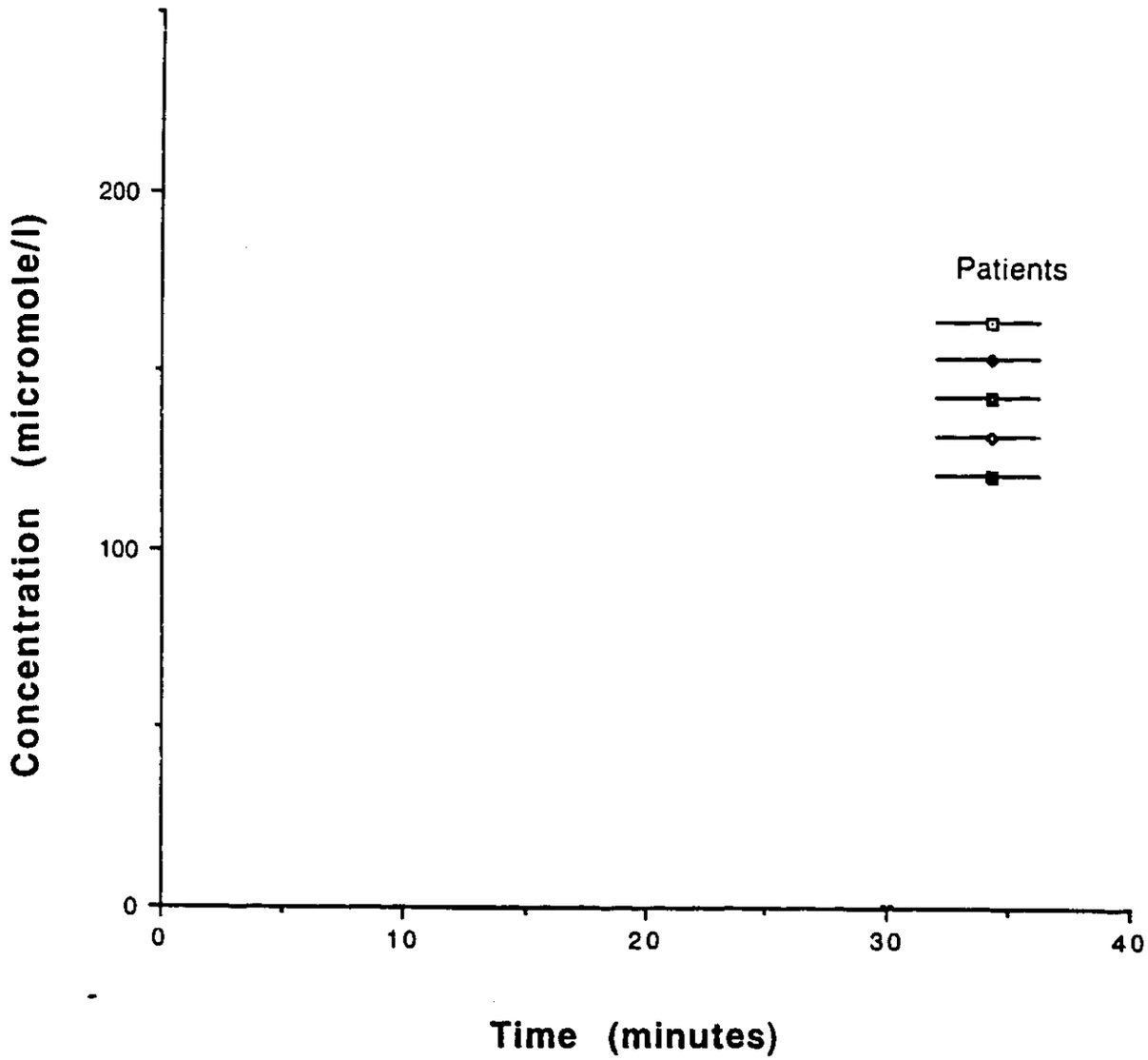
Study	Initial	Age	BSA	Sex	Baseline Performance Status	Disease	Baseline Serum Creatinine
UPCC		60	1.7	M	1	NSCLC	1.0
UPCC		43	1.65	M	1	Melanoma	0.6
RTOG		67	-	F	-	Adeno-carcinoma	-
RTOG		59	1.95	M	80	Mesothelioma	0.9

Table 7.

WR-2127 Pharmacokinetics and Urinary Excretion Data for Patients Receiving minute infusion) at 740 mg/m^2

Initial	AUC (μmole*min*l^{-1})	Cls (l/min)	Vd (l)	Half-life (min)	Cmax (μmole/l)	% Dose Excreted Unchanged in Urine within 45 min
Mean	**1505.8**	**4.31**	**8.72**	**1.49**	**100.2**	**1.1**
std	**664.6**	**1.79**	**2.75**	**0.35**	**44.0**	**1.0**

UPCC & RTOG Protocols
Plasma Amifostine
IV infusion min): 740 mg/m2**



TITLE: **PHARMACOKINETICS OF SINGLE DOSE AMIFOSTINE (WR-2721) AT 910 mg/m²**

REPORT: ETH-PK-3

INVESTIGATOR: Leslie M. Shaw, Ph.D.
Dept. of Pathology and Laboratory Medicine
Hospital of the University of Pennsylvania

STUDY DATES: 1989 -1994

REPORT DATE: September 1994

OBJECTIVE: The objectives of this study is to evaluate the pharmacokinetics of single dose WR-2721 (910 mg/m²) administered as a 15 minute i.v. infusion.

METHODS:

Study Drug

WR-2721 (dihydrogen phosphorothioate, amifostine, Ethyol). WR-2721 was manufactured and stored in vials of 500 mg. Drug from five lots (see attached) were used for this study and the pivotal clinical trial. These included: i) lactated Ringer's solution buffered with sodium bicarbonate to pH 7.4, ii) sodium chloride, 0.9 % and iii) D5W (personal communication with Dr. Shaw on 8-20-95).

Subjects

No subject inclusion/exclusion criteria were incorporated in the study. WR-2721 pharmacokinetics were evaluated in thirteen (5 male, 8 female) patients who were participating in clinical trials at either the University of Pennsylvania (n=7) or University of Wisconsin (n=6). The ages of study participants ranged from 30 to 67 years. Patients were diagnosed as having cancer originating from various sites (see Table 8).

Study Protocol

Each patient received a single 15-minute infusion of WR-2127 at a dose of 910 mg/m². Blood samples were obtained at 0, 3, 6, 9, 12, 15, 16, 16.5, 17, 17.5, 18, 20, 30, 45, and 60 minutes after the start of the infusion.

Assay

All blood samples were sent to the University of Pennsylvania for pharmacokinetic analysis. A high performance liquid chromatographic system equipped with a Hg/Au electrochemical detector was utilized to measure WR-2721 concentrations in blood samples. Based on published literature, an internal standard (WR80855) is added to each sample.

Pharmacokinetic Analysis

Pharmacokinetic parameters were estimated using the Lagran, model-independent non-linear regression software system. Values for area under the curve, clearance, volume of distribution (at steady state), half-life, mean residence time and Cmax were reported.

As a part of the review, a population pharmacokinetics approach was used describe the disposition of WR-2721. Data from this report were combined with data from report ETH-PK-1 (antiemetic drug interaction study).

RESULTS:

The pharmacokinetics of WR-2721 are presented in Table 9. The average observed peak concentration achieved was μmole/l μg/ml). Both a rapid systemic clearance L/min) and relatively small steady-state volume of distribution (7.3 L) resulted in a half-life of only min. In most subjects WR-2721 was not detectable 30 minutes after the end of the infusion.

CONCLUSION:

This clinical trial may be considered the second pivotal study evaluating the pharmacokinetics of WR-2721. It appeared to be well controlled and included an adequate sample size.

Table 8.

PATIENT DEMOGRAPHICS

ID#	Sex	Age	Race	Wt (kg)	Ht (cm)	Surface area (m^2)	Primary site	Stage	Performance status
	M	55					Renal cell		
	F	67		45.5	154.94	1.40	Pancreas	IV	1
	F	55					Colon		
	F	67		57.7	152.40	1.52	Lung		1
	M	36		83.0	175.00	1.90	Pelvis	IV	
	F	46	Caucasian	58.18	165.10	1.68	Melanoma		1
	F	30	Caucasian	52.95	162.56	1.56	Melanoma		1
	F	67	Caucasian	70.2	161.29	1.77	Lung	III	1
	F	42	Caucasian	53.2	157.48	1.52	Lung	I	1
	F	49	Caucasian	72.3	165.1	1.79	Lung	II	1
	M	61	Caucasian	68.9	182.88	1.85	Lung	III	1
	M	48	Caucasian	70.3	162.56	1.77	Lung	I	0
	M	65	Caucasian	77.5	172.72	1.92	Lung	IV	0

Table 9.

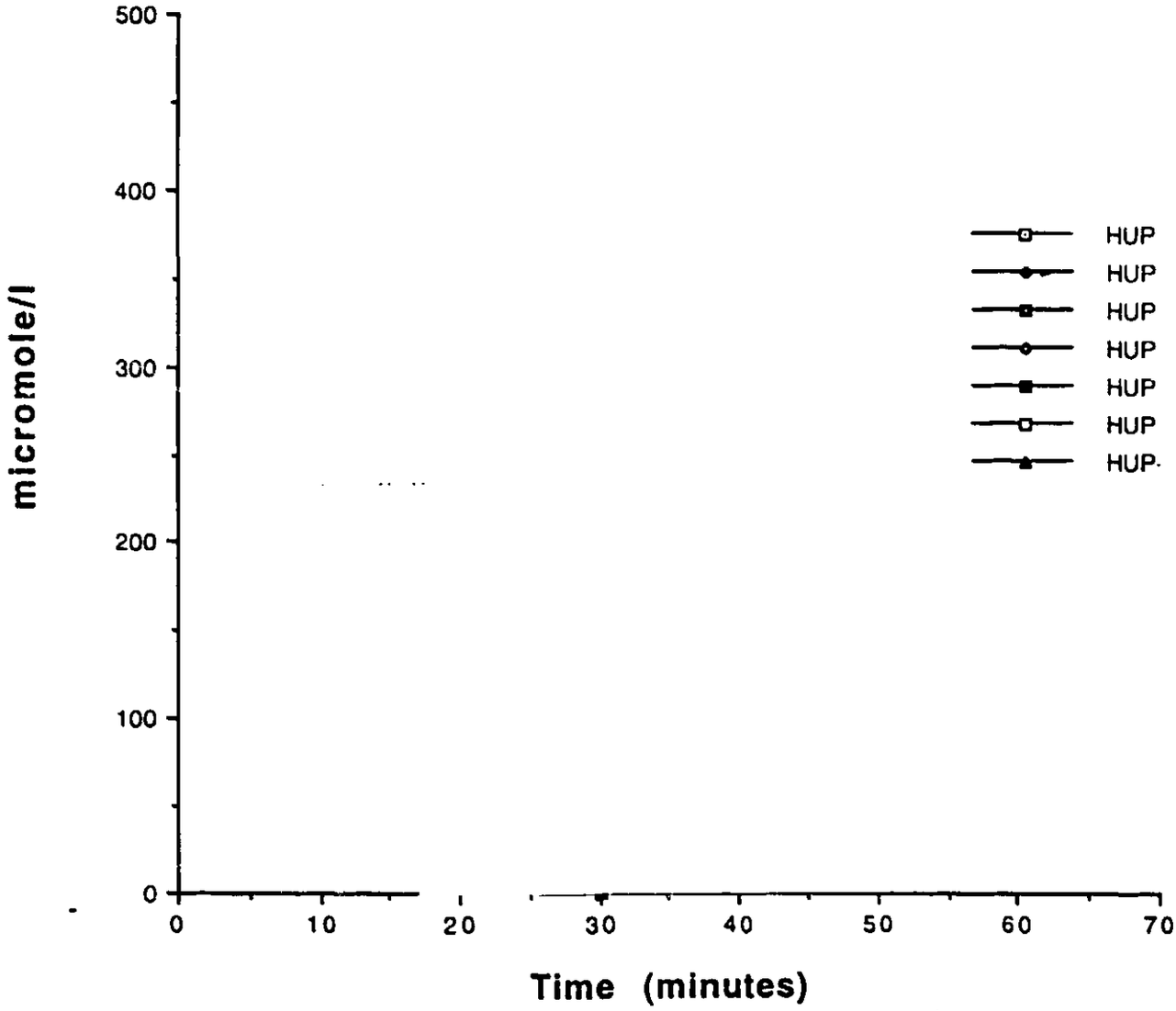
Pharmacokinetic Parameters for Amifostine (910 mg/m²)
Administered as a 15-Minute Infusion

Patient*	AUC (μmol/L x min)	Cmax (μmole/L)	Vd_u (L)	t½ (min)	Cl (L/min)	MRT (min)
HUP						
HUP						
HUP						
HUP						
HUP						
HUP						
HUP						
W.						
W.						
W.						
W.						
W.						
W						
MEAN	3827	238	7.0	2.0	2.0	3.0

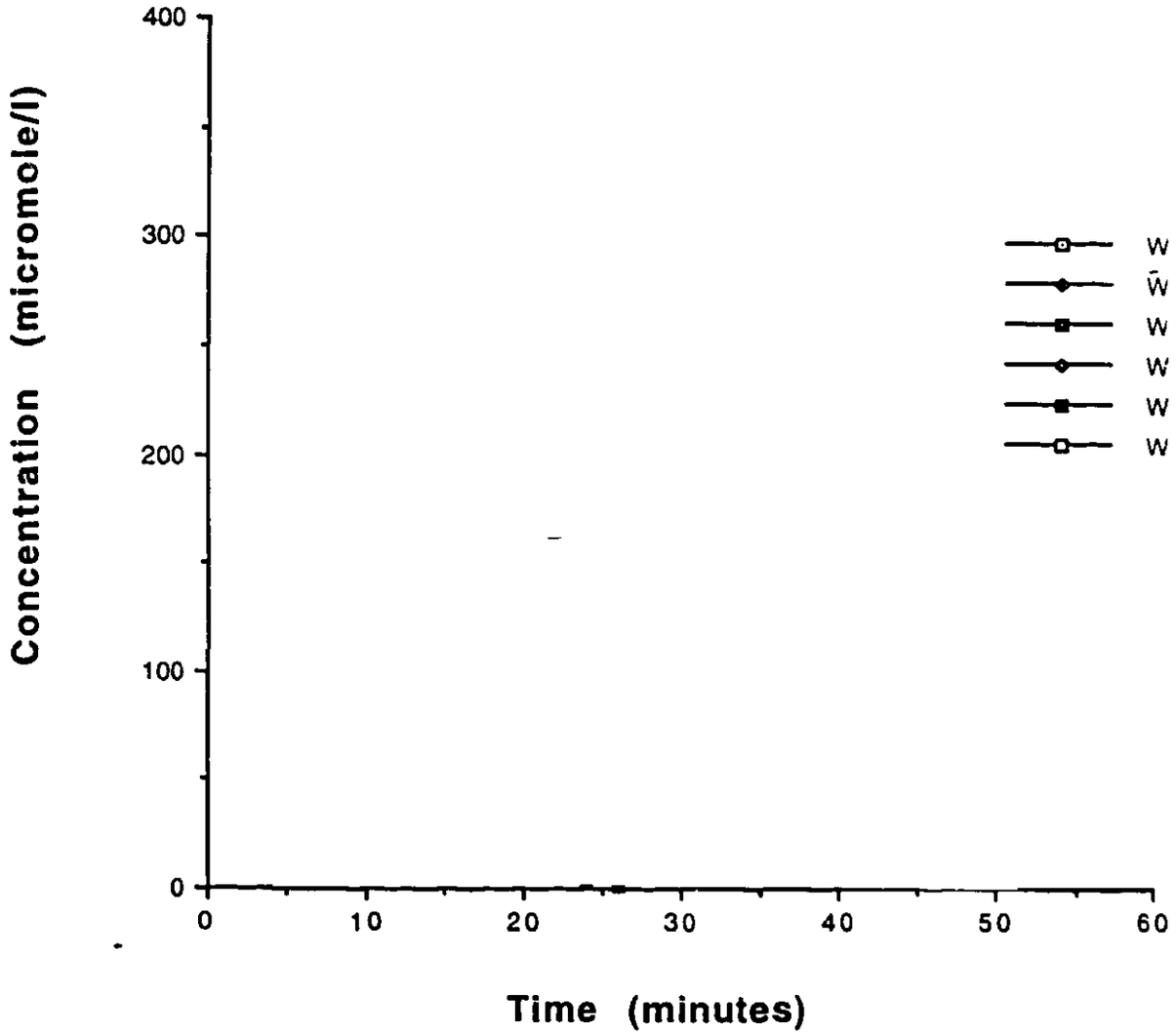
* HUP = patients studied at the Hospital of the University of Pennsylvania
W = patients studied at the University of Wisconsin

AUC = area under the concentration-time curve; CL = plasma clearance; VD_u = volume of distribution at steady-state; MRT = mean residence time; Cmax = maximum concentration; T½ = half-life

ETH-PK-3
Plasma Amifostine
IV infusion (15 min): 910 mg/m2**



ETH-PK-3
Plasma Amifostine
IV infusion (15 min): 910 mg/m2**



TITLE: **PHASE I STUDY OF BONE MARROW PROTECTION BY ETHYOL (AMIFOSTINE) IN PATIENTS WITH SOLID TUMORS TREATED WITH CARBOPLATIN**

PROTOCOL: 04911

INVESTIGATORS: J.B Vermorken
Free University Hospital
Amsterdan, The Netherlands

J. Verwei
Rotterdam Cancer Institute
Rotterdam, The Netherlands

D.J.T. Wagener
University Hospital Ni megan
Ni megen, The Netherlands

STUDY DATES: *October, 1992 - ongoing*

REPORT DATE: July 10, 1995

OBJECTIVES:

i) To determine the effect of multiple dose WR-2721 administration on the maximum tolerated dose of carboplatin

ii) To investigate the safety of WR-2721 following multiple dose therapy

iii) To propose a safe doses for concomitant therapy with WR-2721 and carboplatin

METHODS:

Study Design

This is an open label, nonrandomized study

Study Drugs

WR-2721 (dihydrogen phosphorothioate, amifostine, Ethyol). WR-2721 is manufactured and stored in vials of 500 mg. No specific information regarding WR-2721, batch or lot information was submitted. WR-2721 is to be reconstituted in normal saline (final volume ml) and administered over minutes as a continuous intravenous infusion.

Carboplatin is commercially available in 150 mg vials. The source of carboplatin was not specified. The drug was dissolved in 250 ml 5 % dextrose and administered over 15 minutes as a continuous intravenous infusion.

Subjects

Inclusion Criteria

i) Patients with histologically/cytologically proven solid tumors who may benefit from carboplatin therapy
ii) Age range: 18 - 75 years
iii) ECOG performance status: 0 - 2
iv) Life expectancy: ≥3 months
v) Hematological parameters, renal and liver function within normal ranges (see report)
vi) No evidence of infection
vii)Written consent

Exclusion Criteria

i) More than 2 prior regimens with cytotoxic chemotherapy
ii) Previous treatment with carboplatin, mitomycin-C or nitrosoureas
iii) Pregnancy/lactation
iv) CNS disease or hypertension requiring therapy other than diuretics
v) Treatment with drugs which may affect bone marrow function
vi) Antibiotics that interfere with platinum excretion will be avoided

Study Protocol

Carboplatin

Groups of at least 5 patients will receive increasing doses of carboplatin Dose escalation steps will be taken if 0 or 1 of the 5 patients has shown predefined dose limiting toxicity (DLT). If 2 or more show DLT, then that will be deemed the MTD. Carboplatin will be administered at 4-week intervals (or longer depending on recovery time). The following plan will be used for dose escalation:

Patients	Dose of Carboplatin
	400
	500
	600
	720
	865
	1040

WR-2721

WR-2721 will be administered as a 15 minute i.v. infusion. The dose of WR-2721 will be 910 mg/m^2 at each administration of WR-2721. If 2 or more patients are not able to receive the full WR-2721 dose due to toxicity (hypotension), the next 5 patients (and all additional patients) will be treated at the 740 mg/m^2 dose. Each patient will receive three doses: the first one to complete immediately prior to carboplatin and the second and third, two hours and four hours, respectively, after the commencement of the carboplatin infusion.

Concurrent Medication

Patients will be adequately hydrated before each treatment. At least one liter of normal saline will be given two hours before study drug administration. Metoclopramide will be given prior to WR-2721 to reduce nausea/vomiting. Concurrent medication for non-malignant disease will also be permitted.

Assay

A high performance liquid chromatography procedure with electrochemical detection was developed for WR-2721, its active metabolite, WR-1065 and mixed disulfides. No further details about the assay (validation etc.) were provided.

Pharmacokinetic Analysis

Plasma samples were obtained at the end of each infusion. Peak levels of WR-2721 and metabolites (WR-1065 and disulfides) were determined.

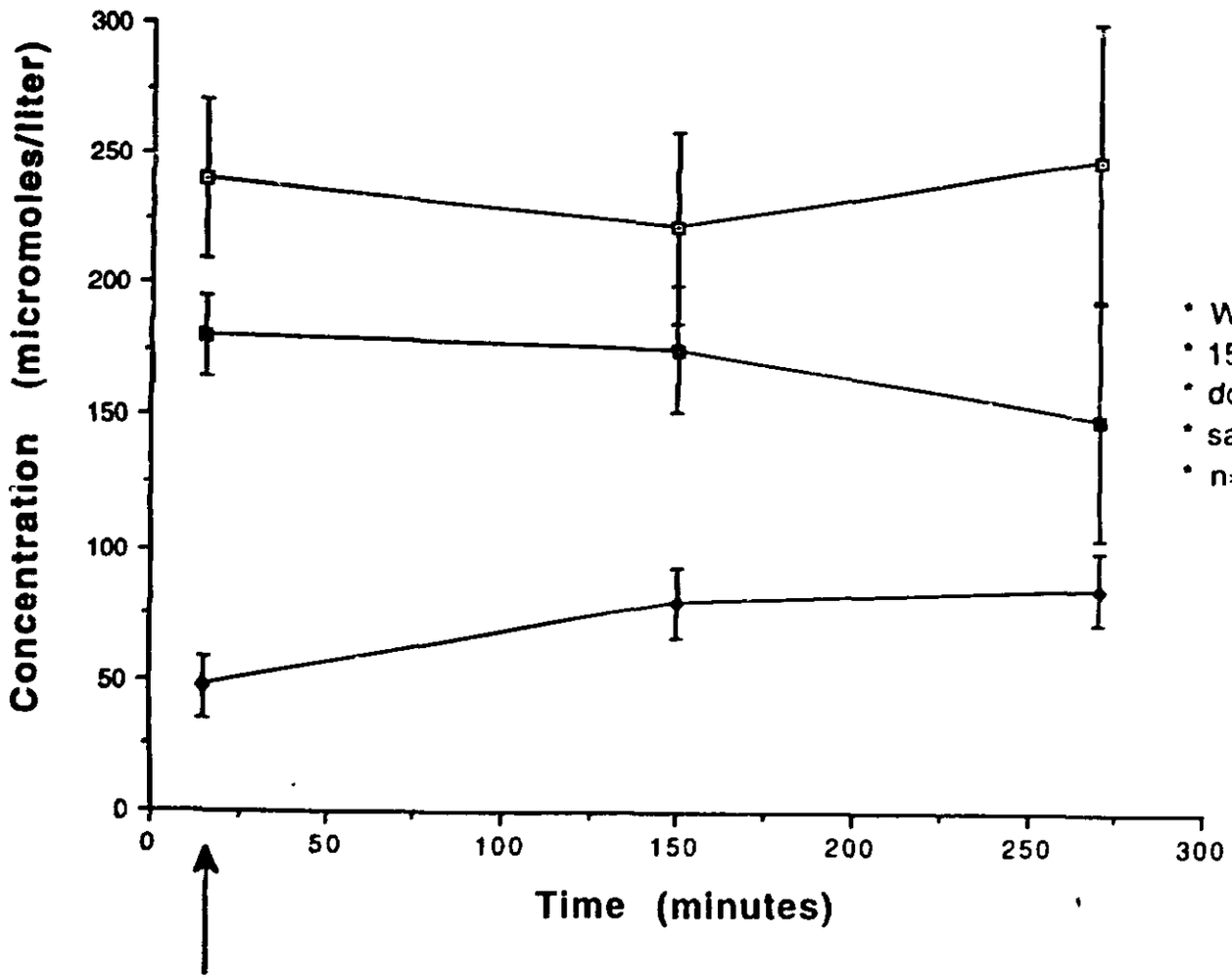
RESULTS:

Data from six patients treated with WR-2721 at 740 mg/m^2 have been submitted. All compounds were detectable at the end of the infusion (see attached graph). No accumulation of parent compound (WR-2721) or disulfides was apparent. In contrast, a 1.5- and 1.8-fold increase in mean WR-1065 levels was observed after the second and third doses, respectively, as compared to the first peak concentration. Limited information regarding the pharmacokinetics of WR-1065 can be extracted from this study, however, the half-life of the active metabolite appears to be longer than the prodrug (WR-2721) and, at this dosing regimen, results in some accumulation.

CONCLUSION:

This is a preliminary report evaluating the safety of combination therapy with carboplatin WR-2721. The data contained in the report was reviewed since it is the only source of information regarding the disposition of WR-2721 and its metabolites (WR-1065 and disulfides). However, given the sample size (n=6), the limited number of samples taken per patient (1 peak level after each of the three WR-2721 doses) and lack of assay methodology/validation data from this group of investigators, this cannot be considered a pivotal study.

Peak Plasma Concentration of WR-2721, WR-1065 & Disulfides



* WR-2721: 3 doses at 740 mg/m**2
* 15 minute IV infusion
* dosed at 0, 135 & 255 minutes
* samples obtained at end of each infusion
* n= 6 patients (mean +/- std)

Carboplatin: 400 mg/m**2, 15 min IV infusion
administered at end of first WR-2721 dose

POPULATION PHARMACOKINETICS ANALYSIS OF WR-2721 (NONMEM)

BACKGROUND:

Pharmacokinetic data submitted by the sponsor were analyzed without assuming any particular model (ie. noncompartmental analysis). As a part of the review, plasma concentration versus time profiles from two separate reports were combined and re-analyzed by a population pharmacokinetics approach. Data were obtained from clinical trials EHT-PK-1 (includes only data from patients without antiemetic premedication) and ETH-PK-3. The data set consisted of a total of 26 patients who were administered WR-2127 (740 or 910 mg/m^2) intravenously over a 15 minute period .

NONMEM ANALYSIS:

The analysis was performed using the NONMEM program (Method 1, double precision, version IV, Level 2.0 developed by Stuart Beal and Lewis Sheiner) with the NMTRAN pre-processor. A two-compartment structural kinetic model with first-order elimination was used (see attached control stream).

The basic parameters were elimination clearance (CL, L/min), volume of distribution of the central compartment (V1, L), volume of distribution in the peripheral compartment (V2, L) and the intercompartmental clearance or flow (Q, L/min).

The PREDPP subroutine ADVAN 3, TRANS 4 was used. The inter-compartmental rate constants K12 and K21, and elimination constant K, were defined as Q/V1, Q/V2 and CL/V1, respectively.

Interpatient variability for clearance and distribution volume were modeled as proportional. The residual error model contained two components, a proportional and additive portion (ie. combination error model).

Bayesian estimates of pharmacokinetic parameters for each individual were made using parameter estimates from the final population model as priors and plasma concentrations (NONMEM POST HOC option, see Table 8).

RESULTS:

This analysis describes WR-2721 disposition with a two compartment model. The magnitude of the standard errors relative to the corresponding estimates of basic pharmacokinetic parameters (clearance and distribution volume) is acceptable. In contrast, estimation of the random effects (inter- and intra-individual variability) were relatively imprecise.

Piots of the observed concentrations (DV) and weighted residuals versus predicted concentrations (PRED) also provide evidence that the two compartment model adequately describes these data. However, these plots also indicate a high degree of variability in the disposition of WR-2721.

The estimated values of the various pharmacokinetic parameters are listed in Table 8. The mean Bayesian estimated systemic clearance was 2.2±0.8 l/min. The average clearance in females was 1.75±0.38 as compared to 2.66±0.90 in males (34 % difference, $p<0.05$, 2-sample t-test). WR-2721 distribution did not appear to be extensive with a mean distribution volume in the central and peripheral compartments of 9.5±8.2 liters and 4.8±7.2 liters, respectively (Vdss = 14.3 L). The distribution and terminal half-lives were determined to be approximately 1.2±1.1 and 7.2±8.7 minutes, respectively.

The coefficient of variation (CV) of clearance (ie. inter-patient variability remaining not explained by the population model) was 36 %. A relatively high inter-patient variability in the volume of distribution was found as evidenced by a CV of 68 % and 147 % for the theoretical volume in the central and peripheral compartments, respectively. Half-life, which is a hybrid parameter dependent on clearance and distribution volume, also exhibited a high degree of variability (see above).

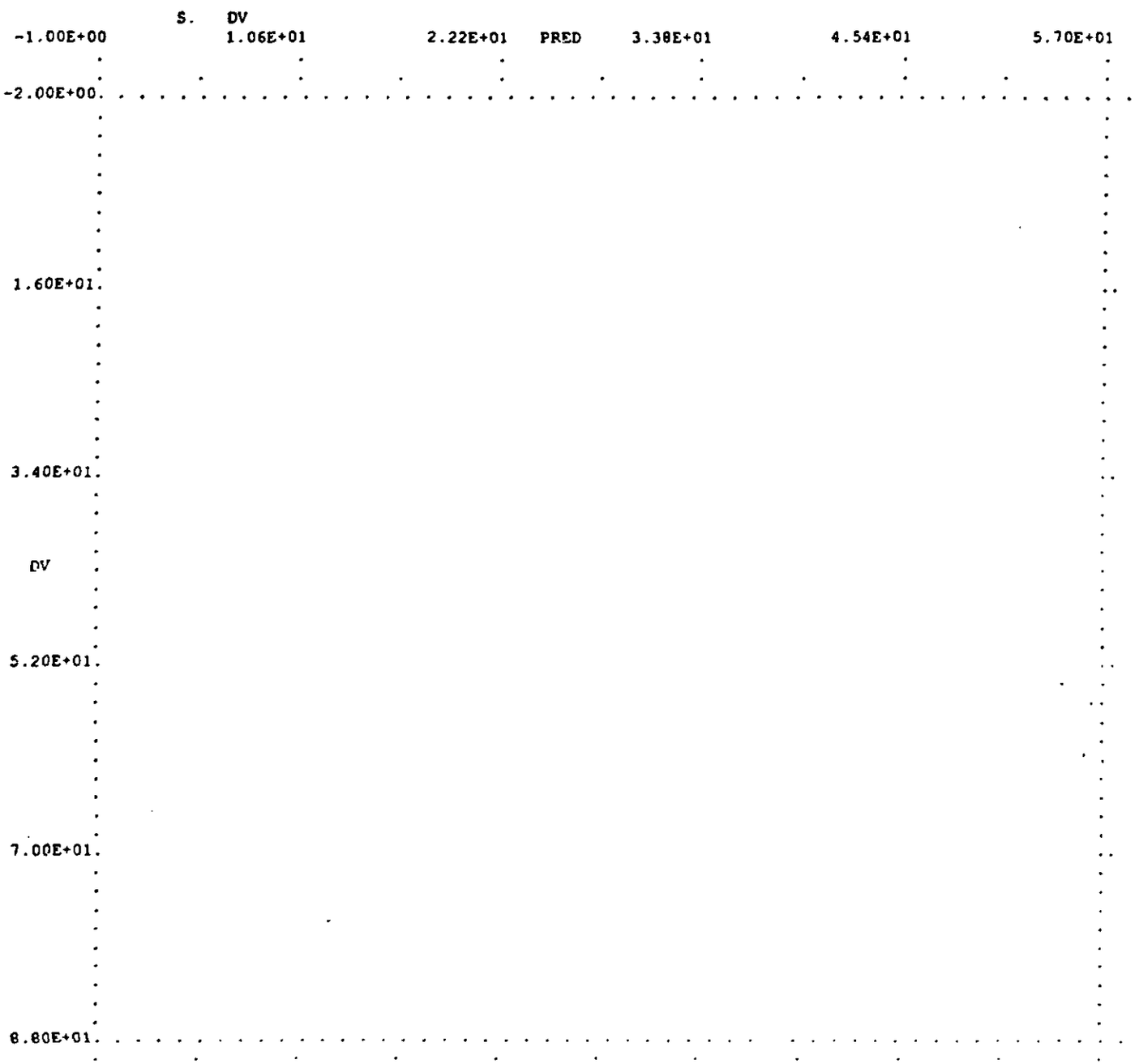
CONCLUSION:

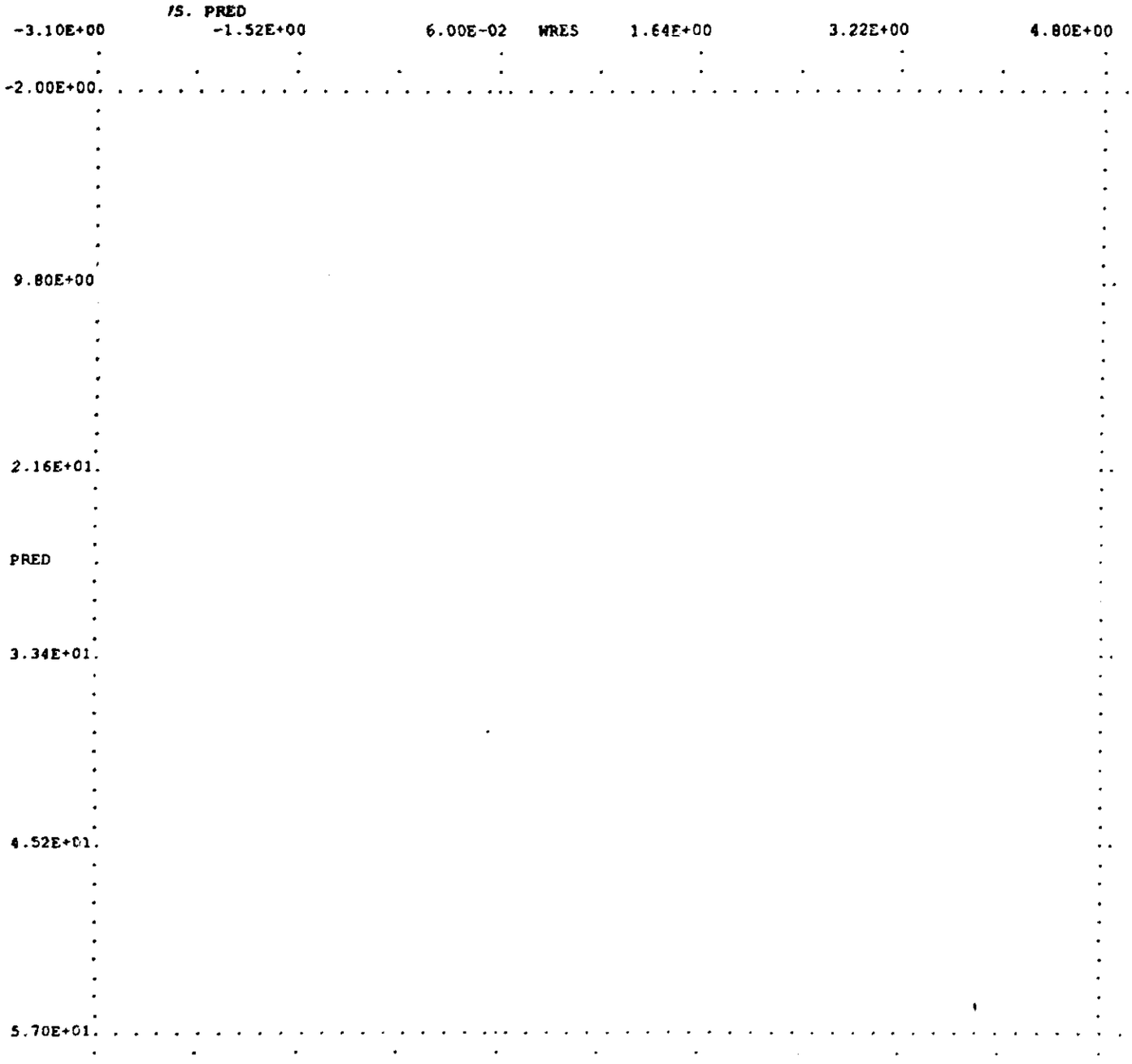
The population approach is useful in that it provides an alternative method to analyze the disposition of WR-2721. In general, results of the NONMEM analysis appears to agree with the noncompartmental analysis performed by the sponsor. For example, estimates of mean systemic clearance, distribution volume and half-life are comparable. A high degree of between-patient variability was observed in distribution volume and half-life of WR-2721 in both the NONMEM and noncompartmental analyses.

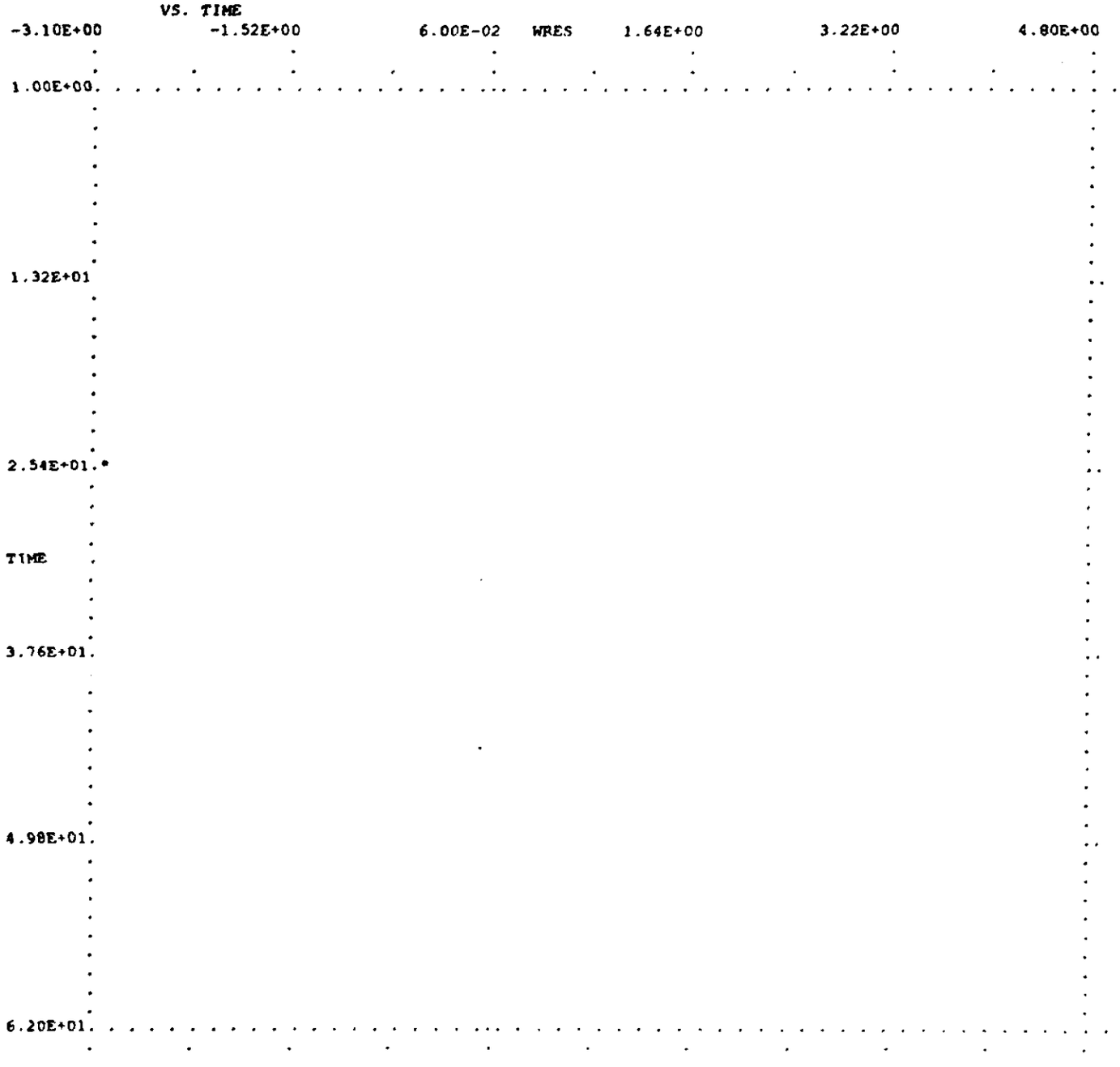
In conclusion, the population approach in estimating pharmacokinetic parameters supports the information on WR-2721 intended to be included in the labeling.

Table 8. Population Pharmacokinetic (NONMEM) Analysis Of WR-2721

Trial/Protocol	Sub ect	Dose mg/m**2	Clearance L/min	Vd1 L	Vd2 L	alpha min	beta min
ETH-PK-1							
		740					
		740					
		740					
		740					
		740					
		740					
		740					
		740					
		740					
		740					
		740					
		910					
		910					
		910					
		910					
		910					
ETH-PK-3							
		910					
		910					
		910					
		910					
		910					
		910					
		910					
		910					
		910					
		910					
		910					
		910					
		910					
mean			2.215	9.459	4.823	1.229	7.158
std			0.829	8.156	7.239	1.077	8.727
geometric mean			2.090	7.660	2.580		


S. DV
-1.00E+00
1.06E+01
2.22E+01
PRED
3.38E+01
4.54E+01
5.70E+01
-2.00E+00
1.60E+01
3.40E+01
DV
5.20E+01
7.00E+01
8.80E+01


/S. PRED
-3.10E+00
-1.52E+00
6.00E-02
WRES
1.64E+00
3.22E+00
4.80E+00
-2.00E+00
9.80E+00
2.16E+01
PRED
3.34E+01
4.52E+01
5.70E+01


VS. TIME
-3.10E+00
-1.52E+00
6.00E-02
WRES
1.64E+00
3.22E+00
4.80E+00
1.00E+00
1.32E+01
2.54E+01
TIME
3.76E+01
4.98E+01
6.20E+01

12 AUG 1992 12 AUG 1992

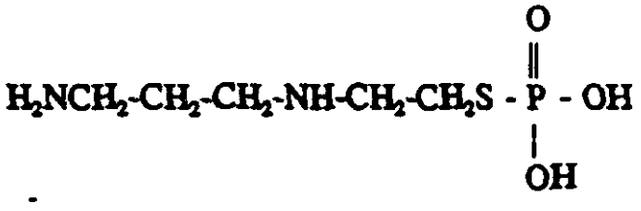
NDA 20-221	SUBMISSION DATES:	September 30, 1991 January 10, 1992 January 23, 1992
Amifostine (ETHYOL®) Lyophilized Powder 500 mg/vial	U.S. Bioscience	
TYPE OF SUBMISSION: NME	REVIEWER: Suresh Mallikaarjun, Ph.D.	

SYNOPSIS: The sponsor has submitted incomplete information on the pharmacokinetics of amifostine at doses of 710 mg/m^2, with and without dexamethasone and metoclopramide. No information has been provided at 910 mg/m^2, the proposed dose.

RECOMMENDATION: The Human Pharmacokinetics Section of NDA 20-221, Amifostine (ETHYOL®) cannot be evaluated since the information requested by the Division of Biopharmaceutics has not been provided by the sponsor.

Please convey the Recommendation and Comments 1-2 to the sponsor.

BACKGROUND: Amifostine is being proposed as a chemoprotective agent for the protection of tissues from toxicities associated with intensive regimens of platinum and alkylating agents. It has the following structure:



Proposed Dosing: The proposed dosage of amifostine is 910 mg/m^2 as a 15 minute infusion. If the dose is not tolerated, subsequent doses should be reduced starting with a dose of 740 mg/m^2, and subsequent reductions, if necessary, to 600 mg/m^2 or 450 mg/m^2. Doses below 450 mg/m^2 have not been adequately studied for efficacy with intensive platinum or alkylating agent chemotherapy and are therefore not recommended. Multiple doses of ETHYOL have been given in conjunction with antineoplastic agents with long half-lives.

The dose and schedule used were ETHYOL 740 - 910 mg/m^2 prior to the administration of the antineoplastic agent and a second dose (740 - 910 mg/m^2) 2 hours later.

The sponsor's original submission (September 30, 1991) contained the following:

1. A graph of conc vs.time after a dose of mg/kg in 1 patient.
2. A graph of conc vs.time after 5 doses of mg/m^2 at minute intervals in 1 patient.
3. Mean and individual PK parameters (Vc, Cl, T½α, T½β, Vss) after a single dose of 150 mg/m^2 in 13 patients.
4. A single graph of conc vs.time following a dose of 740 mg/m^2 in 10 patients.
5. Two tables listing only mean parameters of amifostine (740 mg/m^2) with and without pretreatment with dexamethasone (10 patients) and with metoclopramide (6 patients).
6. A brief reference to a 910 mg/m^2 study.
7. Two published articles on the assay of amifostine by HPLC with EC detection.

The sponsor was asked to provide information on the 910 mg/m^2 study, the 740 mg/m^2 study, the two drug interaction studies, dose proportionality, protein binding and assay validation of the studies. and the request faxed to them. In response to this, the sponsor provided the following information in the January 10, 1992 submission:

1. Six bone marrow levels in 4 patients after a 910 mg/m^2 dose,
2. A statement that the investigator for the 740 mg/m^2 study "is attempting to locate the document".
3. Two tables listing individual and mean parameters of the metoclopramide and dexamethasone drug interaction studies,
4. A statement that no dose proportionality information is available in humans,
5. A literature article about protein binding of amifostine. (There was no information on protein binding in humans in this article).
6. A statement that, "Additional assay techniques to the liquid chromatographic electrochemical method referenced in the Ethyol NDA (Submission Date: September 30, 1991) are described in the attached publication. These assay methods may be used as validation of the assay method described in the NDA". The "attached publication" was an article on the hydrolysis of amifostine.

A meeting was held with the sponsor by the clinical division on January 17, 1992 where the problems with the NDA were reiterated by this reviewer. In reply, the sponsor submitted the following statements (January 23, 1992):

The data on the biopharmaceutics of WR-2721 have been generated by Dr. Leslie Shaw, Professor of Department of Pathology and Laboratory Medicine at the Hospital of the University of Pennsylvania. Dr. Shaw's pharmacokinetic studies were performed independent of U.S. Bioscience. They were pursued as research projects to complement the clinical studies with WR-2721 that were conducted at the Hospital of the University of Pennsylvania. Copies of relevant manuscripts are included in this submission (Attachment #2). These deal with analytical methods for WR-2721 in plasma and WR-1065 in whole blood. Published and unpublished data regarding the pharmacokinetics of i.v. bolus dose of 150 mg/m2 and 15-minute infusions of 740 mg/m2 were provided previously in Volume 5.1 submitted to NDA #20,221 on January 10, 1992. Dr. Shaw has pharmacokinetic data from patients who received 910 mg/m2 of WR-2721 on file which he will share with us. These will be submitted to the NDA as soon as possible. U.S. Bioscience plans to conduct additional clinical pharmacologic studies. These protocols will be submitted to our IND and to NDA #20,221 as they are finalized.

The "copies of relevant manuscripts" refer to two journal articles on the assay of amifostine which are identical to the ones submitted in the original submission (September 30, 1991). The statement that "Published and unpublished data regarding the pharmacokinetics of .... 15-minute infusions of 740 mg/m^2 were provided previously in Vol 5.1 .... on January 10, 1992" is incorrect. In fact the sponsor stated in volume 5.1 that, "U.S. Bioscience contacted Dr. Shaw who stated that the information was presented in an internal document at the University of Pennsylvania. He is attempting to locate the document."

On January 28, 1992, once again a fax was sent to the sponsor listing the information required by this reviewer, essentially repeating the earlier request, this time in more detail (Appendix I). Since then, there has been no communication from the sponsor.

SUMMARY OF BIOAVAILABILITY/PHARMACOKINETICS:

I. PHARMACOKINETICS: The data after 740 mg/m^2 doses of amifostine alone, from the two drug interaction studies was combined and from this data, the mean clearance of amifostine was 3.5 L/min, the mean harmonic T½ 6.4 minutes, and the mean Cmax 208.6 μmoles (44.7 ng/mL) (Table 1).
The sponsor states that amifostine is dephosphorylated to the active metabolite, WR-1065 by alkaline phosphatase. The sponsor presented a single graph indicating the conc-time profile of amifostine and WR-1065 (Fig. 1). No other information has been provided on the metabolism of amifostine. The mean urinary excretion after a 740 mg/m^2 dose was reported to be 1.1% for amifostine, and 1.4% for WR-1065.

II. DRUG INTERACTIONS: Drug interaction studies were performed with antiemetics a) metoclopramide (1 mg/kg IV), and b) dexamethasone (10 mg IV). No statistical analyses were reported. The AUC, Cmax, and CL of amifostine appeared to be similar before and after the concomitant administration of the other drug (Table 1).

12 AUG 1992

COMMENTS (TO BE SENT TO THE SPONSOR):

1. The appropriate mean for the T½ is the harmonic mean, and not the arithmetic mean which has been reported.

2. Since the sponsor intends to administer amifostine with other antineoplastic agents, drug interaction studies should be performed delineating the effect of amifostine on the pharmacokinetics of the co-administered antineoplastic agents.

Suresh Mallikaarjun

Suresh Mallikaarjun, Ph.D.
Pharmacokinetic Evaluation Branch

for

FT initialed by Nicholas Fleischer, Ph.D. 8/12/92

cc: NDA 20-221, HFD-150, HFD-426 (Mallikaarjun, Fleischer, Baweja), Chron, Drug, Reviewer, FOI (HFD-19).

SEP 1 _ 1994

PHARMACOKINETIC SECTION

NDA 20-221 Submission Date: July 12, 1994
Amifostine
Lyophilized powder - IV use
500 mg/vial
Ethyol™
Reviewer: Lydia C. Kaus, M.S., Ph.D.

Type of Submission: 45-day filing meeting to be held 9/1/94

Background:
This is Amendment #17 to NDA 20-221. Prior events concerning this NDA from a Biopharmaceutics point of view is as follows:

Dates:	Notes:
9/30/91 1/10/92 1/23/92	An NDA review of the original submission. Completed by Dr. Mallikaarjun 8/12 /92. Many omissions of data resulting in incomplete conclusions being able to be made. Information requested from the firm.
11/25/92	Review of amendment #13 for meeting. Completed 3/23/93 (see below).
3/19/93	Meeting held with firm . Biopharm comments submitted to firm. Biopharm. still awaiting dose proportionality study, Phase 3 clinical PK studies, drug interaction studies.
11/1/93	Discussion with firm of Amendment #14 submitted 8/30/93. No PK per se mentioned in minutes of meeting.
11/23/93	Memo to file from Biopharm referring to pre-NDA meeting. Promise of PK of cyclophosphamide, cisplatin or carboplatin after pretreatment with drug. Three clinical studies with PK promised still not submitted.

Comments:
1. From telephone conversation held with Christine Smith 8/30/94 it was ascertained that the firm do not intend to submit "any time soon' the complete information on the three pharmacokinetic studies given in summary format alone in the NDA submission Amendment #17.

2. The incorrect cross-referencing in the labeling was pointed out and the firm agreed to FAX a corrected version. The firm were requested to give complete references to pharmacokinetic studies submitted to the Agency to date. From a telephone conversation held with Christine

Smith 8/31/94 it was ascertained that the firm are to submit a complete list of references to pharmacokinetic studies submitted to the Agency with a chronological description of response to Biopharmaceutics. This will be sent to the Agency to arrive 9/2/94.

3. A FAX was received 8/31/94 with the corrected annotated labeling relevant to the PK section. The corrected annotated labeling refers to the pharmacokinetic studies in Amendent #17 volume 41 that are in the form of a summary report without details on assay validation, individual data etc. This is unacceptable. The corrected annotated labeling also refers to pharmacokinetics of amifostine from pharmacokinetic studies in the original submission. These parameters do not agree with the review by Dr. Mallikaarjun.

Recommendation:
Amendment #17 of NDA 20-221 is unacceptable for adequate review by the Division of Biopharmaceutics.

9/1/94

Lydia C. Kaus, M.S., Ph.D.
Pharmacokinetics Evaluation Branch

FT 9/1/94
Mehul Mehta, Ph.D., Section Head.

cc
HFD-150:McCollum
HFD-150:Delap
HFD-150: Div. File
HFD-150:Schmidt
HFD-150:Blumenstein
HFD-426:Biopharm/Drug File
HFD-426: Biopharm/Mehta
HFD-426: Biopharm/Fleischer
HFD-426: Biopharm/ChenL
HFD-340: Viswanathan

Stat

FEB 16 1995

STATISTICAL REVIEW AND EVALUATION

NDA#: 20-221

APPLICANT: U.S. Bioscience

NAME OF DRUG: AMIFOSTINE or ETHYOL (WR-2721)

INDICATION: Cytoprotective agent against the acute hematologic and renal toxicities of cyclophosphamide and cisplatin in advanced ovarian cancer

DOCUMENTS REVIEWED: Volumes 1 - 4 of the submission dated 7/12/94

MEDICAL OFFICER: Robert DeLap, M.D., Ph.D.

RELEVANT STATISTICAL ISSUES:

(1) Data quality for one of the primary efficacy endpoints is the main issue. Ascertainment dates were not consistently provided for the primary efficacy endpoint, viz., development of neutropenic fever ± infection; temperatures were not consistently recorded. The type of antibiotic therapy administered was not standardized, viz., broad spectrum vs. agents for local infections. The reviewing MO's (medical officer) assessment for this endpoint is at variance with that of the sponsor indicating that test results are highly dependent on how these incomplete data are interpreted.

(2) This reviewer's analyses of the MO's assessment of time to first neutropenia associated clinical event and number of such events indicate that new cohort patients did not show statistically significant improvement for this endpoint whereas the original cohort did. Thus the second half of the study does not confirm the first half for this endpoint.

(3) Time to progression (TTP) and survival are the two endpoints used in assessing the tumor protection question. In ovarian cancer patients second look surgery is needed to confirm disease progression. Many patients who were so evaluated received second line therapy for residual disease at the time of this surgery. The sponsor did not provide an analysis censoring these patients on the date of second line therapy. This reviewer's analysis based on the MO so censoring these patients indicates a somewhat improved TTP profile for Amifostine patients.

(4) The total sample size of 242 patients (original + new cohorts) is not large enough to fully resolve whether or not there is a possible tumor protective effect of Amifostine (based on the lower confidence limits of the hazard ratio for survival and time to disease progression). The sponsor has provided bootstrap resampling and simulation studies from an empirically determined parametric distribution which indicate that the estimate of the survival hazard ratio and associated

confidence interval point toward no tumor protective effect for Amifostine.

I BACKGROUND:

The original NDA application for Amifostine was submitted in September 1991. In that application the sponsor sought approval for Amifostine pretreatment to reduce four chemotherapy induced toxicities, viz., hematologic toxicity, nephrotoxicity, neurotoxicity and ototoxicity, without compromising the cyclophosphamide + cisplatin regimen's antitumor activity. In the present submission the neurotoxicity and ototoxicity endpoints have been dropped. The original definitions of the hematologic and renal toxicity endpoints were as follows. Incidence and duration of hematologic toxicity was defined as granulocytes nadir < 500/ml, leukopenia related fever or the need to delay or reduce the dose of cyclophosphamide for safety considerations. The incidence of nephrotoxicity was defined as the need to delay or reduce the dose of cisplatin. One-sided tests of significance were utilized. Interim analysis data from one controlled study, viz., WR-2721-1, were presented. The study was initiated in April, 1988.

Study Design: This was a randomized, parallel group, multicenter study in which patients received Cisplatin (100 mg/M^2 i.v.) and Cyclophosphamide (1000 mg/M^2 i.v.) with or without pretreatment with Amifostine (910 mg/M^2 i.v.). There was no blinding of treatment. Eligible patients were assigned in equal proportions to chemotherapy alone (CP) or chemotherapy + Amifostine (CP + WR). Treatment continued for 6 cycles (one cycle = 21 days) or until disease progression or unacceptable toxicity developed. Patients were stratified by center and extent of residual disease following initial surgery (< 2 cm vs. ≥ 2 cm). Primary efficacy endpoints were incidence and duration of hematologic toxicity, nephrotoxicity and tumor response rate. Secondary outcome measures included survival and time to disease progression (TTP). The protocol specified a 200 patient study with one prespecified interim analysis to be performed approximately halfway through the trial (Pocock boundary). If there was a statistically significant reduction ($p < 0.03$, 1-sided) in the incidence of toxicity on the Amifostine arm and no significant reduction in tumor response rate, no additional patients would be enrolled. The sample size was based on a power > 90% to detect a 50% decrease in the incidence of hematologic toxicity from 50% to 25% utilizing a one-sided significance level of 0.05. In addition, it was stated that this sample size also provides 80% power to detect a 50% decrease in the incidence of renal toxicity from 30% to 15% with a one-sided 0.05 significance level. In a subsequent protocol amendment the following statement regarding the sample size was added: *"Assuming the response rate to be 70%, one hundred patients per treatment arm will enable the calculation of a 95% confidence interval within 10% of the observed response rate. Additionally, the sample size provides 90% power to detect a reduction in response rate of 20% (p=.05, one-sided)."*

First cohort patient data were reviewed by the FDA's Oncologic Drugs Advisory Committee (ODAC) on January 31, 1992. ODAC recommended that accrual to the study be completed to the protocol specified sample size of 200 patients to provide additional follow-up with respect to survival and to further strengthen the data on other endpoints, e.g.,

nephrotoxicity.

The original cohort comprised 121 patients (119 from 23 U.S. centers and 2 from United Kingdom) representing the protocol specified interim analysis group. After a March 1992 meeting with FDA, the protocol was amended such that for the second cohort of 121 additional patients the incidence of Grade 4 neutropenia associated with fevers and/or infections would be the primary endpoint used to assess hematologic toxicity. It is to be noted that the use of granulocyte colony stimulating factor (G-CSF) was permitted for the second cohort. However, only a small number of patients received growth factor. **Data from the second cohort were to be analyzed separately and would serve as a second confirmatory pivotal trial.** As of November 1, 1993 a total of 121 patients (49 European and 72 U.S.) were accrued to the second cohort and completed treatment. Thus, a total of 244 patients had been randomized to receive CP ± Amifostine. One patient was randomized but never treated. Another was still on therapy at the time of this cutoff date. These two patients were excluded from analyses. Per FDA request, all endpoints were analyzed for three cohorts: (1) original interim analysis cohort of 121 patients (2) new cohort of 121 patients accrued subsequent to the interim analysis and (3) all 242 patients. Reviewer's Table I summarizes these patient cohorts:

Reviewer's Table I

COHORT COMPOSITION

COHORT	CP + WR	CP
Original	63	58
New	59	62
All patients	122	120

II EFFICACY RESULTS AND REVIEWER'S COMMENTS:

It was found that for all three cohorts the two treatment arms were well matched with respect to baseline characteristics. These included: age, race, FIGO stage, extent of residual disease, measurable disease, % tumor removed, histology and GOG performance status.

PRIMARY CLINICAL ENDPOINTS:

(1) **Hematologic Toxicity:** The primary endpoint to assess Amifostine's protection against hematologic toxicity was the incidence of Grade 4 neutropenia and fever and/or signs and symptoms of infections requiring antibiotics. Neutropenia was defined as an absolute neutrophil count $\leq 500/mm^3$ associated with fever indicated by a temperature of $\geq$ 100.5° F or 38.0° C and/or other signs or symptoms of infection requiring antibiotic therapy. A June 1992 protocol

amendment states: *"As the use of G-CSF is now p[illegible]ffect the duration of neutropenia and hospitalization for this toxicity. Th[illegible]ary endpoint will be the incidence of Grade 4 neutropenia and fever with or witho[illegible] The incidence and duration of hospitalization for neutropenic fever will also be measured as will the use of G-CSF and antibiotics. These will be compared as secondary endpoints."* The protocol specified that WBC, neutrophil and platelet counts were to be measured prior to therapy, twice weekly during the first two cycles and weekly during the four remaining cycles. However, according to the sponsor: *"because of a change in medical practice which deemphasized nadir counts, blood collections were not collected this frequently unless the patient had clinical signs or symptoms referable to blood count suppression."* In addition the sponsor states: *"Patients having fever and/or infection occurring prior to neutropenia or during non-neutropenic episodes were not counted as "events" in these analyses."* As discussed in the MO's review this strategy may be flawed since such a patient could develop neutropenia while on antibiotics but would not develop a fever and would not consequently be counted as a neutropenic event. Reviewer's analysis of the MO's assessment counting such patients as events will be presented in a later section (Reviewer's Table II).

The sponsor analyzed the following hematologic parameters using the Pearson Chi-square statistic: incidence of neutropenia associated with fever and/or signs and symptoms of infections requiring antibiotics, incidence of Grade 4 neutropenia and proportions of patients whose granulocytes failed to recover to $\geq 1500/mm^3$ by Day 22. For patients who discontinued for hematologic toxicity, their last values are carried forward (LOCF) for subsequent cycles. The mean rates of days in hospital and days on antibiotic therapy for neutropenic fever were compared using a Student's t-test.

(a) **Incidence of Neutropenia Associated Events:** Sponsor's TABLES 19A, 19B and 19C present incidence, by episode and by patient, of neutropenia associated with fever ± signs and symptoms of infections requiring treatment with antibiotics for the original cohort, new cohort and all 242 patients. These summaries indicate that for the original cohort there were significantly fewer patients ($p=0.004$, 2-sided) with and fewer total episodes ($p < 0.001$, 2-sided) of neutropenia; for the new cohort the same pattern persists but not as strongly. Corresponding findings were $p=0.055$, 2-sided and $p=0.066$, 2-sided. It should be pointed out that the Chi-square test of proportions requires independent observations for validity. For the by episode analysis this is clearly not the case since some patients had more than one episode. The relevant analysis is the by patient comparison. Reviewer's Table II presents findings of this reviewer's analysis based on the medical officer's (MO) assessment of this endpoint. In this analysis patients who received antibiotic therapy prior to development of neutropenia are included. The rationale is that-this represents a more conservative approach since such patients would probably not develop fever if they did develop neutropenia. In addition, as previously mentioned, temperatures were not consistently recorded and antibiotic therapy was not standardized.

Reviewer's Table II

MO's Assessment of Neutropenia Associated Clinical Events

I. Original Cohort

CYCLE #	GROUP	EVENTS/PTS. (%)	2-sided p-value	1-sided p-value[a]
1	CP + WR	4 / 60 (6.7)	0.092	0.066
	CP	10 / 56 (17.8)	0.079	0.039*
2	CP + WR	0 / 58 (0.0)	0.473	0.473
	CP	1 / 52 (1.9)	0.303	0.152
3	CP + WR	2 / 56 (3.6)	1.000	0.591
	CP	1 / 44 (2.3)	0.628	0.314
4	CP + WR	0 / 47 (0.0)	0.454	0.454
	CP	1 / 39 (2.6)	0.284	0.142
5	CP + WR	0 / 41 (0.0)	0.209	0.209
	CP	2 / 35 (5.7)	0.155	0.078
6	CP + WR	0 / 39 (0.0)	0.178	0.178
	CP	2 / 29 (6.9)	0.125	0.062

[a] Fisher's Exact test result appears on the 1st line; ChiSquare result is on the 2nd line. For low event frequencies (e.g., < 5) Fisher's Exact test should be used.
* Significant at $\alpha = .05$ via 1-sided test

II. - New Cohort

CYCLE #	GROUP	EVENTS/PTS. (%)	2-sided p-value	1-sided p-value[a]
1	CP + WR	7 / 55 (12.7)	1.000	0.517
	CP	7 / 62 (11.3)	0.757	0.378
2	CP + WR	0 / 50 (0.0)	1.000	0.541

	CP	1 / 59 (1.7)	0.373	0.187
3	CP + WR	0 / 45 (0.0)	1.000	0.536
	CP	1 / 52 (1.9)	0.368	0.184
4	CP + WR	1 / 40 (2.5)	1.000	0.694
	CP	1 / 50 (2.0)	0.597	0.299
5	CP + WR	0 / 36 (0.0)	0.501	0.312
	CP	2 / 46 (4.3)	0.268	0.134
6	CP + WR	0 / 35 (0.0)	-----	-----
	CP	0 / 36 (0.0)	-----	-----

[a] Fisher's Exact test result appears on the 1st line; ChiSquare result is on the 2nd line.

III. All 242 Patients

CYCLE #	GROUP	EVENTS/PTS. (%)	2-sided p-value	1-sided p-value[a]
1	CP + WR	11 / 115 (9.6)	0.315	0.175
	CP	17 / 118 (14.4)	0.259	0.129
2	CP + WR	0 / 108 (0.0)	0.498	0.256
	CP	2 / 111 (1.8)	0.207	0.103
3	CP + WR	2 / 101 (2.0)	1.000	0.670
	CP	2 / 96 (2.1)	0.721	0.361
4	CP + WR	1 / 87 (1.1)	1.000	0.509
	CP	2 / 89 (2.2)	0.530	0.265
5	CP + WR	0 / 77 (0.0)	0.121	0.067
	CP	4 / 81 (4.9)	0.057	0.029*
6	CP + WR	0 / 73 (0.0)	0.220	0.220
	CP	2 / 65 (3.0)	0.168	0.084

[a] Fisher's Exact test result appears on the 1st line; ChiSquare result is on the 2nd line.

* Significant at $\alpha = .05$ via 1-sided test.

IV. Patients With At Least One Event / Includes Cycle 1 Data

COHORT	GROUP	EVENTS/PTS. (%)	2-sided p-value	1-sided p-value[a]
OLD	CP + WR	6 / 60 (10.0)	0.048	0.021*
	CP	14 / 56 (25.0)	0.034	0.017*
NEW	CP + WR	8 / 55 (14.5)	0.802	0.416
	CP	11 / 62 (17.7)	0.639	0.319
ALL PTS.	CP + WR	14 / 115 (12.2)	0.079	0.047*
	CP	25 / 118 (21.2)	0.067	0.033*

[a] Fisher's Exact test result appears on the 1st line; ChiSquare result is on the 2nd line.
* Significant at $\alpha = .05$ via 1-sided test.

V. Patients With At Least One Event / Excludes Cycle 1 Data

COHORT	GROUP	EVENTS/PTS. (%)	2-sided p-value	1-sided p-value[a]
OLD	CP + WR	2 / 58 (3.5)	0.146	0.103
	CP	6 / 52 (11.5)	0.111	0.055*
NEW	CP + WR	1 / 50 (2.0)	0.215	0.146
	CP	5 / 59 (8.5)	0.157	0.079
ALL PTS.	CP + WR	3 / 108 (2.8)	0.050	0.028*
	CP	11 / 111 (9.9)	0.032	0.016*

[a] Fisher's Exact test result appears on the 1st line; ChiSquare result is on the 2nd line.
* Significant at $\alpha = 0.05$ via 1-sided test.

Thus, analysis of the medical officer's evaluation (Part IV of Reviewer's Table II) reveals a statistically significant ($p < 0.021$, one-sided Fisher's Exact test) improvement for the original

cohort Amifostine patients, but no statistically significant improvement for the new cohort Amifostine patients (corresponding p-value is 0.416). The same type of analysis was also performed excluding Cycle 1 data. Part V of Reviewer's Table II summarizes the findings. In this case, there is an indication that new cohort Amifostine patients may do better than new CP controls (one-sided Fisher's Exact test p=0.146), but the result does not approach statistical significance.

(b) Time to Grade 4 Neutropenia Associated with Fever ± Infections: The sponsor compared the two treatment groups with respect to time to first occurrence of Grade 4 neutropenia associated with fever ± infections requiring antibiotic therapy via Logrank testing. Sponsor's TABLE 20 (below) presents testing results for the 3 patient cohorts.

Sponsor's TABLE 20

Analysis of the Time to First Occurrence of Grade 4 Neutropenia Associated With Fever ± Infections Requiring Antibiotic Therapy

		p-value[a]	
COHORT	χ^2	2-sided	1-sided
Original	8.676	0.003	0.002
New	3.541	0.060	0.030
All 242 Patients	11.724	0.001	<0.001

[a]Based on Logrank statistic

As in the sponsor's previous analysis, results significantly favor the CP + WR arm in the original cohort (p=0.003, 2-sided). The effect is not as strong in the new cohort patients, but is close to statistical significance with two-sided testing (p=0.060).

Reviewer's Table III and IV present a summary of this same type of time-to-event analysis utilizing the MO's assessment which includes first antibiotic use as an event. The first analysis utilized data from all cycles and the second analysis excluded Cycle 1 data. These tables indicate that the MO's assessment yields a statistically nonsignificant result ($p > 0.60$) for the new CP + WR group, but a statistically significant improvement for the original cohort CP + WR group ($p = 0.024$) when all the available data are used. When Cycle 1 data are excluded, this pattern persists but not as strongly. While there is a high degree of censoring particularly in the second analysis, the MO's assessment indicates results from the original cohort were not replicated in the new cohort for time to 1st neutropenia associated clinical event.

Reviewer's Table III

MO's Assessment of Time to 1st Neutropenia Associated Clinical Event Logrank Analysis (Includes Cycle 1 Data)

COHORT	CP + WR		CP ONLY		Logrank
	Incidence	Median (cycles)	Incidence	Median (Cycles)	p-value[1]
OLD	6 / 60 (10.0%)	------	14 / 56 (25.0%)	------	0.024
NEW	8 / 55 (14.5%)	------	11 / 62 (17.7%)	------	0.648
ALL	14 / 115 (12.2%)	------	25 / 118 (21.2%)	------	0.054

[1] Mantel-Cox Logrank test (2-sided)

Reviewer's Table IV

MO's Assessment of Time to 1st Neutropenia Associated Clinical Event Logrank Analysis (Excludes Cycle 1 Data)

COHORT	CP + WR		CP ONLY		Logrank
	Incidence	Median (cycles)	Incidence	Median (cycles)	p-value[1]
OLD	2 / 58 (3.4%)	------	6 / 52 (11.5%)	------	0.082
NEW	1 / 50 (2.0%)	------	5 / 59 (8.5%)	------	0.158
ALL	3 / 108 (2.8%)	------	11 / 111 (9.9%)	------	0.028

[1] Mantel-Cox Logrank test (2-sided)

Likewise, for 1st occurrence of Grade IV neutropenia, the MO's assessment indicates statistically nonsignificant findings ($p > 0.38$) for all patient cohorts. The results of this analysis are presented in Reviewer's Table IV below.

Reviewer's Table V

MO's Assessment of Time to 1st Grade IV Neutropenia Logrank Analysis

COHORT	CP + WR		CP ONLY		Logrank
	Incidence	Median (cycles)	Incidence	Median (cycles)	p-value[1]
OLD	58/64 (90.6%)	2.0	47/56 (83.9%)	2.0	0.392
NEW	44/60 (73.3%)	2.0	50/62 (80.6%)	2.0	0.423
ALL	102/124 (82.2%)	2.0	97/118 (82.2%)	2.0	0.992

[1] Mantel-Cox Logrank test (2-sided)

(c) Days in Hospital and Days on Antibiotics Associated With Neutropenia, Fever and/or Infection in Patients Requiring Antibiotic Therapy: These were protocol defined secondary endpoints and will only be briefly discussed. On 11/28/94 the sponsor submitted additional analyses of this data utilizing approximate permutation tests. The rationale for this statistical approach is that the parametric assumptions for their original t-test analysis are tenuous because of the small number of patients hospitalized. The most recent results are presented in ATTACHMENT 3. These permutation testing results, although not statistically significant, are more favorable to the CP + WR arm than were the initial t-test results. It must be pointed out, however, that the issue is data reproducibility.

(d) Treatment-related Nephrotoxicity: Sponsor's TABLE 34 (below) summarizes their analysis

of time to 1st occurrence of a 'nephrotoxic event' defined as either failure of serum creatinine levels to return to ≤ 1.5 mg/dL by Day 22 (± 3 days) or discontinuation of chemotherapy for renal toxicity. The two treatment arms were compared for each of the three patient cohorts via the logrank test.

SPONSOR'S TABLE 34

Analysis of Time to 1st Occurrence of a "Nephrotoxic Event"

Pt. Cohort	Chi Square	2-sided p-value	1-sided p-value
Original	6.013	0.014	0.007
New	1.501	0.221	0.111
All 242 Pts.	6.519	0.011	0.006

Again, as for the other primary endpoint, the sponsor's results are statistically significant (p=0.007, 1-sided test) favoring the original cohort Amifostine patients, but this finding is not confirmed for the new cohort Amifostine patients.

The MO's assessment of this endpoint yielded logrank results presented in Reviewer's Table VI below. These indicate a statistically significant improvement for new cohort Amifostine patients ($p < 0.002$). A detailed discussion of the observed patterns for this toxicity is presented in the MO's review and these results should be interpreted in light of that discussion.

Reviewer's Table VI

MO's Assessment of Time to 1st Serum Creatinine Deterioration (> 1.5 mg/dL) Logrank Analysis

COHORT	CP + WR		CP ONLY		Logrank
	Incidence	Median (cycles)	Incidence	Median (cycles)	p-value[1]
OLD	14/63 (22.2%)	------	17/56 (30.3%)	------	0.269
NEW	24/59 (40.7%)	------	10/62 (16.1%)	------	0.002

ALL	38/122 (31.1%)	-------	27/118 (22.9%)	-------	0.130

[1] Mantel-Cox Logrank test (2-sided)

(2) Response and Time to Progression (TTP): Neither tumor response nor TTP was a protocol specified primary endpoint. Since these were patients with advanced ovarian cancer, dates of disease progression could not be very precisely determined. Both the sponsor's response to treatment and TTP findings are discussed in the MO's review. As noted in that review the progression dates used by the sponsor did not take into account second-line therapies administered to some patients before progression was documented. Thus, the MO performed a TTP evaluation censoring patients so treated at second-look surgery. Reviewer's Tables VII and VIII present results of the TTP analysis for a the full cohort (242 patients).

Reviewer's Table VII

TTP Logrank Analysis Censoring 2nd Look Surgery
Patients Who Received 2nd Line Therapy

COHORT	CP + WR		CP ONLY		Logrank
	Incidence	Median (montl.s)	Incidence	Median (months)	p - value
OLD	30/63 (48%)	15.4	26/58 (45%)	16.9	0.892
NEW	15/59 (25%)	20.2	16/62 (26%)	22.9	0.865
ALL	45/122 (37%)	15.8	42/120 (35%)	18.1	0.911

Reviewer's Table VIII

TTP Hazard Ratios (HR) and 95% C.I.'s
Censoring 2nd Look Surgery Patients Who
Received 2nd Line Therapy

COHORT	(CP + WR) / CP HR	95% C.I. for HR
OLD	1.037	0.613 , 1.755
NEW	0.939	0.519 , 1.924
ALL	0.976	0.641, 1.487

Thus, the FDA analysis indicates that the Amifostine patients were more comparable to the CP controls than the sponsor's analysis. While the point estimate for the TTP hazard ratio is now closer to one, the lower confidence limits are still very low. The available sample size (242 patients) is inadequate to rule out a tumor protective effect for Amifostine in terms of the TTP endpoint with acceptable precision.

(3) Survival: The sponsor recently submitted updated survival analyses through July 1, 1994 (The clinical study report analysis included survival data through November 1, 1993). Sponsor's FIGURE 1 presents Kaplan-Meier plots and results of logrank testing for the most current data available on the full 242 patient cohort. The curves for the two groups are almost superimposable. The point estimate for the survival hazard ratio is 0.971 with a lower confidence limit of 0.688.

In addition, the sponsor also recently provided bootstrap resampling studies and simulations from a parametric model of the actual survival data (Sponsor's ATTACHMENTS 1 and 2). This was done to further investigate the tumor protection question based on the survival endpoint. These studies yielded consistent estimates for the survival hazard ratio and empirically determined lower confidnece limits which do not fall below 0.80. Thus, if the trial had been larger, these studies indicate equivalent anti-tumor activity could have been demonstrated based on the survival endpoint.

Overall Summary and Conclusions:

(1) The major concern with the current application is that the data from the new cohort patients, while pointing in a favorable direction for Amifostine, do not demonstrate a statistically significant improvement as did the original cohort for the major efficacy endpoint, viz., neutropenic event, based on this reviewer's analysis of the reviewing medical officer's by patient assessment. Thus, from a regulatory standpoint, there is no second confirmatory clinical study for the primary efficacy question.

(2) The reviewing medical officer's assessment of this endpoint is at variance with that of the sponsor's. This is in part due to the fact that data needed for evaluation of neutropenia associated clinical events were not always collected in a consistent manner. Specifically, ascertainment dates were not consistently provided for the primary efficacy endpoint, viz., development of neutropenic fever ± infection. Temperatures were not consistently recorded. The type of antibiotic therapy administered was not standardized, viz., broad spectrum vs. agents for local infections. Thus, statistical testing results are highly dependent on how these data are interpreted particularly for the new patient cohort.

(3) Regarding assessment of a possible tumor protective effect for Amifostine, objective response rate and time to progression (TTP) cannot be determined with a great deal of precision in the study group, viz., patients with advanced ovarian cancer. This reviewer's analysis of the medical officer's assessment of TTP (censoring patients who received 2nd line therapy for residual disease at 2nd look surgery) indicates that Amifostine patients appear to be comparable to CP controls (0.976 TTP hazard ratio for the full cohort with associated 95% C.I. of 0.641 to 1.487). The lower confidence limit (LCL) is very low given the sample size available. However, particularly encouraging evidence for this question is the survival experience of the full cohort with very mature data. Kaplan-Meier survival curves are virtually superimposable. The estimated survial hazard ratio point estimate is 0.971 with an associated 95% C.I. of 0.688 to 1.372. Sponsor's bootstrap resampling studies and simulation studies from an empirically determined parametric survival model indicate that, if the study had been sized large enough to definitively address the equivalent antitumor efficacy question, Amifostine would have met the 0.80 lower confidence limit criterion.

In this reviewer's opinion the evidence submitted at this time is very encouraging regarding the tumor protection question. However, the primary question regarding Amifostine's protection against neutropenia associated clinical events has not been answered unequivocally. Analysis of the medical officer's assessment of individual patient data yielded statistical testing results significantly favoring the original cohort Amifostine patients. For the new cohort Amifostine patients, however, there was an indication of improved performance, but this could not be confirmed via statistical significance.

Clare Gnecco

Clare Gnecco, Ph.D.
Mathematical Statistician

Concur: Dr. Wilson 2/16/95

Dr. Dubey 2-16-95

cc: NDA 20-221
HFD-150
HFD-150/Dr. Justice
HFD-150/Dr. DeLap
HFD-344/Dr. Lisook
HFD-713/Dr. Dubey [File: DRU 1.3.2 NDA]
HFD-713/Dr. Wilson
HFD-713/Dr. Gnecco
Chron
Gnecco/12-08-94/WP6.03a/ETHYOL.REV
This review consists of 15 pages of text, 3 Tables, 1 Figure and 1 Attachment.

TABLE 19A

Incidence of Neutropenia Associated With Fever and/or Signs/Symptoms of Infection Requiring Treatment with Antibiotics
- Original Cohort (N=121) -

	Amifostine + CP	CP	χ^2	p-value[a] 2-sided	p-value[a] 1-sided
Cycle 1	2/ 63	10/ 58	6.633	0.010	0.005
Cycle 2	0/ 60	2/ 53	2.285	0.131	0.066
Cycle 3	1/ 57	1/ 45	0.028	0.866	0.433
Cycle 4	0/ 48	1/ 40	1.200	0.273	0.137
Cycle 5	0/ 42	2/ 35	2.432	0.119	0.060
Cycle 6	0/ 40	2/ 29	2.800	0.094	0.047
Total episodes	3/310	18/260	14.108	<0.001	<0.001
Incidence by patient	3/ 63	13/ 58	3.132	0.004	0.002

[a] Based on Pearson Chi-Square test.

TABLE 19B

Incidence of Neutropenia Associated With Fever and/or Signs/Symptoms of Infection Requiring Treatment with Antibiotics
- New Cohort (N=121) -

	Amifostine + CP	CP	χ^2	p-value[a] 2-sided	p-value[a] 1-sided
Cycle 1	4/ 59	9/ 62	1.871	0.171	0.086
Cycle 2	0/ 53	1/ 59	0.898	0.343	0.172
Cycle 3	0/ 47	1/ 52	0.904	0.342	0.171
Cycle 4	1/ 41	1/ 50	0.020	0.888	0.444
Cycle 5	0/ 37	2/ 46	1.629	0.202	0.101
Cycle 6	0/ 34	0/ 36	—	—	—
Total episodes	5/271	14/305	3.384	0.066	0.033
Incidence by patient	5/ 59	13/ 62	3.695	0.055	0.028

[a] Based on Pearson Chi-Square test.

TABLE 19C

Incidence of Neutropenia Associated With Fever and/or Signs/Symptoms of Infection Requiring Treatment with Antibiotics
- All Patients (N=242) -

				p-value[a]	
	Amifostine + CP	CP	χ^2	2-sided	1-sided
Cycle 1	6/122	19/120	7.749	0.005	0.003
Cycle 2	0/113	3/112	3.054	0.081	0.041
Cycle 3	1/104	2/ 97	0.411	0.521	0.261
Cycle 4	1/ 89	2/ 90	0.326	0.568	0.284
Cycle 5	0/ 79	4/ 81	3.976	0.046	0.023
Cycle 6	0/ 74	2/ 65	2.294	0.130	0.065
Total episodes	8/581	32/565	15.613	<0.001	<0.001
Incidence by patient	8/122	26/120	11.389	0.001	0.001

[a] Based on Pearson Chi-Square test.

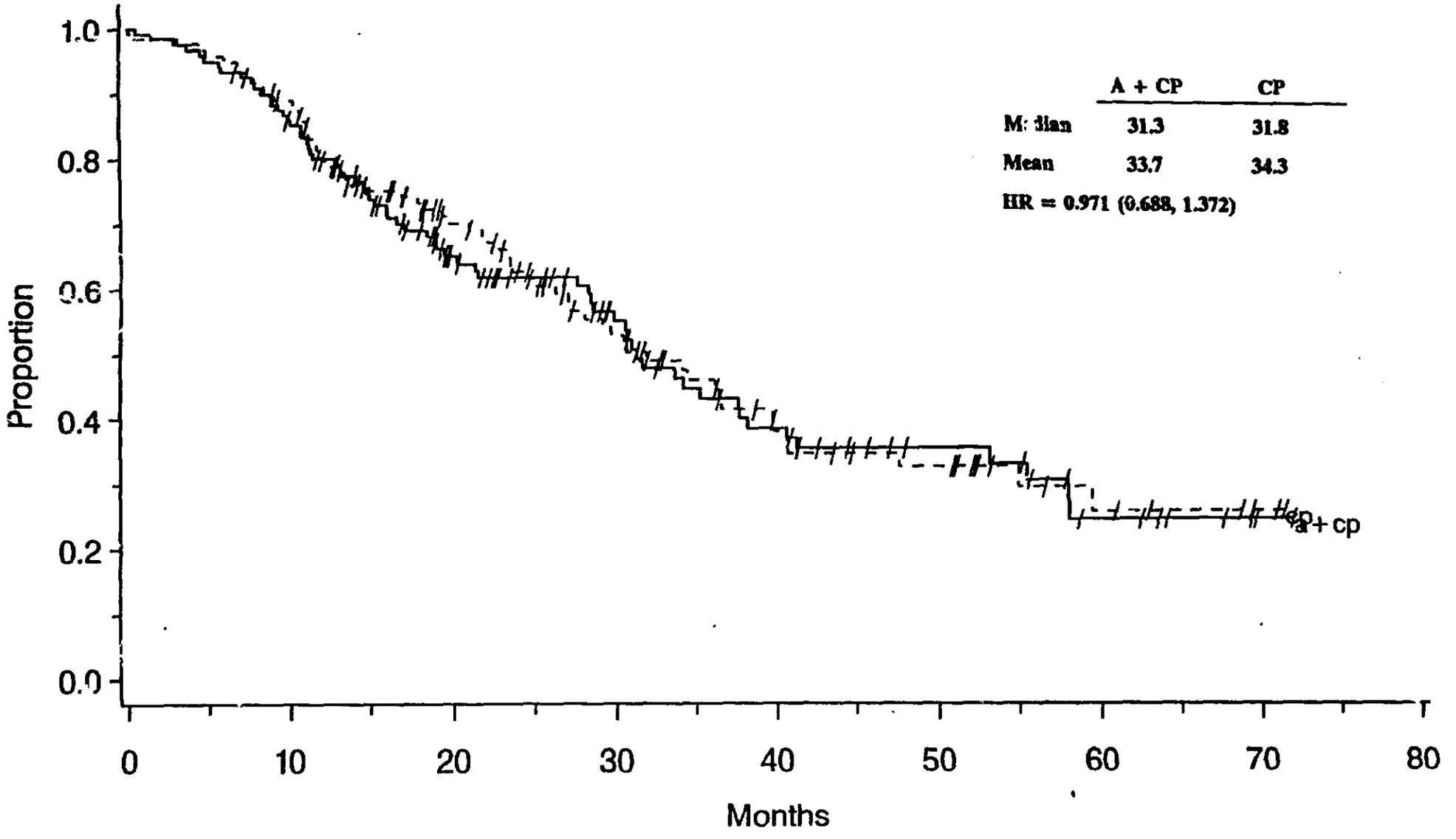
SPONSOR's FIGURE 1

Product Limit Estimate

Survival Time

Cohort: All 242

Amifostine + Cisplatin (n=122) VS Cisplatin (n=120)



	A + CP	CP
Median	31.3	31.8
Mean	33.7	34.3

HR = 0.971 (0.688, 1.372)

Log-Rank Chi-Square: 0.0270 Pr>Chi-Square: 0.8695

Wilcoxon Chi-Square: 0.0952 Pr>Chi-Square: 0.757[illegible]

Data Last Modified on 08NOV94

SAS Program: STAT:[CONLON]SURV[illegible]PH.SAS;47

PWSURV

ETHYOL® FDA STATISTICS

I. Modelling and Simulation Analysis

Modelling Description:

Event data (time to date of death) from each treatment arm were modelled using probit models. For both treatment arms a single probit model provided an inadequate fit to the data. However, when the event data are separated into early and late events and modelled separately, we were able to adequately fit the event data for both treatment arms. To assess the impact of the cutoff point between the early and late event data on the goodness of fit, the data were modelled with cutoffs at 12, 15, 18, 21, and 24 months.

Modelling Results:

Cutoff (Month)	Event Category	Treatment Group	Likelihood Ratio P-value (GOF)	μ	σ
12	Early	Control	>0.999	14.625	6.115
		Amifostine	>0.999	13.940	5.936
	Late	Control	>0.999	18.594	16.544
		Amifostine	>0.999	16.799	18.738
15	Early	Control	>0.999	14.910	6.402
		Amifostine	>0.999	15.003	6.954
	Late	Control	>0.999	19.265	15.574
		Amifostine	>0.999	15.843	20.023
18	Early	Control	0.973	15.947	7.574
		Amifostine	>0.999	15.467	7.461
	Late	Control	>0.999	19.352	15.460
		Amifostine	>0.999	15.876	19.990
21	Early	Control	0.680	17.103	8.920
		Amifostine	0.995	16.202	8.392
	Late	Control	>0.999	19.487	15.294
		Amifostine	>0.999	17.774	17.828
24	Early	Control	0.243	18.843	11.109
		Amifostine	0.980	16.484	8.785
	Late	Control	>0.999	19.115	15.597
		Amifostine	>0.999	21.124	14.099

With the exception of the control arm early event data for 21 and 24 month cutoffs, the probit models demonstrate excellent fit to the observed data. The estimates of μ and σ obtained from the modelling are then used to simulate event data for the two treatment arms.

Simulation Description:

Event data from the models obtained above were simulated for each event category within each treatment group. The number of events simulated for each treatment arm is equivalent to the number actually observed. The chance (probability) of selecting an early or late event within a treatment group is equivalent to the actual rate observed. Censored data is simulated for each treatment arm from a uniform distribution on 0 to 50 months. The number of censored observations in each treatment arm is equivalent to the number actually observed.

For each cutoff point, 500 samples were simulated and the empirical distribution of hazard ratios obtained.

Simulation Results:

Cutoff (Month)	Mean Hazard Ratio	Empirical 95% Confidence Interval
12	1.030	(0.806, 1.292)
15	1.022	(0.795, 1.261)
18	0.994	(0.788, 1.247)
21	0.973	(0.794, 1.190)
24	1.006	(0.814, 1.267)
Mean	1.005	(0.799, 1.251)

- The simulation demonstrates a robustness of results around a hazard ratio of 1.00 and 95% confidence interval of 0.80 to 1.25.

II. Bootstrap Analysis

Bootstrap Description:

The observed data were divided into four groups: (1) Control Arm, censored observations; (2) Control arm, event observations (i.e., deaths); (3) Amifostine arm, censored observations; and (4) Amifostine arm, event observations (i.e, deaths). Bootstrap samples of size 242 were drawn from each group (57 from group 1; 65 from group 2; 56 from group 3; and 64 from group 4), then combined together maintaining treatment group assignment and censoring indicator. The hazard ratio for each sample is then computed using PROC PHREG. A total of 500 bootstrap samples are drawn and the empirical distribution of hazard ratios obtained. This process was performed 20 separate times using 20 different seeds to initiate the random resampling.

Bootstrap Results:

Seed	Mean Hazard Ratio	Empirical 95% Confidence Interval
12354	0.979	(0.803, 1.173)
87349	0.974	(0.818, 1.172)
94886	0.976	(0.822, 1.146)
15354	0.972	(0.794, 1.155)
88349	0.972	(0.815, 1.145)
93886	0.971	(0.809, 1.146)
17354	0.979	(0.824, 1.168)
87349	0.974	(0.818, 1.172)
93486	0.983	(0.813, 1.175)
12561	0.976	(0.806, 1.163)
23349	0.974	(0.807, 1.172)
94566	0.977	(0.817, 1.165)
93785	0.977	(0.808, 1.178)
37445	0.972	(0.803, 1.154)
64686	0.974	(0.820, 1.168)
12234	0.973	(0.812, 1.174)
65423	0.976	(0.813, 1.171)
22552	0.979	(0.821, 1.177)
32995	0.979	(0.811, 1.177)
83483	0.974	(0.813, 1.151)
Mean	0.975	(0.812, 1.165)

The bootstrap analysis demonstrates a robustness of results around a hazard ratio of 0.975 and a 95% confidence interval of 0.812 to 1.165.

III. Analysis of Recovery from Grade 4 Granulocytopenia

Description:

For each patient cycle, we determine from actual laboratory data, if that cycle resulted in a grade 4 granulocyte count. We then restrict the analysis data set to only those cycles in which grade 4 granulocytopenia was present. For those patients who discontinued therapy prior to cycle 6 for granulocytopenia, we augment this data set with a record for each cycle from the patient's last actual cycle of therapy up to 6 cycles of therapy. This resulted in an augmentation of the data set from 486 cycles to 509 cycles for the nine patients who discontinued therapy for granulocytopenia. For each cycle resulting in grade 4 granulocytopenia, a determination is made (where possible) whether recovery to a granulocyte count of 1500 occurred by day 25 of the cycle. For the augmented cycles, the determination was fixed at 'non-recovery.' Once the augmented dataset for grade 4 granulocytopenia has been prepared (with the determination of recovery, non-recovery, or no determination), the analysis by cycle proceeds. The analysis of last cycle and any cycle are performed by creating additional datasets, one for each patient's last grade 4 cycle (for which a determination could be made), and one for each patient's worst recovery (for which a determination could be made). The augmenting of the dataset does not affect these analyses.

To be considered as "recovered" in this analysis, a patient must have demonstrated a recovery value on a cycle day between 16 and 25 inclusive (in addition the day 16 to 25 recovery day could not have been past day 1 of the next cycle. There were eight cases in which a determination could not be made. They are listed below. In all of the eight cases there was evidence that may have supported a determination of recovery by day 25. It was decided instead to treat them as unevaluable.

T Group	Alloc	Cycle	Granulocyte Counts	
			Date	
CP	2003	1	Day 15 Day 27	400 2200
CP	1126	6	Day 15 No Further Counts	1300
CP+WR	0528	1	Day 13 Day 27	11000 6000
CP+WR	0804	1	Day 14 Day 27	130 7000
CP+WR	0920	1	Day 14 Day 29	340 3000
CP+WR	2317	4	Day 15 Day 28	416 2600
CP+WR	0803	5	Day 15 Day 30	100 1500
CP+WR	2317	6	Day 15 Day 31	459 1900

Results: The tables below list number of non-recoveries in the numerator and number of grade 4's for whom a determination could be made in the denominator.

With carried forward data:

Cycle	Amifostine	Control	Chi-square	p-value
1	7/71	13/72	1.982	0.159
2	18/56	25/59	1.273	0.259
3	20/38	20/40	0.053	0.817
4	17/34	18/35	0.014	0.906
5	9/16	29/42	0.825	0.364
6	6/15	16/23	3.170	0.075
Last Cycle	10/26	32/50	4.453	0.035
Any Cycle	43/98	64/99	8.518	0.004

Without carried forward data:

Cycle	Amifostine	Control	Chi-square	p-value
1	7/71	13/72	1.982	0.159
2	18/56	24/58	1.035	0.309
3	20/38	18/38	0.208	0.649
4	16/33	14/31	0.070	0.792
5	8/15	24/37	0.588	0.443
6	5/14	8/15	0.878	0.349
Last Cycle	10/26	32/50	4.453	0.035
Any Cycle	43/98	64/99	8.518	0.004

IV. Analysis of Recovery of Serum Creatinine

Description:

Original dataset contains a record for each patient cycle with determination of recovery to <=1.50mg/dL. Dataset is augmented in the same manner as for granulocyte recovery for the six patients who discontinued for renal toxicity. The dataset was augmented by 13 patient/cycles from 1146 to 1159. Determination of recovery for each cycle, for last cycle, and for any cycle was made in the same manner as for granulocyte recovery.

To be considered for analysis here a patient must have at least one serum creatinine value in the interval between days 16 and 25. The "unk" category refers to those patient cycles for which no determination could be made. There were 208 cases of no determination. For 100 (47 control, 53 amifostine) of these cases, the cycle was the patient's last cycle and follow-up creatinines were not obtained. For the rest of the cases, the physician retreated on day 21 or thereabouts because the patient had not demonstrated any renal toxicity at earlier cycles and had serum creatinine values well within normal range within 15 days after therapy. Analysis was performed using the "unk" observations as recovered as well as using them as missing observations.

Results:

With carried forward data: "unk" treated as recovered

Cycle	Treatment	Unk	Yes	No	Chi-Square	p-value
1	Amifostine	8	112	2	0.320	0.572
	Control	12	107	1		
2	Amifostine	16	97	0	3.054	0.081
	Control	9	100	3		
3	Amifostine	21	82	1	1.118	0.290
	Control	11	85	3		
4	Amifostine	22	67	0	5.970	0.015
	Control	14	72	6		
5	Amifostine	20	59	0	13.204	<0.001
	Control	10	61	13		
6	Amifostine	34	38	2	7.213	0.007
	Control	31	29	11		
Last Cycle	Amifostine	6	111	5	5.609	0.018
	Control	6	99	15		
Any Cycle	Amifostine	6	111	5	6.484	0.011
	Control	6	98	16		

With carried forward data: "unk" treated as missing. (Analyses submitted to original NDA).

Cycle	Treatment	Unk	Yes	No	Chi-Square	p-value
1	Amifostine	8	112	2	0.284	0.594
	Control	12	107	1		
2	Amifostine	16	97	0	2.854	0.091
	Control	9	100	3		
3	Amifostine	21	82	1	0.903	0.342
	Control	11	85	3		
4	Amifostine	22	67	0	5.339	0.021
	Control	14	72	6		
5	Amifostine	20	59	0	11.401	0.001
	Control	10	61	13		
6	Amifostine	34	38	2	7.347	0.007
	Control	31	29	11		
Last Cycle	Amifostine	6	111	5	5.644	0.018
	Control	6	99	15		
Any Cycle	Amifostine	6	111	5	6.525	0.011
	Control	6	98	16		

Without carried forward data: "unk" treated as recovered

Cycle	Treatment	Unk	Yes	No	Chi-Square	p-value
1	Amifostine	8	112	2	0.320	0.572
	Control	12	107	1		
2	Amifostine	16	97	0	3.054	0.081
	Control	9	100	3		
3	Amifostine	21	82	1	0.002	0.961
	Control	11	85	1		
4	Amifostine	22	67	0	4.023	0.045
	Control	14	72	4		
5	Amifostine	20	59	0	10.338	0.001
	Control	10	61	10		
6	Amifostine	34	38	2	1.788	0.181
	Control	31	29	5		
Last Cycle	Amifostine	6	111	5	5.609	0.018
	Control	6	99	15		
Any Cycle	Amifostine	6	111	5	6.484	0.011
	Control	6	98	16		

Without carried forward data: "unk" treated as missing

Cycle	Treatment	Unk	Yes	No	Chi-Square	p-value
1	Amifostine	8	112	2	0.234	0.594
	Control	12	107	1		
2	Amifostine	16	97	0	2.854	0.091
	Control	9	100	3		
3	Amifostine	21	82	1	0.001	0.980
	Control	11	85	1		
4	Amifostine	22	67	0	3.602	0.058
	Control	14	72	4		
5	Amifostine	20	59	0	8.933	0.003
	Control	10	61	10		
6	Amifostine	34	38	2	1.994	0.158
	Control	31	29	5		
Last Cycle	Amifostine	6	111	5	5.644	0.018
	Control	6	99	15		
Any Cycle	Amifostine	6	111	5	6.525	0.011
	Control	6	98	16		

V. Analysis of time to renal toxicity

Description:

Serum creatinine levels were used to assess time to renal toxicity. The cycle at which the patient first experienced the renal toxic event is recorded. Patients who did not experience the event are censored at their last cycle of therapy. Post therapy elevations are assigned to patient's last cycle.

Results:

Elevation to >1.50

	Amifostine	Control
Event Rate	39/122	33/120
Median	ND	ND
Mean	4.876	5.280

Hazard Ratio = 0.842 (0.533, 1.330)
-2 LOG (L) p-value = 0.4594

VI. Assessment of All Serum Creatinine Elevations To >1.5 mg/dL

Description: All cycles in which serum creatinine was elevated to >1.5 mg/dL were assessed for cause. Two categories were considered: Cisplatin toxic effect or effect of dehydration from nausea and vomiting. The first elevation and each creatinine value obtained after the first elevation was assessed individually. For an observation to be classified as a cisplatin toxic effect, either there was no nausea and vomiting associated with the elevation (C-1) or serum creatinine levels were elevated with associated nausea and vomiting but were maintained without associated nausea and vomiting (C-2). For an event to be classified as an effect of nausea and vomiting, one or more of three categories are used: 1) grade >1 nausea and vomiting associated with elevated serum creatine (N-1); 2) mild nausea and vomiting was associated with elevated serum creatinine (N-2), or 3) resolution of nausea and vomiting results in a lowering of serum creatinine (N-3). The determination of overall causality per cycle based on the individual assessments. If the first assessment was C-1, then that cycle elevation was labelled a cisplatin effect with four exceptions:

Patient Cycle 2
- Initial elevation: no nausea and vomiting
- Grade 2 nausea and vomiting produces additional hike in serum creatinine
- Serum creatinine elevation resolves after nausea and vomiting resolves

Patient Cycle 5
- Cycles 1, 4, and 6 produced nausea and vomiting related elevations
- Elevation to 1.8 which resolves within 10 days

Patient Cycle 2
- Cycle 1 produced extreme nausea and vomiting related elevations
- Cycle 2 elevations lower than Cycle 1 elevations

Patient Cycle 4
- Cycle 3 produced nausea and vomiting related elevations
- Elevation to 1.6 which resolves on the following day

In cases where the initial serum creatinine elevation was associated with nausea and vomiting, the assessment was that the elevation was a consequence of nausea and vomiting unless all of the following events occurred. The initial grade of nausea and vomiting was mild, the serum creatinine elevation was less than 3.3 mg/dL, and there were at least 2 stable elevated serum creatinine values following the initial elevation whether or not nausea and vomiting resolved. The following five exceptions are noted:

Patient Cycle 3
- Consistent grade 2 nausea and vomiting associated with constant serum creatinine levels
- Cycle 2 determined to be nausea and vomiting related
- Event classified as nausea and vomiting related

Patient Cycle 3
- Resolution of nausea and vomiting results in immediate lowering of serum creatinine
- Cycle 2 determined to be nausea and vomiting related
- Event classified as nausea and vomiting related

Patient Cycle 4
- Cycle 1 attributed to cisplatin effect
- Pretreatment baseline serum creatinine levels gradually rising
- Mild nausea and vomiting which did not seem to affect serum creatinine levels
- Event classified as a cisplatin effect

Patient . Cycle 2

- Initial elevation to 4.0 mg/dL with no nausea and vomiting recorded for any cycles
- Event classified as a cisplatin effect

Patient . Cycle 1

- Mild nausea and vomiting with persistent elevated serum creatinine levels
- Cycle 2 elevations attributed to cisplatin
- Event classified as a cisplatin effect

Serum creatinine elevations were also categorized as to whether they were transient or protracted. Protracted is defined as persistent elevation of serum creatinine above 1.5 mg/dL beyond day 35 of the cycle. Incidence rates of transient and protracted elevations were analyzed for treatment group differences. The analyses are performed separately for cisplatin-related elevations and for nausea and vomiting-related elevations.

Results

	Cisplatin Effect		p-value		Nausea and Vomiting		p-value	
	Amifostine + CP	CP	2-sided	1-sided	Amifostine + CP	CP	2-sided	1-sided
Transient								
Original Cohort	11	16	0.145	0.073	10	7	0.709	0.355
New Cohort	14	9	0.176	0.088	20	4	<0.001	<0.001
All 242 Patients	25	25	0.920	0.460	30	11	0.003	0.002
Protracted								
Original Cohort	0	9	0.001	0.001	2	1	0.669	0.335
New Cohort	0	5	0.034	0.017	1	1	0.933	0.467
All 242 Patients	0	14	<0.001	<0.001	3	2	0.677	0.339

VII. Analysis of Days of Hospitalization and Days of Antibiotic Use Associated with Neutropenic Fever

- Description:

A dataset with one record for each patient/cycle is created which has the number of hospital days and antibiotic days for those cycles in which an episode of neutropenic fever occurred. Hospital days and antibiotic days are set equal to zero for those cycles in which an event did not occur. For the by patient analysis, one record is created for each patient, representing total days for the entire study. For the by episode analysis, all cycle data is taken together, thus the sample size is the entire set of cycles (with patients

removed from analysis). For the analysis excluding cycle 1, all cycle 1 records are removed from the dataset. This has the effect of removing all patients who had only one cycle of therapy from the by patient analysis. It also eliminates all cycle one data from the by episode analysis.

The incidence of neutropenic fever is only about 20% overall. The presence of 80% or more zeros in the analysis means that the sample is highly skewed. The usual assumptions of the t-test and the Wilcoxon test seem unwarranted. The test chosen is an exact permutation test. Because the number of permutations is too large to be computationally feasible ($>10^{12}$), an approximate p-value is calculated (Agresti *et al*, 1977 and 1979).

For each test a random sample of 1000 permutations is selected and the empirical distribution of the difference in mean days is obtained. The approximate p-value is then calculated from this distribution.

Results:

For the entire cohort (excluding

Analysis	Days Amifostine (Sample Size)	Days Control (Sample Size)	Test	2-sided p	1-sided p
By Patient (Hospital)	129 (N=119)	226 (N=119)	T-test	0.164	0.082
			Permutation	0.144	0.101
By Episode (Hospital)	129 (N=568)	226 (N=563)	T-test	0.087	0.049
			Permutation	0.093	0.047
By Patient (Antibiotic)	146 (N=119)	284 (N=119)	T-test	0.115	0.058
			Permutation	0.093	0.059
By Episode (Antibiotic)	146 (N=568)	284 (N=563)	T-test	0.059	0.029
			Permutation	0.059	0.031

Entire Cohort (Excluding

Analysis	Days Amifostine (Sample Size)	Days Control (Sample Size)	Test	2-sided p	1-sided p
By Patient (Hospital)	89 (N=116)	226 (N=119)	T-test	0.026	0.013
			Permutation	0.025	0.012
By Episode (Hospital)	89 (N=560)	226 (N=563)	T-test	0.012	0.006
			Permutation	0.012	0.006
By Patient (Antibiotic)	111 (N=116)	284 (N=119)	T-test	0.033	0.016
			Permutation	0.027	0.014
By Episode (Antibiotic)	111 (N=560)	284 (N=563)	T-test	0.014	0.007
			Permutation	0.012	0.006

The results indicate that the t-test is relatively robust in this situation, but looses some precision because of the lack of symmetry.

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JUN 23 1995

STATISTICAL REVIEW AND EVALUATION

NDA#: 20-221 / Amendment #21

APPLICANT: U.S. Bioscience

NAME OF DRUG: AMIFOSTINE or ETHYOL® (WR-2721)

INDICATION: Cytoprotective agent against the cumulative renal toxicities associated with cisplatin and the cumulative hematologic toxicity associated with cyclophosphamide.

DOCUMENTS REVIEWED: Volume 19.1 of Amendment #21 submission dated 1/31/95
Correspondence to Dr. DeLap of 4/28/95
Correspondence to Dr. DeLap of 5/22/95
Sponsor's Overview Package for 6/9/95 ODAC

MEDICAL OFFICER: Robert DeLap, M.D., Ph.D.

RELEVANT STATISTICAL ISSUES:

(1) **Current Submission:** The original protocol specified efficacy endpoints were hematologic toxicity, neurotoxicity, nephrotoxicity, ototoxicity, and tumor response rate. The current amendment focuses on analyses of two efficacy endpoints, viz., nephrotoxicity (in terms of creatinine and creatinine clearance) and magnesium levels.

(2) Magnesium was not a protocol specified endpoint. The statistically significant improvement reported for amifostine patients is based on a retrospective analysis of an unblinded study.

(3) This submission, as well as the prior one, is based on the second of two planned analyses. The protocol specified interim analysis design was a one-sided Pocock plan with stopping boundary p-values < .03 for the interim and final looks. The sponsor has carried out all statistical analyses using more stringent two-sided testing. However, no adjustment was made to this α-level for testing multiple endpoints. Reported p-values should be interpreted in that light when assessing the statistical significance of efficacy findings.

(4) Consistency of data collection has been a problem in both the earlier and later portions of the ovarian cancer pivotal trial. In particular, as was pointed out at previous ODAC sessions, ascertainment dates and temperature data were not consistently provided for one of the primary

endpoints, viz., neutropenic fever ± infection (12/94 ODAC). Ototoxicity data were too incomplete to evaluate; 45% were missing on the CP + WR arm and 53% on the CP alone arm (1/92 ODAC). For magnesium determinations approximately 33% of patients had missing baseline data (current submission).

(5) Several of the original study endpoints were not well defined and some of the sponsor's analyses are data driven, i.e., post hoc. If reported improvements in serum creatinine, creatinine clearance and magnesium levels are clinically relevant, another confirmatory study is needed. In this case perhaps a conditional approval could be considered.

I BACKGROUND:

First Submission: Sponsor's APPENDIX 2 is taken from Section 7 of the original study protocol for the pivotal ovarian cancer study (WR-2732-1) and presents the efficacy criteria for the 6 protocol specified endpoints. Hematologic toxicity, neurotoxicity and ototoxicity findings have been presented at two previous ODAC meetings, viz., January 1992 and December 1994. At the first of these sessions, which reviewed the interim analysis results for the first half of this study, some of the problems discussed were: (1) a wide confidence interval for the response rate endpoint which raised concerns about a possible amifostine tumor protective effect (2) multiple protocol violations (3) some study endpoints poorly defined (4) some post-hoc decisions on surrogate endpoints (4) incomplete audiology data, i.e., 45% missing data on the CP + WR arm and 53% on the CP alone arm and (5) the study was not blinded creating a concern about the amount and pattern of missing data. At the second ODAC in December 1994 the tumor protection question was satisfactorily resolved based on survival analysis after completion of the second half of the trial (survival curves were superimposable) and simulation studies which indicated that the lower confidence limit for the survival hazard ratio would not be expected to fall below 0.8.

Second Submission: In the previous submission (7/12/94) the major statistical issues were as follows: (1) Data quality for one of the primary endpoints, viz., development of neutropenic fever ± infection, was the main issue. Ascertainment dates were not consistently provided; temperatures were not consistently recorded. The type of antibiotic therapy administered was not standardized, viz., broad spectrum vs. agents for local infections. The reviewing medical officer's (MO) assessment for this endpoint was at variance with that of the sponsor indicating that test results were highly dependent on how these incomplete data were interpreted. (2) This reviewer's analyses of the MO's assessment of time to first neutropenia associated clinical event and number of such events indicated that the new cohort patients did not show statistically significant improvement for this endpoint whereas the original cohort did. Thus, the second half of the study did not confirm the first half for this endpoint. (3) Although the total sample size of 242 patients was not large enough to fully resolve the tumor protection question, the sponsor's simulation studies for the survival endpoint indicated that, if the study had been sized large enough to definitively address the equivalent antitumor efficacy question, Amifostine

would have met the 0.80 lower confidence limit criterion for equivalence.

II EFFICACY RESULTS AND REVIEWER'S COMMENTS:

This review will focus on only the nephrotoxicity improvement question in terms of serum creatinine and creatinine clearance parameters and magnesium level analysis. To assess cumulative renal toxicity, the sponsor's analyses focused on cycles 4, 5 and 6 or the last treatment cycle received for those patients who received at least 4 cycles.

(a) Delay or Discontinuation of Cisplatin Therapy in Patients Whose Post-Treatment Serum Creatinine Levels Had Failed to Return to 1.5 mg/dL: In the pivotal ovarian cancer trial the protocol defined endpoint to assess nephrotoxicity was the need to delay or discontinue the dose of cisplatin. According to the protocol, cisplatin was to be held if serum creatinine levels remained elevated ≥ 1.5 mg/dL. If serum creatinine was > 1.5 mg/dL at day 22 of a cycle, cisplatin dose was to be delayed; if it was still elevated > 1.5 mg/dL at day 35, cisplatin was to be discontinued. The latter is the **definition of protracted elevation of serum creatinine.** Regarding the 1.5 mg/dL cutoff the sponsor states: *"This level of serum creatinine (1.5 mg/dL) was selected since the upper limit of normal for serum creatinine in women as measured in most hospitals is 1.0 mg/dL; consequently a rise in serum creatinine to 1.5 mg/dL would represent a substantial deterioration in renal function."* Sponsor's TABLE 1 (from 1/31/95 submission) presents their analysis of patients discontinued for unacceptable serum creatinine elevations. Note that for cycles 5 and 6 both the original and new patient cohorts show that a statistically significantly greater proportion in the control vs. amifostine arms were not eligible to receive cisplatin as scheduled because of unacceptably high serum creatinine levels ($p \leq .05$, 2-sided). Both arms received comparable cumulative cisplatin doses. TABLE 9 (6/9/95 ODAC packet) summarizes median cumulative doses received for both cohorts and all patients.

Reviewer's Comments: Recall that the protocol specified group sequential design called for 1-sided α-level boundaries of $< .03$ at each look. The sponsor has reported more stringent 2-sided testing throughout this latest submission. Thus, the corresponding 1-sided values would be half of those reported. However, it should be noted that no statistical adjustment to the p-values was made for testing multiple endpoints. The Clinical Studies section of the proposed label cites improvement in renal toxicity based on statistically significant reductions in serum creatinine and creatinine clearance, improvement in severity of hypomagnesemia, decreased hematologic toxicity, and decreased cumulative incidence of neurologic and/or ototoxicity. The type I (false positive) error rate can be substantially inflated depending upon the number of endpoints tested. Since several of these endpoints would appear to be correlated, a Bonferroni type correction, viz., α/(# endpoints tested), is overly conservative. However, some adjustment appears to be warranted given the multitude of endpoints tested.

The sponsor states that this analysis: *"includes all patients who had serum creatinine levels measured 19 to 25 days following chemotherapy. Patients who were withdrawn from*

therapy in earlier cycles due to renal toxicity or nausea/vomiting associated with protracted elevations in serum creatinine at Day 35 were also included; their last serum creatinine value was carried forward." If this is indeed a true LOCF (last observation carried forward) analysis, it can be problematic depending on the pattern of missing data. The sponsor did not provide stratified serum creatinine analyses utilizing baseline serum creatinine status, nephrotoxic antibiotics, hypertension or diabetic history. However, such analyses were provided for creatinine clearance and will be discussed in that section.

(b) **Protracted Elevations in Serum Creatinine:** The cumulative incidence of treatment-limiting cisplatin-related nephrotoxicity (i.e., incidence of protracted elevations) is presented for all patients (including those who discontinued prior to cycle 4) in Sponsor's TABLE 4 (1/95 submission). The difference between the two treatment arms was statistically significant in both cohorts as well as for all patients ($p \leq .03$, 2-sided). Sponsor's corresponding Table (from 4/28/95 correspondence) presents the corresponding analysis for patients who received ≥ 4 cycles:

Sponsor's Table (4/28/95)

Cumulative Incidence of Treatment Limiting Nephrotoxicity (Protracted Elevations in Serum Creatinine for Patients Who Received ≥ 4 Cycles of Therapy)

	Amifostine + CP	CP	p-value (2-sided)
Original Cohort	1 / 48	9 / 40	0.003
New Cohort	0 / 41	5 / 50	0.038
All Patients	1 / 89	14 / 90	0.001

Testing results are very similar to those for the all patients analysis in TABLE 4.
Attachments #1, #2, and #3 present sponsor's scatter plots of serum creatinine levels following the last cycle of chemotherapy relative to baseline values for the original cohort, new cohort and both cohorts combined for those patients who received at least four cycles of chemotherapy. Protracted elevations are those points lying above the horizontal 1.5 mg/dL line. Also indicated on these plots is a 45 degree (slope = 1) "identity line". Points lying on this line would correspond to identical baseline and post treatment values. Points above indicate a patient's post treatment values increased over baseline; points below indicates a patient's post treatment values decreased relative to baseline. All three of these plots indicate more CP arm alone values lying above both lines.

Reviewer's Comment: While the analysis of those patients who received ≥ 4 treatment cycles appears to be clinically meaningful, it was not prospectively specified in the protocol and could be partially data driven given the study was not blinded.

(c) Changes in Creatinine Clearance Values: For this assessment the sponsor estimated creatinine clearance values via the formula of Cockcroft and Gault which incorporates a patient's age, body weight and gender in addition to serum creatinine level. The sponsor compared the proportion of patients in each treatment arm who had ≥ 40% reduction in creatinine clearance. The sponsor's justification for this cutpoint is as follows: *"This represents a clinically significant decrease in renal function and generally requires dosage adjustment for drugs whose main route of elimination are the kidneys: relevant cancer drugs include carboplatin, ifosfamide, methotrexate and bleomycin amongst others."* Sponsor's TABLE 4A (from 6/95 ODAC packet) summarizes baseline creatinine clearance values in cc/min by arm for old and new cohorts as well as for both cohorts combined. This table indicates an imbalance in the two treatment arms favoring the CP alone control arm in the original cohort, although the difference is not statistically significant (p=0.148). This table indicates that more control arm patients had normal baseline creatinine clearance values, i.e., ≥ 100 cc/min. Sponsor's TABLE 2 (from 1/95 submission) presents the results of this analysis for old cohort, new cohort and all patients. For the old cohort there is one statistically significant finding (p=.008, 2-sided) favoring the amifostine arm, viz., at the last cycle for those patients who received ≥ four cycles of therapy. For the new cohort, on the other hand, statistically significant differences favoring the amifostine arm were found for cycle 4 and last cycle for all patients and for patients receiving ≥ four cycles of therapy. Corresponding 2-sided p-values were 0.008, 0.018, and 0.014. For both cohorts combined all tests yielded statistically significant results with the exception of cycle 5 (p=0.086, 2-sided). The sponsor reports that more patients in the control CP alone arm had persistent reductions in creatinine clearance of at least 40%.

A similar table appears in the sponsor's 6/95 ODAC package, viz., TABLE 4B. In this table, the findings are qualitatively similar to those of the earlier table, but many of the counts are different, both in the numerators and the denominators. In the earlier TABLE 2 the sponsor performed a true LOCF analysis which can be highly problematic depending on the pattern of missing data since it utilizes the last recorded value and imputes that value for remaining ascertainment periods after the patient has discontinued protocol therapy. In a recent correspondence packet (5/22/95 letter/data listings to Dr. DeLap) the sponsor explains how the analysis in TABLR 4B was conducted: *"We have also performed the analyses comparing the proportion of patients who had ≥ 40% reductions in creatinine clearance following their last cycle of therapy where we utilized the last creatinine level during days 19-35 following the next to last cycle of therapy rather than a value occurring > 35 days after the last cycle."* This is the relevant analysis since no values are imputed if the patient did not receive therapy for a cycle. Here, those patients with incomplete last cycle data, i.e., nothing recorded for the last half of their cycle, were assigned a value from the last portion of their previous cycle or a > 35 day value obtained after going off study.

In addition, in a recent conversation with John Conlon, Ph.D., the firm's statistician, on 5/25/95, he pointed out that a computational error had been made in the Cockcroft and Gault determined creatinine clearance value for several patients. The sponsor is in the process of correcting this error as of this review's date and will provide any needed changes to TABLE 4B.

An additional stratified analysis was performed on post-therapy creatinine clearance values using the Cochran Mantel Haenszel Chi Squared test stratifying on the baseline creatinine clearance categories. This appears in sponsor's TABLE 5 (6/95 ODAC package). The results in TABLE 5 (all patients analysis) indicate amifostine pretreatment significantly reduced the occurrence of creatinine clearance reduction in the new cohort patients ($p=0.050$, 2-sided) when adjusting for baseline status category. The effect for the old cohort is weaker, reaching only borderline significance ($p=0.061$, 2-sided). Sponsor's FIGURE 5 (1/95 submission) presents a by patient descriptive display of creatinine clearance reductions seen over cycles 4, 5 and 6. This display indicates the higher frequency and more persistent (i.e., occurring in two to three cycles consecutively) nature of these reductions in the CP alone control arm.

Sponsor's TABLE 6 (6/95 ODAC packet) presents the corresponding stratified by baseline clearance category analysis for those patients who received ≥ 4 treatment cycles. The difference between the two treatment groups is more pronounced in those patients who received ≥ 4 cycles of chemotherapy ($p < .03$, 2-sided). In this package the sponsor has also provided the results of additional stratified analyses based on the use of nephrotoxic antibiotics, presence of diabetes and presence of hypertension. These analyses indicate that for all patients and for the subgroup who received ≥ 4 chemotherapy cycles the creatinine clearance improvement observed for amifostine patients remains statistically significant ($p < .03$) after controlling for these factors.

(d) Cisplatin-Induced Hypomagnesemia: Sponsor's TABLE 3 (1/95 submission) presents analysis results of serum magnesium levels prior to each patient's last cycle of therapy. The sponsor claims: *"....that pretreatment with amifostine statistically significantly reduced the severity of hypomagnesemia in both the original and new cohorts as well as overall."*

Reviewer's Comment: Magnesium was never mentioned as an endpoint in the original protocol. While the improvement reported for amifostine patients is statistically significant ($p \leq 0.017$, 2-sided), it must be borne in mind that this analysis was performed post hoc in an unblinded study. In addition, approximately 33% of the patients had missing baseline measurements for this endpoint.

Overall Summary and Conclusions:

(1) This submission is based on the second of two planned analyses. The protocol specified interim analysis design was a one-sided Pocock plan with stopping boundary p-values $< .03$ for the interim and final looks. The sponsor has carried out all statistical analyses using more stringent two-sided testing for serum creatinine, creatinine clearance and serum magnesium endpoints in the current submission. However, no adjustment was made to this α-level for testing multiple endpoints. The type I (false positive) error rate can be substantially inflated depending upon the number of endpoints tested. Since several of these endpoints would appear to be correlated, a Bonferroni type correction, viz., $\alpha/(\#$ endpoints tested), is overly conservative. However, some adjustment appears to be warranted given the multitude of endpoints tested and

efficacy findings should be interpreted in this context.

(2) Magnesium was never mentioned as an endpoint in the protocol. While the improvement reported for amifostine patients is statistically significant, it must be borne in mind that this analysis was performed post hoc in an unblinded study.

(3) Both protracted serum creatinine and creatinine clearance analyses indicate a statistically significant advantage particularly for those amifostine patients who received ≥ 4 cycles of chemotherapy. While this appears to be a clinically important subgroup, it was never prospectively defined in the protocol and could be partially data driven given the study was not blinded.

In this reviewer's opinion, if the reported improvements in serum creatinine, creatinine clearance and magnesium levels are clinically relevant, this application could be considered for conditional approval. Given the problems of changes in endpoint definition over time and the multiplicity of endpoints tested, a commitment to carry out another controlled study is warranted. This, however, is subject to the concurrence of the medical division.

Clare Gnecco

Clare Gnecco, Ph.D.
Mathematical Statistician

Concur: Dr. Wilson 6/14/95

Dr. Dubey 6-23-95

cc: NDA 20-221
HFD-150/Dr. Justice
HFD-150/Dr. DeLap
HFD-344/Dr. Lisook
HFD-713/Dr Dubey [File DRU 1.3.2 NDA]
HFD-713/Dr. Wilson
HFD-713/Dr. Gnecco

Chron
Gnecco/06-22-95/WP6.0Ja/ETHYOLAM.REV
This review consists of 7 pages of text, 1 Appendix, 9 Tables, 1 Figure and 3 Attachments.

APPENDIX 2

CRITERIA FOR EFFICACY
(Section 7 of protocol WR-2721-1)

To determine if pretreatment with WR-2721 reduces the toxicities of cisplatin and cyclophosphamide without loss of antitumor activity, the two treatment arms will be compared with respect to the following end points.

(1) incidence and duration of hematologic toxicity - defined as granulocytes nadir (<500/ml) leukopenia related fever or the need to reduce or delay the dose of cyclophosphamide for safety considerations

(2) incidence of nephrotoxicity defined by the need to delay or reduce the dose of cisplatin

(3) incidence of neurotoxicity defined by the occurrence of grade 1 or worse neuropathy (GOG criteria) and the cumulative dose of cisplatin at the onset of neuropathy.

(4) incidence of ototoxicity defined by moderate or severe hearing loss or the need to reduce or delay the dose of cisplatin.

(5) incidence of dose limiting toxicity - defined as the need for dose reduction, delay or discontinuation of cisplatin or cyclophosphamide due to toxicity.

(6) tumor response rate.

TABLE 1

Proportion of Patients Who Required a Delay or Discontinuation of Cisplatin Therapy Due to Nephrotoxicity[a]

	Amifostine + CP	CP	p-value[b]
Original Cohort			
Cycle 4	2/36	5/33	0.190
Cycle 5	2/32	9/35	0.033
Cycle 6	4/27	11/23	0.012
Last Cycle[c]	4/61	11/53	0.026
Last Cycle[d]	3/48	9/40	0.028
New Cohort			
Cycle 4	0/33	1/45	0.392
Cycle 5	0/29	5/40	0.050
Cycle 6	0/15	5/21	0.045
Last Cycle[c]	2/55	6/61	0.190
Last Cycle[d]	0/39	6/50	0.026
All Patients			
Cycle 4	2/69	6/78	0.203
Cycle 5	2/61	14/75	0.006
Cycle 6	4/42	16/44	0.003
Last Cycle[c]	6/116 (5%)	17/114 (15%)	0.014
Last Cycle[d]	3/87 (3%)	15/90 (17%)	0.004

[a] Cisplatin was to be delayed if serum creatinine >1.5mg/dL at Day 22; cisplatin was to be discontinued if serum creatinine >1.5mg/dL at Day 35.
[b] Based on Pearson's Chi Square Test (2-sided).
[c] All patients
[d] Patients who had at least four cycles of therapy.

TABLE 9

Median Cumulative Dose of Cisplatin Received (mg/m^2)
(All Patients)

	Amifostine + CP	CP
Original Cohort	600	488
New Cohort	525	500
Entire Study	555	500

TABLE 4

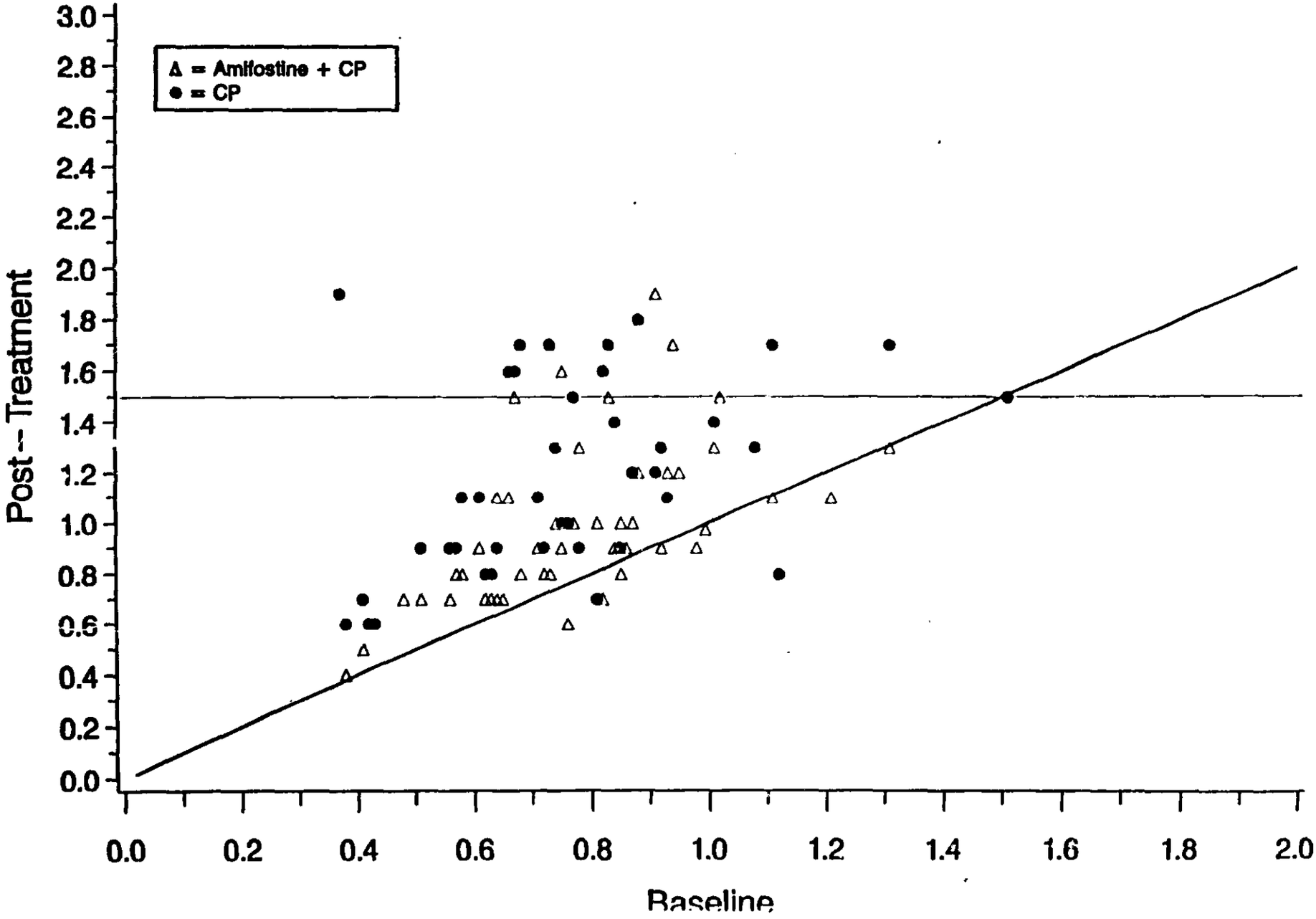
Cumulative Incidence of Treatment-Limiting Cisplatin-Related Nephrotoxicity[a]

	Amifostine + CP	CP	p-value (2-sided)
Original Cohort[a]	2/63	11/58	0.005
New Cohort	0/59	5/62	0.027
All Patients	2/122	16/120	0.001

[a] Three of the patients in the original cohort (2 amifostine + CP: 301 and 320 and 1 CP: 305) who had protracted elevations in serum creatinine also had severe nausea and vomiting and clinical dehydration which resulted in withdrawal from the study. These three patients were treated prior to the allowance of high dose dexamethasone as an antiemetic.

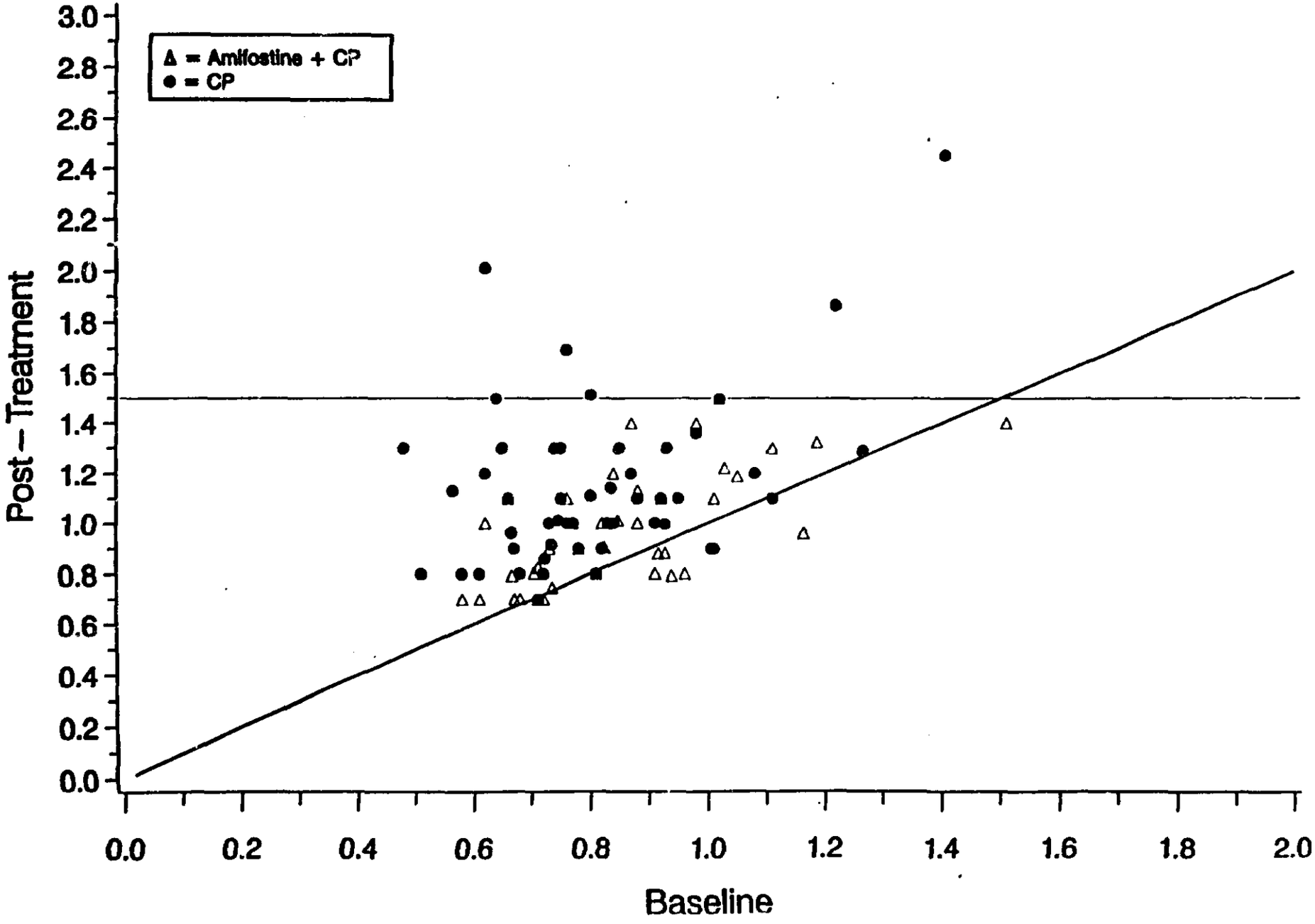
← Note

Serum Creatinine: >= 4 Cycles Only
Original 121



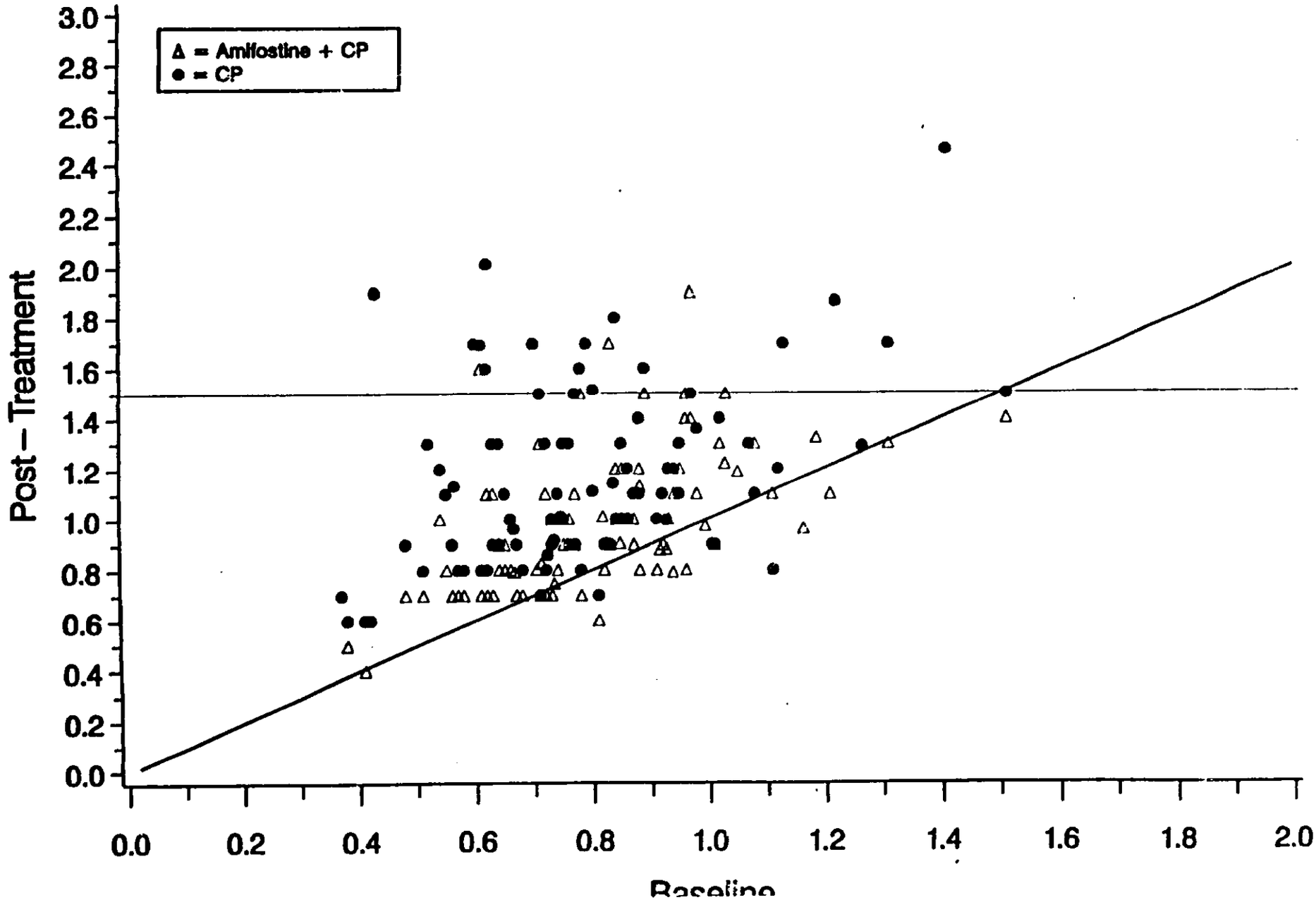
ATTACHMENT #1

Serum Creatinine: > = 4 Cycles Only
New 121



ATTACHMENT # 2

Serum Creatinine: >= 4 Cycles Only
All 242



ATTACHMENT #3

TABLE 4A

Baseline Creatinine Clearance Values (cc/min)

	<60	60-79	80-99	≥100	p-Value
Original Cohort					
Amifostine + CP	13	18	15	17	
CP	6	16	14	20	0.148
New Cohort					
Amifostine + CP	11	22	13	11	
CP	15	14	13	19	0.404
Entire Study					
Amifostine + CP	24	40	28	28	
CP	21	30	27	39	0.124

TABLE 2

Proportion of Patients With ≥40% Reduction in Creatinine Clearance

	Amifostine + CP	CP	χ^2(2-sided)
Original Cohort			
Cycle 4	5/49	9/42	0.141
Cycle 5	7/44	9/38	0.379
Cycle 6	7/37	13/35	0.087
Last Cycle[a]	10/63	18/56	0.038
Last Cycle[b]	6/48	15/41	0.008
New Cohort			
Cycle 4	0/40	8/49	0.008
Cycle 5	2/34	9/45	0.075
Cycle 6	2/25	10/31	0.029
Last Cycle[a]	6/57	17/61	0.018
Last Cycle[b]	3/40	14/50	0.014
All Patients			
Cycle 4	5/89	17/91	0.008
Cycle 5	9/78	18/83	0.086
Cycle 6	9/62	23/66	0.008
Last Cycle[a]	16/120 (13%)	35/117 (30%)	0.002
Last Cycle[b]	9/88 (10%)	29/91 (32%)	<0.001

[a] All patients

[b] Patients who had at least four cycles of therapy

TABLE 4B

Proportion of Patients With ≥40% Reduction in Creatinine Clearance

	Amifostine + CP	CP	χ^2(2-sided)
Original Cohort			
Cycle 4	4/48	10/40	0.034
Cycle 5	4/42	8/35	0.111
Cycle 6	5/35	11/29	0.031
Last Cycle[a]	10/63	20/58	0.018
Last Cycle[b]	6/48	17/41	0.002
New Cohort			
Cycle 4	0/40	7/49	0.013
Cycle 5	2/34	7/44	0.172
Cycle 6	2/25	6/28	0.177
Last Cycle[a]	6/59	16/62	0.025
Last Cycle[b]	3/40	13/50	0.023
Entire study			
Cycle 4	4/88	17/89	0.003
Cycle 5	6/76	15/79	0.044
Cycle 6	7/60	17/57	0.015
Last Cycle[a]	16/122 (13%)	36/120 (30%)	0.001
Last Cycle[b]	9/88 (10%)	30/91 (33%)	<0.001

[a] All patients
[b] Patients who had at least four cycles of therapy

The results following the last cycle of therapy in TABLE 4B are displayed graphically in FIGURES 4A and 4B for all patients and for patients who received at least 4 cycles of therapy, respectively.

TABLE 5

Proportion of Patients With Creatinine Clearance <60 cc/min
Following the Last Cycle of Chemotherapy
Stratified by Pretreatment Creatinine Clearance
(All Patients)

Pretreatment Creatinine Clearance	Amifostine + CP		CP	χ^2 adj	p-value (2-sided)
Original Cohort					
<60	8/13		6/6		
60-79	10/18		11/16		
80-99	3/15		6/14		
≥100	3/17		4/20	3.504	0.061
New Cohort					
<60	11/11		14/15		
60-79	9/22		10/14		
80-99	2/13		6/13		
≥100	2/11		4/19	3.828	0.050
Entire Study					
<60	19/24		20/21		
60-79	19/40	p=0.062*	21/30		
80-99	5/28	p=0.035*	12/27		
≥100	5/28		8/39	8.509	0.004

* 2-sided p-values based on Pearson's Chi Square statistic

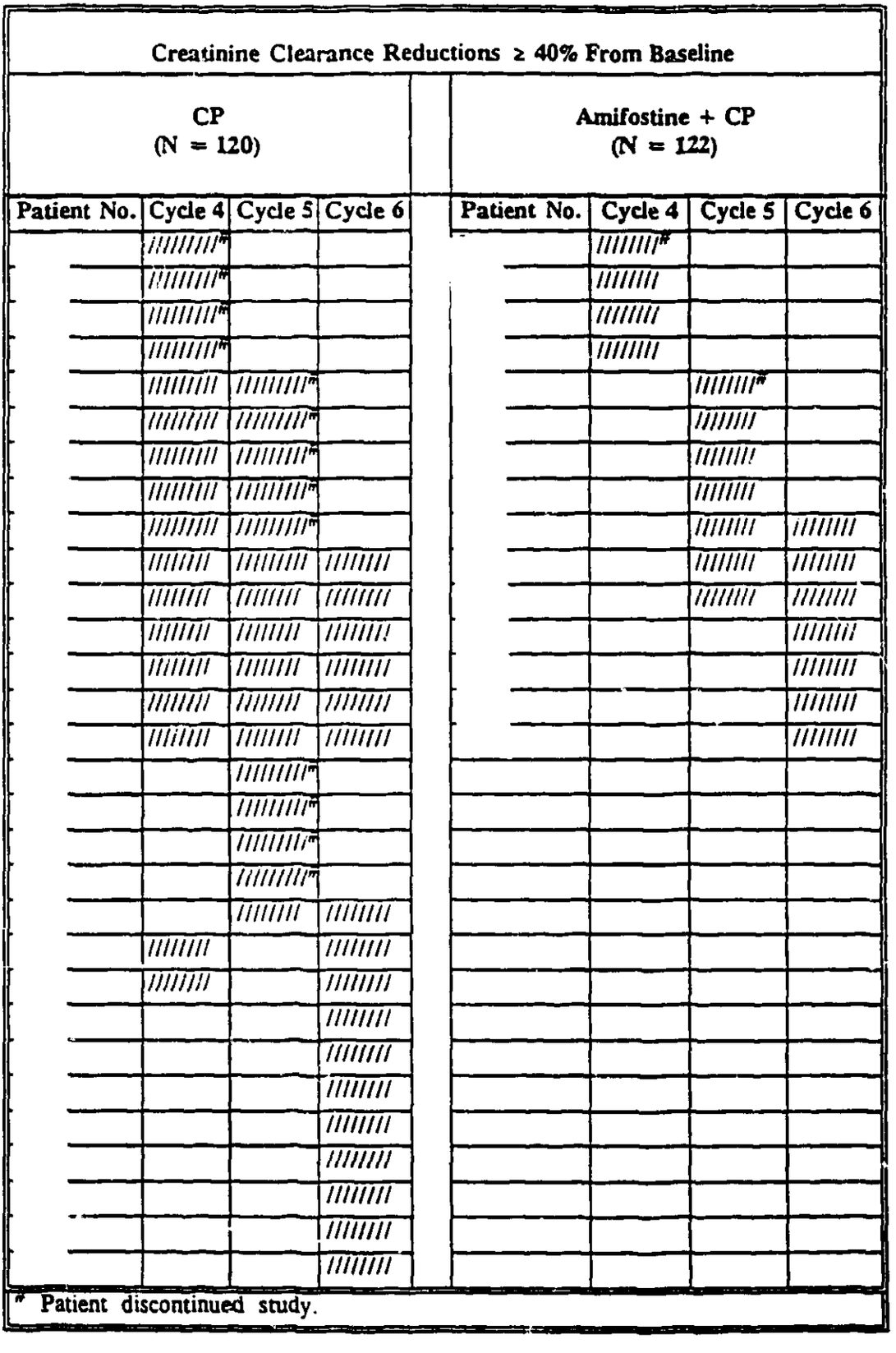


FIGURE 5: Pattern of reductions in creatinine clearance of ≥40%.

TABLE 6

Proportion of Patients With Creatinine Clearance <60 cc/min Following the Last Cycle of Chemotherapy Stratified on Pretreatment Creatinine Clearance (Patients Who Received at Least 4 Cycles of Chemotherapy)

Pretreatment Creatinine Clearance	Amifostine		CP	χ^2 CMH	p-value (2-sided)
Original Cohort					
<60	6/9		5/5		
60-79	8/11		7/8		
80-99	3/14		6/11		
≥100	2/14		4/17	4.936	0.026
New Cohort					
<60	8/8		12/13		
60-79	7/15		9/10		
80-99	0/10		4/8		
≥100	1/7		4/19	6.913	0.009
Entire Study					
<60	14/17		17/18		
60-79	15/26	p=0.027*	16/18		
80-99	3/24	p=0.005*	10/19		
≥100	3/21		8/36	12.389	<0.001

* 2-sided p-values based on Pearson Chi Square statistic

TABLE 3

NCI Toxicity Grades of Serum Magnesium Levels for Each Patient's Last Cycle of Therapy

NCI-CTC Grade: (mEq/L)	0 >1.4	1 ≤1.4->1.1	2 ≤1.1->0.8	3 ≤0.8->0.5	4 ≤0.5	p-value[a]
Original Cohort						
Amifostine+ CP	49	10	3	0	0	0.017
CP	35	8	6	3	1	
New Cohort						
Amifostine + CP	43	3	0	0	0	0.012
CP	38	10	1	2	0	
All Patients						
Amifostine + CP	92	13	3	0	0	0.001
CP	73	18	7	5	1	

[a] Based on 2-sided Mantel-Haenszel Chi-Square statistic.

APPENDIX 4A presents an analysis of serum magnesium levels for each of the three cohorts of patients; APPENDIX 4B presents a by-patient listing of serum magnesium levels for each patient.

Origional

Statistical Review and Evaluation

DATE: AUG 11 1995

NDA#: 20-221

APPLICANT: US Bioscience

NAME OF DRUG: Ethyol (amifostine) for Injection

DOCUMENTS REVIEWED: Volumes 1 and 2 of 06/06/95 Desk Copy to Dr. S. Koepke.

I. Background

The above volumes contain the sponsor's responses to the Agency's 02/27/95 deficiencies letter. The reviewing chemists, Drs. Dietze and Koepke (HFD-150), requested the Division of Biometrics to review the sponsor's stability data in Volume 2, Attachment 4, in particular the +5 degree Celsius data of amifostine stored in clear vials, the thiol impurity data with an upper limit of 3.5 %, the sponsor's response to deficiency 32, and his statistical analyses in Attachment 9.

II. Sponsor's Results

In Attachment 4 the sponsor reported the stability data of various assays and various temperature conditions for three drug substance lots and for six drug product lots. All six drug product lots were stored in amber vials. Two drug product lots were also stored in clear vials for 18 months. The requested expiration date is 18 months.

In Attachment 9 the sponsor presented the statistical analyses and graphs of the various data sets obtained at +5 degrees C storage. Though the sponsor used the FDA stability program the batches were pooled without testing for potential batch differences. Based on this approach an estimated 67-month expiration date for potency was obtained for the data of the two batches in clear vials. For the impurity thiol the sponsor created a new variable, (100 - %impurity). In his analysis he again disregarded any possible batch differences and estimated a 23-month expiration dating period for thiol with an upper limit of 4.5%.

III. Reviewer's Results

In none of his analyses had the sponsor tested for the poolability of batch slopes or batch intercepts. He had formed simple regression lines with the observations at a given time point being simply repeat assays. Rerunning these analyses properly the

amifostine data regressed to a model of parallel lines and estimated extrapolated expiration dating periods of 63 and 70 months for batches 1015A and 3015A, respectively. This reviewer modified the Division stability program which had been also used by the sponsor, such that it analyzed the actual thiol data rather than the variable (100 - %thiol) created by the sponsor. The data from the two batches of the clear vials again regressed to a parallel lines model. The estimated expiration dating periods are 22 and 23 months when the upper limit is set at 4.5 %, but only 16 and 15 months when the upper limit is set at 3.5 %.

It is noted that there are only two batches filled into clear vials and there is no reasonable estimate of between batch variability.

It is also noted that the sponsor had a typographical error in his answer to question 32 where he described the formation of the tiol (100 - %thiol) limit, namely 100-4.5=95.5, not 94.5. His analyses and graphs used the correct number 95.5.

The sponsor's response to question 32 is satisfatory to this reviewer except that he apparently did not understand how to input the data properly to the Division stability program to allow for testing the poolability of batches.

This reviewer observed that the sponsor had analyzed the results of old and new analytic methodologies by forming the ratio of the average results, averaging the ratios, and apparently testing the results using a t-test against a ratio of 1. The documents did not contain any details of the exact design of this study. Application of normal theory methods to ratios, such as a t-test, is inappropriate. As the sponsor calls it a cross-over study, details of the study design including whether aliquots were taken from the same or from separate vials, are necessary prior to a proper statistical analysis. Without a review of this documentation of the "cross-over" design and the statistical methodologies applied to it the sponsor's finding of no significant differences should not be accepted.

IV Summary and Conclusion

The sponsor's statistical analyses were inappropriate. One of the main purposes of the Division's stability program is to be able to test whether batches can be pooled in their slopes or intercepts. The sponsor obviated this purpose by inputting the data without batch identifyers. Properly analyzing the +5 degree Celsius amifostine data from the clear vials resulted in extrapolated estimated expiration dating periods of 63 and 70 months. The corresponding thiol data estimated expiration dating periods of 22 and 23 months when the upper limit is set at 4.5 %, but only of 16 and 15 months when the upper limit is set at 3.5 %.

A cursory review of the sponsor's crossover study to test the old and new analytic methodologies showed great lack in documentation of the study design and statistical methods applied. It is almost certain that the sponsor's statistical methods are inappropriate and the results should not be accepted without full description of the study design and the statistical methods used.

In summary, only two batches of clear vials are available for estimating the expiration dating period for the product. The amifostine data support an expiration dating period of 18 months, but the % thiol data do not support an 18 months expiration date if the upper limit is set at 3.5%. Additionally, the sponsor's findings on the comparison of old and new methodologies cannot be accepted until proper documentation of the design and the statistical analyses can be evaluated.

Roswitha E. Kelly

Concur: Karl K. Lin 8/9/95

Karl K. Lin, Ph.D.

cc: HFD-150/NDA 20-221 Original
HFD-150/Dr. Tolygesi
HFD-150/Dr. Dietze
HFD-150/Dr. Koepke
HFD-710/Chron
HFD-715/Dr. Fairweather
HFD-715/Dr. K. Lin
HFD-715/R. Kelly
HFD-715/DRU 2.2.1 Ethyol (amifostine) for Injection, U.S. Bioscience.
HFD-715/RKELLY/08/09/95/wp-ethyol.rev

Chem

Division of Oncology and Pulmonary Drug Products
Review of Chemistry, Manufacturing and Controls

NDA #: 20-221 **CHEM. REVIEW #:**4 **REVIEW DATE:** November 2, 1995

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment (AC)	27-Oct-95	30-Oct-95	31-Oct-95

NAME AND ADDRESS OF SPONSOR:
US Bioscience
One Tower Bridge; 100 Front Street
West Conshoohocken, PA 19428

DRUG PRODUCT NAME:

Proprietary:	Ethyol® For Injection
Nonproprietary/USAN:	Amifostine
Code Name/#:	WR-2721
Chem. Type/Ther. Class:	1-P

PHARMACOL. CATEGORY/INDICATION: Protective agent against hematologic side-effects of alkylating cytotoxic oncologic drugs

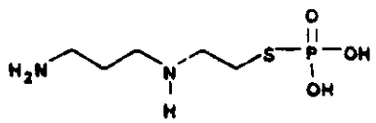
DOSAGE FORM: Lyophilized powder for injection

STRENGTHS: 500 mg/vial

ROUTE OF ADMINISTRATION: IV

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:
Ethanethiol,2-[(3-aminopropyl)amino]dihydrogen phosphate (ester)
$C_5H_{15}N_2O_3PS$ (anhydrous) MW 214.22
$C_5H_{15}N_2O_3PS \cdot 3H_2O$ (trihydrate) MW 268.27



CONSULTS:

Consult	Status	Comments
EER	Acceptable	Found acceptable on 9/28/95
Trademark	Acceptable	Found acceptable by Trademark Cmmt on 12/24/91.
Methods Validation	Hold	Will be initiated once all methods deficiencies have been addressed.
Statistics	Received	Received on August 11, 1995
EA	Acceptable	Submitted 11/19/91. FONSI issued

		on 12/13/94.
Microbiology	Approved	Submitted 9/29/94.Found acceptable on 12/16/94.

REMARKS/COMMENTS:

This amendment is a response to deficiencies cited in our review of the amendment dated 6/6/95. The current amendment is submitted as a response to the October 6, 1995 approvable letter from the Agency. Method Validation will be requested for FDA labs once all cited deficiencies are adequately addressed.

CONCLUSIONS AND RECOMMENDATIONS:
The application is APPOVABLE. However, there are still issues that need to be addressed before the NDA can be approved. The remaining deficiencies concern the regulatory specifications for Thiol levels and for Total Related Substances in the drug product. These issues should be communicated to the sponsor.

11/13/95

Paul Dietze, Ph.D.
Review Chemist

CC:
Orig. NDA
HFD-150 Division File
HFD-150/P. Dietze
HFD-150/E. Tolgyesi
HFD-151/L. McCollum
R/D Init. by:

n20221r4.001

NDA #: 20-221 **ADDENDUM TO CHEM. REVIEW #3** **DATE: 9/20/95**

DRUG PRODUCT NAME: ETHYOL (amifostine) for Injection

NAME OF SPPLICANT: US Bioscience

CONCLUSIONS/RECOMMENDATIONS:

The objective of the summary below is to clarify the current status of this NDA:

a. Although the amendment provided adequate responses to most of the major chemistry issues, there are still some deficiencies which need to be addressed before an APPROVAL letter may issue. There are deficiencies pertaining to the regulatory specifications, reference standard, degradation studies, expiration dating period and labeling.

b. I consider the NDA APPROVABLE based on the submitted safety and efficacy data and the fact that the Applicant will be able to address the remaining chemistry deficiencies in the near future.

Eva Tolgyesi, Ph.D.
Supervisory Chemist
9/20/95

cc:
Orig. NDA 20-221
HFD-150/Div. File
HFD-150/ETolgyesi
HFD-150/PDietze
HFD-151/LMcCollum
R/D Init. by:

File: C:\WPFILES\N20221.ADD

Division of Oncology and Pulmonary Drug Products
Review of Chemistry, Manufacturing and Controls

NDA #: 20-221 **CHEM. REVIEW #:**3 **REVIEW DATE:** July 7, 1995

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment (AC)	06-Jun-95	07-Jun-95	09-Jun-95

NAME AND ADDRESS OF SPONSOR:
US Bioscience
One Tower Bridge; 100 Front Street
West Conshoohocken, PA 19428

DRUG PRODUCT NAME:

Proprietary:	Ethyol® For Injection
Nonproprietary/USAN:	Amifostine
Code Name/#:	WR-2721
Chem. Type/Ther. Class:	1-P

PHARMACOL. CATEGORY/INDICATION: Protective agent against hematologic side-effects of alkylating cytotoxic oncologic drugs

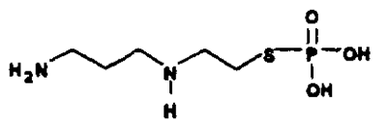
DOSAGE FORM: Lyophilized powder for injection

STRENGTHS: 500 mg/vial

ROUTE OF ADMINISTRATION: IV

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:
Ethanethiol,2-[(3-aminopropyl)amino]dihydrogen phosphate (ester)
$C_5H_{15}N_2O_3PS$ (anhydrous) MW 214.22
$C_5H_{15}N_2O_3PS \cdot 3H_2O$ (trihydrate) MW 268.27



CONSULTS:

Consult	Status	Comments
EER	Pending	Submitted 6/20/95.
Trademark	Acceptable	Found acceptable by Trademark Cmmt on 12/24/91.
Methods Validation	Hold	Will be initiated once all methods deficiencies have been addressed.
Statistics	Hold	Submitted to Biometrics on 6/6/95 for evaluation of the stability data.

EA	Acceptable	Submitted 11/19/91. FONSI issued on 12/13/94.
Microbiology	Approved	Submitted 9/29/94.Found acceptable on 12/16/94.

REMARKS/COMMENTS:

This amendment, dated 6/6/95, addresses issues concerning CM&C that were communicated to the sponsor by Agency letter dated February 27, 1995. There are still deficiencies that need to be addressed before the NDA can be approved. Method Validation will be requested for FDA labs once all cited deficiencies are adequately addressed.

CONCLUSIONS AND RECOMMENDATIONS:
The application is considered deficient and NOT APPOVABLE. However, the application is nearing approval. While there are still some remaining deficiencies most of the deficiencies have been addressed in a satisfactory manner. Most of the remaining are relatively minor.

8/2/95

Paul Dietze, Ph.D.
Review Chemist

8/2/95

CC:
Orig. NDA
HFD-150 Division File
HFD-150/S. Koepke
HFD-150/E. Tolgyesi
HFD-151/CSO LMcCollum
R/D Init. by:

n20221r3.001

1-3-95

Division of Oncology and Pulmonary Drug Products
Review of Chemistry, Manufacturing and Controls

NDA #: 20-221 CHEM. REVIEW#:2 **REVIEW DATE:**November 21,1994

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Correspondence	27-May-93	01-Jun-93	04-Aug-94
Amendment #14	30-Aug-93	01-Sep-93	04-Aug-94
Amendment #15	10-May-94	11-May-94	04-Aug-94
Amendment #19	20-Oct-94	21-Oct-94	24-Oct-94
Amendment #20	24-Oct-94	25-Oct-94	25-Oct-94

NAME AND ADDRESS OF SPONSOR:
US Bioscience
One Tower Bridge; 100 Front Street
West Conshoohocken, PA 19428

DRUG PRODUCT NAME:

Proprietary:	Ethyol® For Injection
Nonproprietary/USAN:	Amifostine
Code Name/#:	WR-2721
Chem. Type/Ther. Class:	1-P

PHARMACOL. CATEGORY/INDICATION: Protective agent against hematologic side-effects of alkylating cytotoxic oncologic drugs

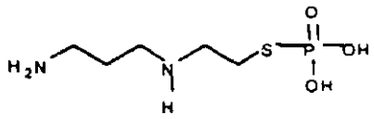
DOSAGE FORM: Lyophilized powder for injection

STRENGTHS: 500 mg/vial

ROUTE OF ADMINISTRATION: IV

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:
Ethanethiol,2-[(3-aminopropyl)amino]dihydrogen phosphate (ester)
$C_5H_{15}N_2O_3PS$ (anhydrous) MW 214.22
$C_5H_{13}N_2O_3PS \cdot 3H_2O$ (trihydrate) MW 268.27



SUPPORTING DOCUMENTS:

Type/Number	Subject	Holder	Status	Review Date	Letter Date
IND					
IND					
IND		US Biosciences			
DMF					
DMF			Deficient	11/21/94	

Type/Number	Subject	Holder	Status	Review Date	Letter Date
DMF					
DMF		Ben Venue Laboratories, Inc.			

CONSULTS:
EER sent 11/8/91, Pending
Trademark cmmt sent 11/8/91
EA sent 11/19/91
Micro found deficient 9/29/94

1. The dissolution method and specifications were submitted to the Division of Biopharmaceutics 9/30/93. Currently pending.
2. The statistical methodology was submitted to the Division of Biometrics 9/30/93. Currently pending.
3. An amendment (NDA 20-140, 2/17/92) concerning the Environmental Assessment was forwarded to HFD-102, 3/16/92 (A. Schroeder). Currently pending.

REMARKS/COMMENTS:

Amendments -014, -015 and -017 were submitted in order to address deficiencies cited in the original review of NDA # 20-221. Amendment -019 was requested to provide updated stability data for the DP. Steve Koepke is the reviewer of issues in Amendment -014 that are related to the drug substance and Paul Dietze is the reviewer of issues in Amendment -014 that are related to the drug product. P. Dietze will review amendment -015 and -019 since amendment -015 and -019 deals only with the drug product.

With respect to the drug product there are still numerous deficiencies that need to be addressed before the NDA can be approved.

CONCLUSIONS & RECOMMENDATIONS: Amendments -013, -014, -017 and -019 to NDA #20-221 dated 8/30/93, 5/10/94, 7/21/94 and 10/20/94 have not satisfactorily addressed the issues raised in the initial review of NDA #20-221. From a chemistry manufacturing and controls perspective NDA #20-221 is still **not approvable** and a deficiency letter should be conveyed to the sponsor. The deficiencies will need to be addressed before the NDA can be approved. Method Validation will be requested for FDA labs once all cited deficiencies are adequately addressed.

CONCLUSIONS AND RECOMMENDATIONS:
The application is considered deficient and NOT APPOVABLE.

DRUG SUBSTANCE:

1/3/95
Steven R. Koepke, Ph.D.
Review Chemist

DRUG PRODUCT:

12/29/94
Paul Dietze, Ph.D.
Review Chemist

CC:
Orig. NDA
HFD-150 Division File

1/3/95

HFD-150/S. Koepke
HFD-150/E. Tolgyesi
HFD-151/CSO LMcCollum
R/D Init. by:
□Reviews\n20221r2.000

DONE
JAN 29 1992

Division of Oncology and Pulmonary Drug Products
Review of Chemistry, Manufacturing, and Controls

NDA #:20-221 **DATE REVIEWED:** 29 January 1992

REVIEW #: 1 **RECOMMEND ACTION:** Not Approvable

REVIEW TEAM MEMBERS: Guiragos Poochikian,Ph.D.
Jeffrey Blumenstein, Ph.D.
Michael V. Ganey, Ph.D.

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	9/30/91	9/30/91	10/15/91

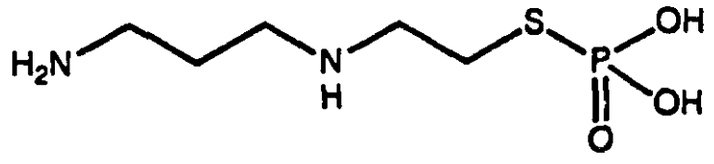
NAME & ADDRESS OF APPLICANT:
US Bioscience
One Tower Bridge; 100 Front Street
West Conshohocken, PA 19428

DRUG PRODUCT NAME
Proprietary: Ethyol®
Established: Amifostine for injection
Code Name/#: WR-2721
Chem.Type/Ther.Class: 1A

PHARMACOL. CATEGORY/INDICATION: Protective agent against hematologic side-effects of alkylating cytotoxic oncologic drugs
DOSAGE FORM: Lyophilized powder for injection
STRENGTHS: 500 mg/vial
ROUTE OF ADMINISTRATION: IV
Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOLECULAR WEIGHT:

Ethanethiol,2-[(3-aminopropyl)amino]dihydrogen phosphate (ester)
$C_5H_{15}N_2O_3PS$ (anhydrous) MW 214.22
$C_5H_{15}N_2O_3PS \cdot 3H_2O$ (trihydrate) MW 268.27



SUPPORTING DOCUMENTS:

IND		WR-2721
IND		WR-2721
IND	US Biosciences	WR-2721
DMF		Foreign Facilities
DMF		Manufacture of Amifostine
DMF		Formulation B0857, 2/9/84
DMF	Ben Venue Laboratories, Inc.	DMF and all amendments in processing Ethyol

RELATED DOCUMENTS (If applicable): NONE

CONSULTS:
EER sent 11/8/91
Trademark cmmt sent 11/8/91
EA sent 11/19/91
Micro sent on 2/3/92

REMARKS:

Method Validation will be requested for FDA labs once all cited deficiencies are adequately addressed.

DRUG SUBSTANCE:

Michael V. Ganey, Ph.D.
Review Chemist

DRUG PRODUCT:

11/24/92
Jeffrey J. Clumenstein, Ph.D.
Review Chemist

CONCLUSIONS & RECOMMENDATIONS:

NDA 20-221 is not approvable from a chemistry perspective and the chemistry deficiency letter should be conveyed to the sponsor.

1/29/92

Guiragos Poochikian, Ph.D.
Chemistry Project Manager

cc:
Org. NDA 20-221
HFD-150/Division File
HFD-150/J. Blumenstein
HFD-150/M. Ganey
HFD-150/G. Poochikian
HFD-150/CSO K. Downs
filename:

Fonsi

and

E. A

FINDING OF NO SIGNIFICANT IMPACT

AND

ENVIRONMENTAL ASSESSMENT

FOR

NDA 20-221

Ethyol (amifostine) For Injection

CENTER FOR DRUG EVALUATION
AND RESEARCH

HFD-150

FINDING OF NO SIGNIFICANT IMPACT
NDA 20-221

The Food and Drug Administration (FDA) recognizes the National Environmental Policy Act of 1969 (NEPA) as the national charter for protection, restoration, and enhancement of the environment. NEPA establishes policy, sets goals (section 101), and provides procedures (section 102) for carrying out the policy. Environmental information is to be available to the public and the decisionmaker before decisions are made about actions that may significantly affect the quality of the human environment; FDA actions are to be supported by accurate scientific analyses; and environmental documents ar to concentrate on timely and significant issues, not to amass needless detail.

FDA's Center for Drug Evaluation and Research (CDER) has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for Ethyol (amifostine) for Injection, U.S. Bioscience prepared an abbreviated environmental assessment (EA) (21 CFR 25.31a(b)(3)) (attached) based on the fact that this is an orphan drug which will be manufactured in small quantities and for infrequent use. The abbreviated EA evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

CDER has concluded that the product can be manufactured and used without any expected adverse environmental effects. The small amounts to be manufactured, the infrequent use of the drug product, and precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Any residues of Ethyol For Injection or its degradation product entering the environment as a result of administering the drug to humans are expected to be in such low concentrations as to not be toxic to organisms. Accidental spill control procedures are available. Disposal will be in accordance with appropriate medical waste procedures.

12/12/94
DATE

Christina L. Good, Esq.
Regulatory Counsel
Center for Drug Evaluation and Research

12/13/94
DATE

Phillip G. Vincent, Ph. D.
Environmental Assessment Officer
Center for Drug Evaluation and Research

12/13/94
DATE

Charles S. Kumkumian, Ph. D.
Assistant Director (Chemistry)
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Attachment:
FOI Copy of Environmental Assessment for Ethyol, NDA 20-221

file:fonsi\no20221

F01

ENVIRONMENTAL ASSESSMENT

Ethyol® (Amifostine) for Injection

Prepared for:

U.S. Bioscience
One Tower Bridge
100 Front Street
West Conshohocken, Pennsylvania 19428

Prepared by:

N.W. Gabel & Assoc.
8201 Corporate Drive, Suite 620
Landover, Maryland 20785

CONTENTS

CONTENTS, continued

FIGURES

TABLES

1. **DATE**

July 30, 1993

2. **NAME OF APPLICANT**

U.S. Bioscience

3. **ADDRESS**

One Tower Bridge
100 Front Street
West Conshohocken, Pennsylvania 19428

4. DESCRIPTION OF THE PROPOSED ACTION

4.1 REQUESTED APPROVAL

Approval is sought for the manufacture and use of the new drug product known as Ethyol® (amifostine) for Injection. On May 30, 1990, the United States Food and Drug Administration (FDA) determined that Ethyol® (ethiofos) qualified as an orphan drug when it is used as a chemoprotective agent during cyclophosphamide therapy for the treatment of advanced ovarian carcinoma (ethiofos is a synonym for amifostine).

The format of this Environmental Assessment (EA) is arranged as required by 21 CFR 25.31(a). Although Items 7 through 11 and 15 may not be required for products that have qualified for an orphan drug designation, these items have been addressed to facilitate the review of this EA.

4.2 NEED FOR ACTION

Amifostine provides significant protection against cyclophosphamide-induced hematologic toxicity. In clinical trials with patients suffering from advanced ovarian cancer, concurrent administration of amifostine with cyclophosphamide resulted in a reduction of the incidence of fever and neutropenia as well as a reduction of neurotoxicity. However, no reduction occurred in the antitumor effect of the cyclophosphamide. The mechanism of amifostine's selective protection appears to be related in part to its preferential absorption by normal tissues as compared to malignant tissues.

4.3 LOCATIONS OF MANUFACTURE

The bulk drug substance--amifostine--will be synthesized by SIPSY, Route de Beaucouzé, 49240 Avrillé, France. The drug product--Ethyol® (amifostine) for Injection--will be prepared by Ben Venue Laboratories, Inc., 270 Northfield Road, Bedford, Ohio 44146.

4.4 LOCATIONS OF USE AND DISPOSAL

This drug will be administered with cyclophosphamide to patients in hospitals and clinics, as well as in the offices of physicians, specializing in the care and treatment of cancer patients, located throughout the United States. The number of hospitals and clinics (including physicians offices) likely to use this drug is estimated at 30% and 70%, respectively. Very small amounts of the unchanged drug and its metabolite will then be eliminated to municipal sewage systems. Unused vials (past the expiration date) would be returned to the U.S. Bioscience distributor--Ogden BioServices Corporation (Rockville, Maryland).

4.5 ENVIRONMENTAL SETTING OF FACILITIES

4.5.1 Preparation of the Drug Substance

The facility where amifostine will be synthesized is in France. A letter from the French agency responsible for enforcing environmental and safety regulations has been provided.

4.5.2 Preparation of the Product

Ben Venue Laboratories is located in an industrial section of Bedford in Cuyahoga County approximately 17 miles south of Cleveland, Ohio. There are 15 buildings on the 8.37-acre site Topography is generally flat with drainage either to the street or to a runoff ditch along an adjacent railway track. This site is the highest point in a ¾-mile radius.

The facility is bordered by other industries to the north and south and by residences to the east. Railway tracks are located approximately 100-300 feet to the southwest. A group of chemical manufacturing plants are situated on the opposite side of the railway tracks. Potable water and sewage system service are provided by the City of Bedford.

A wooded park is located beyond the industrial area to the west and northwest. Its runoff drains into small brooks which, within ¾ mile, empty into Tinkers Creek, a tributary of the Cuyahoga River. All local sewage treatment plants discharge effluent into Tinkers Creek or the Cuyahoga River. The area experiences generally moderate weather conditions with extremes in summer (June) of 97°F and winter (January) of 15°F. Average yearly rainfall is 35.13 inches and average yearly snowfall is 56 inches.

5. IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

The product that is the subject of the proposed action is Ethyol® (amifostine) for Injection. The corresponding drug substance is amifostine.

5.1 NOMENCLATURE

5.1.1 Chemical Name (CAS Eleventh Collective Index)

Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester)

5.1.2 United States Adopted Name (USAN)

Amifostine

5.1.3 CAS Registry Number

20537-88-6 (anhydrous)
63717-27-1 (monohydrate)
112901-68-5 (trihydrate)

5.1.4 Code Names and Synonyms

WR-2721; ethiofos; gammaphos; NSC 296961

5.1.5 Trade Name

Ethyol®

5.2 PHYSICAL DESCRIPTION

Amifostine is an organic amine as well as a thiophosphate ester (Figure 5-1). In its final purification, the drug substance is crystallized as a trihydrate. The chemical and physical properties are listed in Table 5-1. As a substance with both acidic and basic functional groups in the same molecule, amifostine will be ionic in aqueous solutions at all pH values. The molecular structures of amifostine and its degradates are similar to the structures of several sewage constituents (Metcalf & Eddy, Inc., 1979).

5.3 ADDITIVES

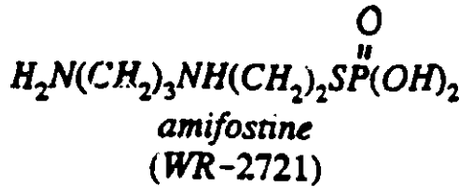
In addition to amifostine, each vial will contain a polyhydroxy excipient.

5.4 IMPURITIES

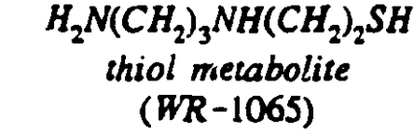
Degradation of amifostine can proceed via hydrolysis to the corresponding thiol (Figure 5-1). Subsequent conversion of the thiol to the disulfide can occur by oxidation under analytical conditions. However, conversion to the disulfide has not been observed with bulk chemical stored in a closed container for prolonged periods of time at room temperature or at 40°C through 6 months.

Density was not determined for amifostine because this property is colligative rather than molecular. Colligative properties are those that are characteristic of a group of molecules, i.e., these properties depend on the spatial distribution of the molecules in that specific group. Thus, the density of amifostine (as a separate solid phase) is irrelevant to the behavior of the dissolved molecules because the molecules will have become dissipated in the aqueous medium.

Similarly, the vapor pressure of amifostine was not determined, because it cannot be related to the volatility of the drug substance in the aquatic environment. From Table 5-1, the pK_a values of <2, 4.4, 9.3 and 11.2 mean that, within the pH range of any aqueous system, amifostine will be present as an ionic substance. Estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant) assume that the dissolved chemical is not ionic (Mackay and Wolkoff, 1973; Lyman et al., 1982). If the chemical is ionic--as is amifostine--its volatilization to the atmosphere cannot be regarded as an operative process and, therefore, can no longer be estimated.



amifostine
(*WR*-2721)



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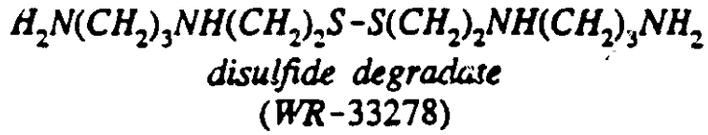
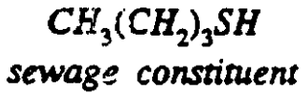
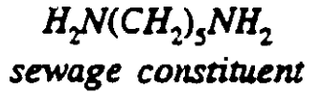
(*WR*-1065)



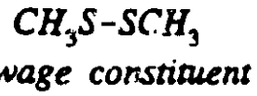
disulfide degradate
(*WR*-33278)



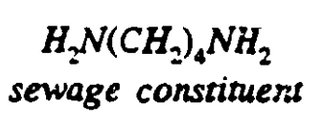
sewage constituent



sewage constituent



sewage constituent



sewage constituent

Figure 5-1
Structures of Amifostine and Related Chemicals

Table 5-1

Chemical and Physical Properties of Amifostine Trihydrate[a]

Molecular formula	$C_5H_{15}N_2O_4PS \bullet 3H_2O$
Molecular weight	268.27
Melting range[b][c]	Not determined
Solubility in water[b]	450,000 mg/L
Density[d]	Not determined
Vapor pressure[e]	Not determined
Log octanol-water partition coefficient[f]	-1.6
Dissociation (pK_a)[b]	<2, 4.4, 9.3, and 11.2
Electromagnetic absorption (>290 nm)[b]	None

(a) According to 21 CFR 58.3(d) and (i), determinations of physical or chemical characteristics are not included in the determinations that must be carried out under conditions designated as Good Laboratory Practice.

(b) New Drug Application, Section 3: Drug Substance.

(c) Physical changes in amifostine are observed over a temperature range of 138°C to 165°C with no sharp melt. It was concluded that a simple melting point determination by capillary methods is irreproducible and would not be useful as a specification for the drug substance.

(d) Not determined for amifostine because density is a colligative property rather than a molecular property. Colligative properties are those that are characteristic of a group of molecules, i.e., these properties depend on the spatial distribution of the molecules in that specific group. Thus, the density of amifostine (as a separate solid phase) is irrelevant to the behavior of the dissolved molecules because the molecules will have become dissipated in the aqueous medium.

(e) Not determined for amifostine because the vapor pressure cannot be related to the volatility of the drug substance in the aquatic environment. Estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant) assume that the dissolved chemical is not ionic (Mackay and Wolkoff, 1973; Lyman et al., 1982). If the chemical is ionic--as is amifostine--its volatilization to the atmosphere cannot be regarded as an operative process and, therefore, can no longer be estimated.

(f) Calculated from the solubility using Equation 2-3 ($\log S = 4.184 - 0.922 \log K_{ow}$) from Lyman et al. (1982).

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

6.1 PREPARATION OF THE DRUG SUBSTANCE

A declaration of responsiblity has been issued by the French governmental agency that would regulate environmental and occupational safety pertaining to the manufacture of amifostine at Avrillé. This agency regularly inspects the SIPSY facility at that location. Certification for safety of workers, safety of installations, and proper waste removal has also been provided by the General Manager of SIPSY.

6.2 PREPARATION OF THE PRODUCT

6.2.1 Substances Generated During Manufacturing of the Product

Production losses of the drug substance that occur during the manufacturing of Ethyol® (amifostine) for Injection by Ben Venue Laboratories, Inc., are carefully containerized and transported to incinerators licensed for disposal of medical wastes (Subsection 6.2.2). In fact, all wastes (aqueous, nonaqueous and solid) will be incinerated.

Atmospheric Emissions: Losses of drug substance or its excipient to the atmosphere are obviated by their physical properties. Amifostine (Figure 5-1) is a substance with both acidic and basic functional groups in the same molecule (see Table 5-1 for pK_a values). Substances of this type are ionic under all conditions (similar to amino acids such as glycine). Ionic organic substances do not volatilize from the solid state or from solution in water. Mannitol, the sole excipient of this product, is a polyhodroxy alcohol. It too will not volatilize under the conditions of manufacturing.

Dust will not be emitted during the weighing of chemicals, because this activity is carried out in a negative pressure room. Containers, vent filters and cleaning materials--as well as any spills--will be removed from this room in double-bagged containers for incineration as medical waste (Subsection 6.2.2).

Aqueous/Solid Wastes: All wastes (whether aqueous, nonaqueous, or solid) are carefully removed in double-bagged containers by the operating personnel for incineration as medical waste. The manufacturing of this drug product will be carried out in a monitored and carefully controlled system that transports the formulation to the vials and ultimately produces the finished goods, i.e., it is a closed system. Quality control samples, water from equipment cleaning, and aqueous tailings, are collected at their point of origin by the operating personnel. The other portions of formulation for which disposal is required will be in vials. Again, all such wastes are placed in absorbent-containing double-bagged drums for transport to an incinerator. Aqueous wastes from the manufacturing of this product are not sewered.

6.2.2 Controls Exercised on Residuals and Emissions

The manufacturing of this drug product will be carried out in a monitored and carefully controlled system that transports the formulation to the vials and ultimately (via lyophilization) produces the finished goods. Contact with drug substances is minimal. Liquid wastes--in this case, the aqueous wastes--are transported by Chemical Analytics, Inc., (29959 Beverly Road, Romulus, Michigan) to an incinerator (Permit No. 16-88I and 13-89IB), operated by Valley City Disposal (1040 Market Street, S.W., Grand Rapids, Michigan). Solid wastes, vials and contaminated protective clothing are removed from the premises by Medical Tracking Systems (607 Freedlander Road, Wooster, Ohio) for incineration at Castle Medical Disposal, Inc. (3900 Christopher Avenue, Hamtramck, Michigan) (Permit No. C9435 through C9438). The incinerators are operated for disposal of medical waste under the regulatory jurisdiction of the Michigan Department of Natural Resources.

Employee safety at Ben Venue Laboratories is ensured by adherence to a Chemical Hygiene Plan and implementation of a formal policy on Hazard Communication. Employee education on health and safety is provided through lectures and hands-on training in the proper operation of equipment. Operating protocols include the checking of safety and personal protection equipment. Material Safety Data Sheets must be reviewed by personnel for all chemicals handled in the manufacturing area. Industrial hygienists monitor the exposure of employees to hazardous materials.

Access to drug substances and products is restricted to authorized personnel. In addition, access to the Bedford site is carefully controlled and the premises are protected by 24-hour security. The facility is serviced by the Bedford Fire Department. The latter would respond to large spills and other emergencies. If small spills occur, they will be remediated by personnel who have received appropriate training. Essentially, the spills will be absorbed with the absorbents normally placed in waste drums. The used absorbents will be drummed while fresh water is applied to the area of the spill. This cleaning water is then also absorbed and drummed. The procedure is generally repeated several times. The spill area is then monitored by an industrial hygienist.

6.2.3 Compliance of Proposed Action with Applicable Emission Requirements

All chemicals used in the manufacture of the drug product are regulated by the Occupational Safety and Health Administration under its responsibility for (1) permissible exposure limits, (2) safe handling of flammable liquids, (3) safe handling of corrosives, and (4) hazard communication. To comply with these areas of concern, the management of Ben Venue Laboratories has instituted a Chemical Hygiene Plan (Appendix D-1) and a formal policy on Hazard Communication (Appendix D-2).

Air emissions from the production of Ethyol® (amifostine) for Injection are limited to water vapor. According to Ohio Administrative Code and EPA requirements, air permits must be obtained if releases are generated that constitute over 100 tons per year of air contaminants including ozone, carbon monoxide, particulates, SO_x, NO_x and lead for non-attainment area. Ben Venue Laboratories (BVL) does not generate any of these in permitting quantities. Permits must also be filed in Ohio if over 10 tons per year of Hazardous Air Pollutants (HAPs) are released. BVL is in the process of investigating its permit status in regard to the release of HAPs. A new regulation in under development which will require controls of emissions of ethylene oxide from sterilization operations. A study is underway to determine how these regulations will impact BVL's operations and permitting status.

Aqueous wastes that contain amifostine will be transported to a -waste incinerator (Permit No. 16-881 and 13-89IB) operated by Valley City Disposal (Grand Rapids, Michigan). Solid wastes, vials, and contaminated protective clothing will be incinerated at Castle Medical Disposal, Inc., (Hamtramck, Michigan) (Permit No. C9435 through C9438). These permits are issued by the Michigan Department of Natural Resources.

Wastewater that does not contain drug substances is discharged to the Publicly Owned Treatment Works of the City of Bedford under permit from the Ohio Environmental Protection Agency (Appendix D-4). The Indirect Discharge Permit (#3PD00005101*BP) was renewed at the end of 1993 and became effective on

January 3, 1994. A copy of the first two pages of the permit showing the discharge limitations are provided in Appendix D-4.

Certification of compliance with applicable emission requirements is provided in Appendix D-5.

6.2.4 Effect of the Proposed Action on Compliance with Current Emission Requirements

Manufacturing of the drug product does not generate air emissions that must be permitted. All liquid and solid wastes generated from manufacturing will be incinerated offsite as medical waste. BVL is calssified under RCRA as a large quantity generator (Generator #OHD091625749) but because it does not store hazardous waste over 90 days on site, BVL is not required to have permits to treat, dispose or store these wastes (40 CFR 262.43). The proposed action does not generate waste that is considered hazardous, and will therefore certainly not affect compliance with current requirements.

6.3 AMOUNT OF AMIFOSTINE ENTERING THE ENVIRONMENT

The only route by which amifostine or its degradates can enter the general environment of the United States is through its use and elimination by human patients. After injection, elimination occurs via the urine. The amounts detected are 0.69, 2.64, and 2.22 percent of the administered dose as unchanged amifostine, the thiol, and the disulfide, respectively (Figure 5-1) (Shaw et al., 1986). As pointed out in Item 5 (Subsection 5.4), the disulfide can be generated from the thiol during laboratory analysis, and thus may be an artifact. Moreover, amifostine itself is hydrolytically unstable (Risley et al., 1986). Therefore, calculations for the amount of substances entering the environment are combined as if the thiol metabolite (alone) were being released.

The estimated concentration of thiol metabolite at a typical wastewater treatment plant would be less than 1 ng/L. This concentration is, of course, a worst-case estimate, because it is calculated as if further degradation of the metabolite were not occurring. The effect of degradative processes on the concentration and dissipation of the thiol metabolite is discussed in Item 7 (Subsection 7.2).

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

In Item 7, information is presented that is relevant to the environmental transport and fate of amifostine and its thiol metabolite. Assessment of their transport and fate is accomplished by an evaluation of processes affecting transport (between air, water, and soil) and processes affecting structural degradation. The methodology involved in this evaluation and its application to specific chemicals is discussed in Water-Related Environmental Fate of 129 Priority Pollutants (USEPA, 1979).

7.1 AIR

There are no releases of amifostine to the atmosphere during preparation of the sealed vials. Volatilization from aquatic systems to the atmosphere also does not occur, because the drug substance and its metabolite will be present as ionic molecular species throughout the pH range of surface water.

7.2 WATER

Amifostine and its metabolite will be introduced to the aqueous environment via elimination by patients to wastewater treatment plants throughout the United States. Degradation is expected to decrease their concentration before the sewage effluent is released to surface water.

In the water of a sewage treatment facility, or in the surface water that dilutes the effluent, amifostine and its metabolite could be affected by environmental processes that include photolysis, oxidation, hydrolysis, volatilization, adsorption, bioaccumulation, and biodegradation. These processes are individually evaluated in this subsection before a concluding statement is made on the probable fate and concentration of released substances in the aqueous environment.

7.2.1 Photolysis

The electromagnetic absorption spectrum of amifostine exhibits no absorption within the wavelength range of terrestrial sunlight (Table 5-1). Therefore, its photodegradation in surface water is not expected.

7.2.2 Oxidation

Photochemically produced hydroxyl radicals in water have been observed to oxidize many similar organic chemicals, including ethylene diamine (Dorfman and Adams, 1973). Hydroxyl radicals (and alkylperoxy radicals) are generated in surface water from the photolysis of naturally occurring substances that absorb terrestrial sunlight (Mill et al., 1980). These oxidants may be significant for the degradation of amifostine and its metabolite. Oxidation by this pathway would be limited, however, by the low concentration of the radical species, estimated to be present at levels of 10^{-9}M (Mill et al., 1980). The half-life for similar chemicals would be about 80 days.

The disulfide that is detected in urine and in other samples for analysis is th oxidative coupling product of the thiol (Figure 5-1). However, it may be an analytical artifact (Subsection 5.4). In the environment of a sewage treatment plant, the concentration of amifostine's thiol metabolite will be so much smaller than the concentration of other organic thiols that (if oxidative coupling occurs) the metabolite would not be expected to couple with another identical molecule.

7.2.3 Hydrolysis

The hydrolytic instability of amifostine has been studied in several buffers at a pH range of 1.0 to 10.3 (Risley et al., 1986). At pH 6 (30°C), the half-life for hydrolysis to the thiol (Figure 5-1) is reported as 3,736 min (or 2.6 days). At 20°C, the half-life would be about twice that number.

7.2.4 Volatilization

Transport from water (or soil) to the atmosphere is not a relevant process for the environmental disposition of amifostine or its metabolite. The pK_a values from Table 5-1 indicates that these substances will be ionic in aqueous solutions at all pH

values. The volatility of such ionic species from water--or the solid state--is zero. In addition, estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant) assume that the dissolved chemical is not ionic (Mackay and Wolkoff, 1973; Lyman et al., 1982). If the chemical is ionic--as are amifostine and its metabolite--volatilization to the atmosphere cannot be regarded as an operative process.

7.2.5 Sorption/Desorption

Partitioning into the lipophilic organic matter of soil or sediment can be estimated by calculating the log soil adsorption coefficient, log K_{oc}, from an experimentally derived relationship (Lyman et al., 1982):

$$\log K_{oc} = 3.64 - 0.55 \log \textit{Solubility } (mg/L)$$
$$(r^2 = 0.71;\ n = 106)$$

where:

r^2 = the coefficient of determination (proportionate reduction in error).

n = the number of chemicals from which the regression was developed.

The log K_{oc} calculated with this equation is 0.53. The distribution coefficient, K_d, for partitioning between surface water and sediment can now be estimated by assuming a 4 percent organic content in the sediment. This is the assumption used in the ENPART model recommended in USFDA (1987). The relationship between K_{oc} and K_d is given by the following equation:

$$K_d = K_{oc} (OC)$$

where:

OC = the fractional amount of organic carbon in sediment.

The distribution coefficient, K_d, estimated by this method is 0.13, indicating no tendency for partitioning into the organic matter of sewage sludge or sediment.

7.2.6 Bioaccumulation/Bioconcentration

A surface water bioconcentration factor (BCF) can be calculated for amifostine from the following equation (Lyman et al., 1982):

$$\log BCF = 2.791 - 0.564 \log Solubility\ (mg/L)$$
$$(r^2 = 0.49;\ n = 36)$$

where:

r^2 = the coefficient of determination (proportionate reduction in error).

n = the number of chemicals from which the regression was developed.

Using the solubility from Table 5-1, the log BCF calculated by this method is -0.40. The BCF itself is 0.39. This value indicates that there is no tendency for amifostine to become absorbed into aquatic life.

7.2.7 Biodegradation

Amines, diamines, mercaptans (i.e., thiols), and organic sulfides are normal constituents of sewage treatment facilities (Metcalf & Eddy, Inc., 1979). The thiol metabolite of amifostine can be classified as a member of this group of chemicals to which sewage constituents belong (Figure 5-1). Under aerobic conditions of sewage treatment, these chemicals are biologically oxidized to carbon dioxide, nitrogen, sulfate ion, and water before the treated effluent is released.

7.2.8 Probable Fate of Amifostine and Its Degradates

The processes that affect the environmental fate of amifostine and its degradates are summarized in Table 7-1. Based on the environmental behavior of similar chemicals, biodegradation in the sludge of sewage treatment facilities is the expected fate of these substances. If any molecules of amifostine or its thiol metabolite survive sewage treatment, hydrolysis and continued biodegradation in the surface waters that receive the effluent would ensure their destruction. Thus, the Expected Environmental Concentration (EEC) (PMA, 1991) is zero.

7.3 SOIL

Amifostine and its manufacturing wastes will not be deposited in landfills. If any intact amifostine is present in sewage sludge that is landfilled, its degradation is expected to proceed as described in Subsection 7.2.8.

Table 7-1

Summary of Environmental Transport and Fate of Amifostine and Its Degradates

Environmental Process	Summary Statement	Confidence of Data[a]
Photolysis	The drug substance does not absorb radiation in the wavelength range of terrestrial sunlight. Therefore, this process does not contribute to degradation.	High
Oxidation	Oxidation of the released substances by both nonenzymatic and enzymatic catalysis will be the process responsible for their ultimate destruction.	High
Hydrolysis	Hydrolysis of amifostine will occur under ambient conditions.	High
Volatilization	Amifostine and its metabolite cannot volatilize to the atmosphere because they are ionic under environmental conditions.	High
Sorption/Desorption	There is no tendency for adsorption.	Medium
Bioaccumulation/ Bioconcentration	There is no tendency for bioconcentration in aquatic organisms.	Medium
Biodegradation	Amifostine and its degradates will be oxidized biologically by sewage cultures to carbon dioxide, nitrogen, sulfate ion, and water.	Medium

(a) Levels of confidence are based on criteria discussed in USEPA (1979). High confidence requires that the data are quantitative; rate constants and half-lives are either explicitly described or can be calculated from the results. Medium confidence is assigned to quantitative data reported for a different but structurally related compound. A low confidence ranking is given to theoretical estimates or to speculative statements.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

In surface waters that receive sewage effluent, the estimated worst-case concentration of released substance (without any degradation occurring) would be less than 1 pg/L, because the typical dilution factor for rivers in the United States is 10^3 (Linsley et al., 1975; Metcalf & Eddy, Inc. 1979). However, based on the degradation of similar chemicals during sewage treatment, the Expected Environmental Concentration (EEC) (PMA, 1991) is zero. (Manufacturing of the drug product at Ben Venue Laboratories does not release any amifostine.) In addition, amines and thiols are normal constituents of surface water and sediment (Conroy et al., 1981; Mitchell et al., 1984). It is concluded, therefore, that the environmental effects of amifostine and its degradates are negligible. These substances will not be persistent in the environment and will not affect the future viability of any ecosystem.

9. USE OF RESOURCES AND ENERGY

The proposed action does not require a large commitment of resources. Moreover, no irreversible or irretrievable commitment of resources will be involved.

Because environmental effects of releases are negligible, no threatened or endangered species can be affected.

The State of Ohio does not regard any property in the vicinity of the manufacturing location identified in Section 4.3 to have historical or archaeological importance.

10. MITIGATION MEASURES

The manufacturing of this drug product will be carried out in a monitored and carefully controlled system (Figure 6-1) that transports the formulation to the vials and ultimately produces the finished goods, i.e., it is a closed system. An examination of Figure 6-1 discloses that quality control samples, water from equipment cleaning,

and aqueous tailings, are collected at their point of origin by the operating personnel. The other portions of formulation for which disposal is required will be in vials. Again, all such wastes are paced in absorbent-containing double-bagged drums for transport to an incinerator. Aqueous wastes from the manufacturing of this product are not sewered. Moreover the physical properties of amifostine and its excipient preclude their emission to the atmosphere.

11. ALTERNATIVES TO THE PROPOSED ACTION

No potential adverse environmental impacts have been identified for the proposed action. Amifostine is carefully controlled during manufacture (Item 6). The small amount of amifostine and its degradates that will be eliminated from patients will be degraded in sewage sludge (Item 7). Because no environmental impact is expected, alternatives to the proposed action are not being considered. A no-action alternative would prevent the use of amifostine with cyclophosphamide in the treatment of ovarian cancer.

12. PREPARER

Norman W. [illegible]
Senior Scientist
N.W. Gabel & Assoc.
8201 Corporate Drive, Suite 620
Landover, Maryland 20785

13. CERTIFICATION

The undersigned certifies that the information available to us and provided to N.W. Gabel & Assoc. (preparer) by U.S. Bioscience, One Tower Bridge, 100 Front Street, West Conshohocken, Pennsylvania (applicant) is true, accurate, and complete to the best of our knowledge.

Signature Mats Hogner Date August 23, 1993
Title Director of Pharmaceutical Chemistry

The undersigned certifies that the information presented is as true, accurate, and complete (with the omission only of proprietary material) as provided to N.W. Gabel & Assoc. for preparation of the EA in accordance with 21 CFR 25.31(a).

Signature Norman W. Gabel Date July 21, 1993
Title Senior Scientist

14. REFERENCES

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15. APPENDICES

A Fate and Effects Studies for Environmental Assessment of Amifostine

APPENDIX A

Fate and Effects Studies for Environmental Assessment of Amifostine

Fate and Effects Studies for Environmental Assessment of Amifostine

FDA Guideline	Type of Study	Result	Significance	Citation
3.01	Water Solubility	450,000 mg/L	Baseline data for other studies.	NDA Section 3A
3.02	Log Octanol-Water Partition Coefficient	-1.6	Estimation of partitioning into lipophilic material from surface water.	Table 5-1
3.03	Vapor Pressure	ND	Vapor pressure of amifostine cannot be related to its environmental volatility. Estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant) assume that the dissolved chemical is not ionic and does not become adsorbed to suspended particulates or sediment (Mackay and Wolkoff, 1973; Lyman et al., 1982). (These restrictive conditions are often overlooked in the widespread application of this model.) If the chemical is ionic--as are amifostine and its principal degradate (Item 7, Subsection 7.2.5)--its volatilization to the atmosphere cannot be regarded as an operative process and, therefore, can no longer be estimated.	EA Item 5.2
3.04	Dissociation (pK_a)	<2, 4.4, 9.3, and 11.2	Amifostine and its principal degradate will be ionic under all environmental conditions.	NDA Section 3A
3.05	UV-Visible Spectrum	No absorption above 290 nm	The drug substance is not photolabile.	NDA Section 3A
3.06	Melting Temperature	ND	Variable range for decomposition without specific melting point.	NDA Section 3A

FDA Guidel..ie	Type of Study	Result	Significance	Citation
3.07	Density	ND	The density of the solid phase of a substance cannot be related to the behavior of its dissolved molecules.	EA Item 7.2
3.08	Sorption and Desorption	Amifostine wi!' not be adsorbed by natural sediments.	Adsorption to sediment decreases distribution of the drug substance within the aqueous phase of surface water systems.	EA Item 7.2.5
3.09	Hydrolysis	At pH 6 (30°C) the half-life is 2.6 days.	Amifostine hydrolyzes to its corresponding thiol.	EA Item 7.2.3
3.10	Photodegradation in Water	ND	Amifostine is not photolabile.	EA Item 7.2.1
3.11	Biodegradation in Water	Similar chemicals are degraded in sewage.	Breakdown of released substances will occur during sewage treatment.	EA Item 7.2.7
3.12	Biodegradation in Soil	ND	Disposal on soil (outside of landfills) is not expected.	--
4.01	Algal Toxicity	ND	The expected environmental concentration (EEC) is zero.	--
4.02	Microbial Growth Inhibition	ND	The expected environmental concentration (EEC) is zero.	--
4.06	Seed Germination	ND	The expected environmental concentration (EEC) is zero.	--
4.07	Seedling Growth	ND	The expected environmental concentration (EEC) is zero.	--
4.08	Daphnia Acute Toxicity	ND	The expected environmental concentration (EEC) is zero.	--
4.09	Daphnia Chronic Toxicity	ND	The expected environmental concentration (EEC) is zero.	--
4.10	Hyalella Toxicity	ND	The expected environmental concentration (EEC) is zero.	--
4.11	Freshwater Fish Acute Toxicity	ND	The expected environmental concentration (EEC) is zero.	--
4.12	Earthworm Subacute Toxicity	ND	The expected environmental concentration (EEC) is zero.	--

ND - Not Determined. Levels of concern (e.g., NOEL and LC_{50}) were not determined because the expected environmental concentration (EEC) is zero.

Date : JULY 1991 MSDS : 7182 A

SIPSY Route de BEAUCOUZE 49240 AVRILLE Tél : 41.43.32.11 Telex: 720911 Fax : 41.42.76.55	**MATERIAL SAFETY DATA SHEET** **SPECIFIC RISKS**
1 IDENTIFICATION 1.1. Common name 1.2. Use 1.3. Additional information	AMIFOSTINE Active ingredient Syn. : Ethiofos
2. CHEMICAL NATURE OF PRODUCT - Substances - Principal hazardous component(s)	CAS name : Ethanethiol,2-(3-aminopropylamino)-, dihydrogen phosphate CAS N° : 20537-88-6 Mol.Form.: $C_5H_{15}N_2O_3PS$
3. PHYSICAL CHARACTERISTICS 3.1. Appearance 3.2. Characteristic 3.3. Solubility 3.4. pH 3.5. Vapor pressure 3.6. Specific gravity 3.7. Additional information	White crystalline powder Soluble in water ; almost insoluble in organic solvents. 6-8 for a 5 % aqueous solution Hygroscopic product
4. HANDLING AND STORAGE 4.1. Precautions to be taken in handling and storage. 4.2. Packaging material 4.3. Hazardous reactions with 4.4. Hazardous decomposition products 4.5. Protective equipment 4.6. Special precautions 4.7. Steps to be taken in case material is spilled or released 4.8. Other precautions	Store in tigh closed container under nitrogen in a temperate or cooled to +4 °C place. Handle in a ventiled area. Polyethylene bags and cardboard drums Gloves ; goggles ; dust mask

5. FIRE AND EXPLOSION DATA 5.1. Flash point 5.2. Auto-ignition temperature 5.3. Unusual fire and explosion hazards 5.4. Extinguisher media 5.5. Special fire fighting 5.6. Other precautions	
6. TOXICOLOGICAL DATA	DL50 (o.mouse) : 842 mg/kg DL50 (i.musc.) : 396 mg/kg DL50 (i.p.rat) : 418 mg/kg Protective agent (chimio- and radiotherapy) Non mutagen and non teratogen.
7. EMERGENCY AND FIRST AID PROCEDURES	Contact with eyes or skin : Flush with water.
8. PROTECTION OF ENVIRONMENT	Do not discard in natural environment.
9. SPECIAL PRECAUTIONS Precautionary labeling Shipment RTMD : Shipment RID/ADR : Shipment IMDG : Shipment IATA :	Not dangerous for shipment.

The information contained herein are based on our present state of knowledge regarding the concerned product, as on :
Users must be aware that use of product for other purposes than which it has been meant for may incur hazardous risks.

Date : JUILLET 1991 MSDS : 7182 A

SIPSY Route de BEAUCOUZE 49340 AVRILLE Tél : 41.43.32.11 Telex: 720911 Fax : 41.42.76.55	**FICHE DE DONNEES DE SECURITE** RISQUES PROFESSIONNELS

1 IDENTIFICATION	
1.1. Nom usuel :	AMIFOSTINE
1.2. Types d'utilisation	Principe actif pharmaceutique
1.3. Autres données :	Cytostatique : Ethiofos
2. NATURE CHIMIQUE	
- Substances	Nom CAS : Ethanethiol,2-(3-aminopropylamino)-,dihydrogen phosphate N° CAS : 20537-88-6 F.brute : $C_5H_{15}N_2O_3PS$
- Composants apportant un danger	
3. PROPRIETES PHYSIQUES	
3.1. Etat physique	Poudre cristalline blanche
3.2. Températures caractéristiques	
3.3. Solubilité	Soluble dans l'eau ; pratiquement insoluble dans les solvants organiques.
3.4. pH	6-8 pour une solution aqueuse à 5 %
3.5. Pression de vapeur	
3.6. Masse volumique	
3.7. Autres données	Hygroscopique
4. STOCKAGE ET MANIPULATION	
4.1. Précautions en cours de stockage et de manipulation.	Stocker en emballages bien clos sous azote dans un local tempéré ou réfrigéré à +4 °C. Manipuler dans un local ventilé.
4.2. Matériaux d'emballage	Sacs polyéthylène et fûts carton
4.3. Réactions dangereuses avec	
4.4. Produit(s) de décomposition dangereux.	
4.5. Mesures individuelles de prévention	Lunettes, gants, masque à poussières
4.6. Mesures spéciales de protection	
4.7. Mesures après fuite ou déversement accidentel	
4.8. Autres recommandations	

5. INFLAMMATION ET EXPLOSION 5.1. Point éclair : 5.2. Température d'auto-inflammation 5.3. Dangers particuliers d'incendie ou d'explosion 5.4. Moyen d'extinction : 5.5. Mesures particulières de protection dans la lutte contre l'incendie 5.6. Autres recommandations	
6. RENSEIGNEMENTS TOXICOLOGIQUES	DL50 (oral/souris) : 842 mg/kg DL50 (i.musc/rat) : 396 mg/kg DL50 (i.p. rat) : 418 mg/kg Agent protecteur en chimiothérapie et radiothérapie. Non mutagène et non tératogène.
7. MESURES DE PREMIERS SECOURS	Contact cutané et oculaire : laver abondamment à l'eau.
8. PROTECTION DE L'ENVIRONNEMENT	Ne pas rejeter en milieu naturel.
9. INDICATIONS PARTICULIERES Etiquetage volontaire : Transports RTMD : Transports RID/ADR : Transports INDG : Transports IATA :	Non réglementé ; produit non dangereux

Libellé : AMIFOSTINE BROYE COND
Approuvé le : 16/7/91
RECHERCHE et DEVELOPPEMENT

Abrégé : AM275 BYC
Par :
PRODUCTION :

Les renseignements contenus dans cette fiche sont basés sur l'état de nos connaissances relatives au produit concerné à ce jour. Ils sont donnés de bonne foi

DRIRE Direction Regionale de l'Industrie de la Recherche et de l'Environnement

PAYS DE LA LOIRE

DIVISION ENVIRONNEMENT ET SURETE INDUSTRIELS

FC/SR/ENV/92/400

Nantes, le 12 juin 1992

ATTESTATION

La production d'AMIFOSTINE s'effectue sur le site de la société SIPSY à Avrillé (Maine et Loire). Cette usine respecte les lois et les règles français[illegible] l'environnement et la sécurité et fait l'objet d'inspections régulières de notre [illegible]. Le procédé de production d'AMIFOSTINE repond également aux lois et réglements [illegible] en vigueur en matière d'environnement et de sécurité.

The manufacture of AMIFOSTINE occurs at the site of the company SIPSY in Avrillé (Maine et Loire). This facilities complies with local french environmental and safety regulations and is regularly inspected by ourself. The manufacturing process of AMIFOSTINE is also in accordance with local french environmental and safety regulations.

Pour le directeur et par délégation
Le chef de la division environnement
et sûreté industrielle

François COPIGNEAUX

DIRECTION
3, rue Marcel Sembat - 44048 NANTES Cédex 04 - Tél. 40 73 74 70 - Télécopie 40 73 46 84 - Télétex 40 73 82 23

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

May 30, 1990

U.S. Bioscience, Inc.
Attn: Philip Schein, M.D.
President and CEO
920-B Harvest Drive, Suite 200
Blue Bell, PA 19422

Dear Dr. Schein:

Reference is made to your orphan drug application of December 19, 1989, submitted pursuant to section 526 of the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 360bb) for the designation of Ethyol (ethiofos) as an orphan drug (application #89-83-428-6).

We have completed our review of the information submitted in accordance with the Food and Drug Administration Interim Guideline implementing section 526 of the FFDCA and have determined that Ethyol (ethiofos) qualifies for orphan designation for use as a chemoprotective agent for cyclophosphamide in the treatment of advanced ovarian carcinoma. Please refer to this letter as official notification of designation.

Prior to marketing approval, sponsors of drugs that have been designated as orphan drugs are requested to submit written notification to the Office of Orphan Products Development of their intention to exercise orphan drug exclusivity if they are the first sponsor to obtain such approval for the drug. This notification will assist FDA in assuring that approval for the marketing of the same drug is not granted to another for the statutory period of exclusivity. In addition, please inform this office annually as to the status of the development program, and at such time as a marketing application is submitted to the FDA for the use of Ethyol as designated. If you need further assistance in the development of your product for marketing, please feel free to contact Dr. John McCormick at (301) 443-4903.

Congratulations on obtaining your orphan drug designation.

Sincerely yours,

Marlene E. Haffner, M.D.
Director, Office of Orphan
Products Development (HF-35)

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18/NE

Issue Date: December 3, 1993

Effective Date: January 3, 1994

Expiration Date: December 31, 1999

Ohio Environmental Protection Agency

Indirect Discharge Permit

In compliance with the provisions of the Federal Water Pollution Control Act, as amended (33 U.S.C. 1251 et. seq., hereinafter referred to as "the Act"), and the Ohio Water Pollution Control Act (Ohio Revised Code 6111),

Ben Venue Laboratories, Incorporated

is authorized by the Ohio Environmental Protection Agency, hereinafter referred to as "Ohio EPA," to discharge wastewater from its facility located at 300 Northfield Road, City of Bedford, Ohio. Cuyahoga County into the Publicly Owned Treatment Works of the Bedford located 65 Columbus Road, Bedford, Ohio in accordance with the conditions specified in Parts I, II, and III of this permit.

The permit is issued to apply and enforce pretreatment rules of the state of Ohio. The rights granted by this permit shall not supersede the primacy of the above authority in the regulation of its publicly owned treatment works.

This permit is conditioned upon payment of applicable fees as required by Section 3745.11 of the Ohio Revised Code.

This permit and the authorization to discharge shall expire at midnight on the expiration date shown above. In order to receive authorization to discharge beyond the above date of expiration, the permittee shall submit such information and forms as are required by the Ohio EPA no later than 180 days prior to the above date of expiration.

Donald R. Schregardus

Donald R. Schregardus
Director

006 159
006 08

PART I, A - FINAL EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

1. During the period beginning on the effective date of this permit and lasting until the expiration date, the permittee is authorized to discharge in accordance with the following limitations and monitoring requirements from outfalls as described below in Part I, A.

EFFLUENT CHARACTERISTIC			DISCHARGE LIMITATIONS				MONITORING REQUIREMENTS	
			Concentration		Loading			
Reporting Code	Units	Parameter	Daily Max	Monthly Avg.	Daily Max.	Monthly Avg.	Meas. Freq.	Sample Type
00056	GPD	Flow Rate	-	-	-	-	3/6 Month	24 Hr Total
00402	S.U.	pH, Minimum	Not to be less than 5.0 at anytime				3/6 Month	Grab
00720	mg/l	Cyanide, Total	9.14	2.56	-	-	3/6 Month	See Part II

2. The sampling location is the manhole located on the sidewalk just north of the main driveway into the facility.

PART II. - OTHER REQUIREMENTS

1. The permittee shall comply with all applicable rules, regulations and ordinances of the City of Bedford. Any violation of those provisions shall also be considered a violation of this permit. If the authority to discharge is revoked by the POTW, this permit shall also be considered to be revoked.

2. In addition to the report submitted to Ohio EPA under Part III, item 3 of this permit, a copy of each discharge monitoring report shall be submitted to the POTW at the following address:

City of Bedford
65 Columbus Road
Bedford, Ohio 44146

3. Any slug loading shall be reported to the POTW at (216) 232-1600 pursuant to requirements in Part III, Item 10.

4. Cyanide

If the permittee does not use or generate cyanide, they may certify to the Ohio EPA that they are not using or generating cyanide. The permittee shall make the following certification statement with each compliance monitoring report: "I certify to the best of my knowledge and belief that cyanide is not used or generated as a result of our activities." The certification statement shall be signed by the individual responsible for managing compliance with the requirements of this permit. If the permittee does not certify then they must monitor for cyanide.

006 085
161

PART III GENERAL CONDITIONS

1. DEFINITIONS

"Absolute Limitations". Compliance with limitations having descriptions of "shall not be less than," "nor greater than," "shall not exceed," "minimum," or "maximum," shall be determined from any single value for samples and/or measurements collected.

"Composite" means a combination of individual samples collected at periodic intervals of the entire discharge day. The composite must be flow proportional; either the time interval between each individual sample or the volume of each individual sample must be directly proportional to either the wastestream flow at the time of the sampling or the total wastestream flow since the collection of the previous sample. Samples may be collected manually or automatically.

"Grab" means an individual sample collected at such time and location as to be representative of the discharge.

"Interference" means a discharge which, alone or in conjunction with a discharge or discharges from other sources, both: 1) inhibits or disrupts the POTW, its treatment processes or operations, or its sludge processes, use or disposal; and (2) therefore is a cause of a violation of any requirement of the POTWs NPDES permit (including an increase in the magnitude or duration of a violation) or of the prevention of sewage sludge use or disposal in compliance with the following statutory provisions and regulations or permits issued thereunder (or more stringent local regulations): Section 405 of the Clean Water Act, the Solid Waste Disposal Act (SWDA) (including Title II, more commonly referred to as the Resource Conservation and Recovery Act (RCRA), and including state regulations contained in any state sludge management plan prepared pursuant to Subtitle D of SWDA), the Clean Air Act, and the Toxic Substances Control Act.

"mg/l" means milligrams per liter

"pass through" means a discharge which exits through the POTW to waters of the State in quantities or concentrations which, alone or in conjunction with a discharge or discharges from other sources, is a cause of a violation of any requirement of the POTW's NPDES permit.

"POTW" or "publicly owned treatment works" means a treatment works owned or operated by a public authority. This definition includes any devices and systems used in the storage, treatment, recycling and reclamation of municipal sewage or industrial wastes of a liquid nature. It also includes sewers, pipes and other conveyances only if they convey wastewater to a POTW treatment plant. The term also means the public authority which has jurisdiction over the indirect discharges to and the discharges from such a treatment works.

"Pollutant" means sewage, industrial waste, or other waste as defined by divisions (B), (C) and (D) of Section 6111.01 of the Revised Code.

"Reporting Code" is a five digit number used by the Ohio EPA in processing reported data. The reporting code does not imply the type of analysis used nor the sampling techniques employed.

"Slug loading" means any pollutant, including oxygen demanding pollutants, released in a discharge at a flow rate and/or pollutant concentration as to cause interference in the POTW.

"ug/l" means micrograms per liter

2. GENERAL EFFLUENT LIMITATIONS

A. All users of a POTW shall comply with the requirements of 40 CFR Part 403, the Federal "General Pretreatment Regulations for Existing and New Sources of Pollution," as appropriate.

B. The permittee shall not introduce the following pollutants into a POTW:

1. Pollutants which create a fire or explosion hazard in the POTW;
2. Pollutants which will cause corrosive structural damage to the POTW, but in no case discharges with pH lower than 5.0, unless the POTW is specifically designed to accommodate such discharges;
3. Solid or viscous pollutants in amounts which will cause obstruction to the flow in sewers, or other interference with the operation of the POTW;
4. Any pollutant, including oxygen demanding pollutants (BOD, etc.) released in a discharge at a flow rate and/or pollutant concentration as to cause interference in the POTW;
5. Heat in amounts that will inhibit biological activity in the POTW resulting in interference or causing damage, but in no case heat in such quantities that the temperature exceeds 40° C (104° F) at the POTW unless the Director, upon request of the POTW, approves an alternate temperature limit;
6. Any liquids, gases, or solids which either singly or by interaction prevent entry into sewers for their maintenance and repair.

C. The permittee shall not achieve any effluent concentration by dilution. The permittee shall not increase the use of potable water, process water or cooling water or otherwise attempt to dilute a discharge as a partial or complete substitution for adequate treatment to achieve compliance with the limitations contained in this permit.

3. REPORTING

A. Monitoring data required by this permit shall be reported on the Ohio EPA report form (4500) on a semi-annual basis, unless specified otherwise in Part II - Other Conditions. Reports for each sampling period are to be received no later than the 15th day of June and December. The original plus first copy of the report form must be signed and mailed to:

Ohio Environmental Protection Agency
Pretreatment Unit
1800 WaterMark Drive
P. O. Box 1049
Columbus, Ohio 43266-0149

B. If the permittee monitors any pollutant at the location(s) designated herein more frequently than required by this permit, using approved analytical methods as specified below, the results of such monitoring shall be included in the calculation and reporting of the values required in the reports specified above.

C. Analyses of pollutants not required by this permit, except as noted in the preceding paragraph, shall not be reported on Ohio EPA report form (4500) but records shall be retained as specified in the paragraph entitled "Records Retention."

D. A copy of the Ohio EPA report form (4500) shall be sent to the POTW authority as specified in Part II, Other Requirements.

4. SAMPLING AND ANALYTICAL METHODS

A. Samples and measurements taken as required herein shall be representative of daily operations. Test procedures for the analysis of pollutants shall conform to regulation 40 CFR 136, "Test Procedures For The Analysis of Pollutants" unless other test procedures have been specified in this permit. The permittee shall periodically calibrate and perform maintenance procedures on all monitoring and analytical instrumentation at intervals to ensure accuracy of measurements.

B. Unless otherwise specified in Part II - Other Requirements, samples shall be obtained through use of flow-proportional composite sampling techniques; where composite sampling is not physically possible or contrary to the approved methods set forth in 40 CFR 136, a grab sample is acceptable.

C. The permittee is responsible for providing a sampling location suitable for obtaining a representative sample.

5. RECORDING OF RESULTS

For each measurement or sample taken pursuant to the requirements of this permit, the permittee shall record the following information:

A. The exact place and date of sampling;
B. The person(s) who performed the sampling or measurements;
C. The date the analyses were performed on those samples;
D. The person(s) who performed the analyses;
E. The analytical techniques or methods used; and
F. The results of all analyses and measurements

6. RECORDS RETENTION

The permittee shall retain all of the following records for a minimum of three years, including:

A. All sampling and analytical records (including internal sampling data not reported);
B. All original recordings for any continuous monitoring instrumentation;
C. All instrumentation, calibration and maintenance records; and
D. All plant operation and maintenance records.
E. All reports required by this permit.
F. Records of all data used to complete the application for this permit for a period of at least three years from the date of the sample, measurement, report or application.

7. AVAILABILITY OF REPORTS

Except for data determined by the Ohio EPA to be entit' confidential status, all reports prepared in accordance with the terms of this permit shall be available for public inspection at the appropriate District Office of the Ohio EPA. Both the Clean Water Act and Section 6111.05 Ohio Revised Code state that effluent data shall not be considered confidential. Knowingly making any false statement on any such report may result in the imposition of criminal penalties as provided for in the Ohio Revised Code Section 6111.99.

8. DUTY TO PROVIDE INFORMATION

The permittee shall furnish to the Director, within a reasonable time, any information which the Director may request to determine whether cause exists for modifying or revoking the permit, or to determine compliance with this permit. The permittee shall also furnish to the Director, upon request, copies of records required to be kept by this permit.

9. RIGHT OF ENTRY

The permittee shall allow the Director, or an authorized representative upon presentation of credentials and other documents as may be required by law to:

A. Enter upon the permittee's premises where a regulated facility or activity is located or conducted, or where records must be kept under the conditions of this permit.

B. Have access to and copy, at reasonable times, any records that must be kept under the conditions of this permit.

C. Inspect at reasonable times any facilities, equipment (including monitoring and control equipment), practices, or operations regulated or required under this permit.

D. Sample or monitor at reasonable times, for the purposes of assuring permit compliance or as otherwise authorized by the Clean Water Act, any substances or parameters at any location.

10. NOTIFICATION OF SLUG LOADING

A. The permittee shall notify the POTW at the telephone number provided in Part II - Other Conditions and the Ohio EPA by telephone at 1-800-282-9378 within one hour of discovery of any slug loading and provide the following:

1. A description of the discharge and the cause of the slug loading;
2. The period of slug loading including exact dates and times and, if not corrected, the anticipated time the noncompliance is expected to continue;
3. The steps taken or planned to reduce, eliminate and prevent reoccurrence of the slug loading.
4. The POTW affected by the discharge.

B. A written report containing the above information shall be filed with the POTW at the address provided in Part II - Other Conditions, and the Ohio EPA, at the address provided in Part III, Paragraph 3 entitled "REPORTING", within five business days of the day when the slug loading occurred.

11. DISCHARGE CHANGES

The following changes must be reported to the Ohio EPA as soon as practicable.

A. Any significant change in character of the discharge which the permittee knows or has reason to believe has occurred or will occur which would constitute cause for modification or revocation. The permittee shall give advance notice to the Director of any planned changes in the process line or treatment works from which the permitted discharge originates which may result in noncompliance with permit requirements. These changes include, but are not limited to, increases or decreases in production rates from which categorical standards are calculated, discharge flow rates, and the addition or deletion of wastestreams. Notification of permit changes or anticipated noncompliance does not stay any permit conditions.

Following this notice, modifications to the permit may be made to reflect any necessary changes in permit conditions, including any necessary effluent limitations for any pollutants not identified and limited herein. Sections 6111.44 and 6111.45, Ohio Revised Code, require that plans for treatment works or improvements to such works be approved by the Director of the Ohio EPA prior to construction.

12. TOXIC POLLUTANTS

The permittee shall comply with effluent standards or prohibitions under Section 307(a) of the Clean Water Act or Section 3745-3 of the Ohio Administrative Code for toxic pollutants within the time provided in the regulations that establish these standards or prohibitions, even if the permit has not yet been modified to incorporate the requirement. Following establishment of such standards or prohibitions, the Director shall modify this permit and so notify the permittee.

13. PERMIT MODIFICATION OR REVOCATION

A. After notice and opportunity for a hearing, this permit may be modified or revoked, by the Ohio EPA, in whole or in part during its term for cause including, but not limited to, the following:

1. Violation of any terms or conditions of this permit;
2. Obtaining this permit by misrepresentation or failure to disclose fully all relevant facts; or
3. A change in any condition that requires either a temporary or permanent reduction or elimination of the permitted discharge; or

B. Pursuant to rule 3745-36-08, Ohio Administrative Code the permittee may at any time apply to the Ohio EPA for modification of any part of this permit. The filing of a request by the permittee for a permit modification or revocation does not stay any permit condition. The application for modification should be received by the Ohio EPA Pretreatment Unit at least ninety days before the date on which it is desired that the modification become effective. The application shall be made only on forms approved by the Ohio EPA.

14. TRANSFER OF OWNERSHIP OR CONTROL

This permit cannot be transferred or assigned nor shall a new owner or successor be authorized to discharge from this facility, until the following requirements are met:

A. The permittee shall notify the Ohio EPA Pretreatment Unit at least sixty days in advance of the proposed transfer date;

B. The notice include a written agreement containing a specific date for transfer of permit responsibility and coverage between the current and new permittee (including acknowledgement that the existing permittee is liable for violations up to that date, and that the new permittee is liable for violations from that date on); and

C. The Director does not exercise his right to notify the current permittee and the new permittee of his or her intent to modify or revoke the permit and to require that a new application be filed.

15. STATE LAWS AND REGULATIONS

Nothing in this permit shall be construed to preclude the institution of any legal action nor relieve the permittee from any responsibilities, liabilities, or penalties established pursuant to any applicable state law or regulation under authority preserved by Section 510 of the Act.

16. SEVERABILITY

The provisions of this permit are severable, and if any provision of this permit, or the application of any provision of this permit to any circumstance, is held invalid, the application of such provision to other circumstances, and the remainder of this permit, shall not be affected thereby.

17. PROPERTY RIGHTS

The issuance of this permit does not convey any property rights in either real or personal property, or any exclusive privileges, nor does it authorize any injury to private property or any invasion of personal rights, nor any infringement of federal, state, or local laws or regulations.

18. SIGNATORY REQUIREMENTS

A. All applications and reports submitted to the Ohio EPA must be signed by an authorized representative of the permittee. An authorized representative may be:

1. In the case of a corporation, by a principal executive officer of at least the level of vice president, or his duly authorized representative, if such representative is responsible for the overall operation of the facility from which the discharge originates.

2. In the case of a partnership, by a general partner.

3. In the case of a sole proprietorship, by the proprietor.

19. NEED TO HALT OR REDUCE ACTIVITY

It shall not be a defense for a permittee in an enforcement action that it would have been necessary to halt or reduce the permitted activity in order to maintain compliance with conditions of this permit.

20. APPLICABLE FEDERAL RULES

All references to 40 CFR in this permit mean the version of 40 CFR which is effective as of the effective date of this permit.

21. AUTHORIZED DISCHARGES

All discharges authorized herein shall be consistent with the terms and conditions of this permit. The discharge of any pollutant identified in this permit more frequently than, or at a level in excess of, that authorized by this permit shall constitute a violation of the terms and conditions of this permit. Such violations may result in the imposition of civil and/or criminal penalties as provided for in Ohio Revised Code Sections 6111.09 and 6111.99.

22. DISPOSAL OF RESIDUALS

The storage and disposal of collected screenings, slurries, sludge or other solids shall be in accordance with Section 405 of the Clean Water Act and Subtitle C and D of the Resource Conservation and Recovery Act.

23. CIVIL AND CRIMINAL LIABILITY

Except as exempted in the permit conditions on unauthorized discharges, nothing in this permit shall be construed to relieve the permittee from civil or criminal penalties for noncompliance.

24. OTHER INFORMATION

A. Where the permittee becomes aware that it failed to submit any relevant facts in a permit application, or submitted incorrect information in a permit application or in any report to the Director, it shall promptly submit such facts or information.

B. ORC 6111.99 provides that any person who falsifies, tampers with, or knowingly renders inaccurate any monitoring device or method required to be maintained under this permit shall, upon conviction, be punished by a fine of not more than $25,000 per violation.

C. ORC 6111.99, states that any person who knowingly makes any false statement, representation, or certification in any record or other document submitted or required to be maintained under this permit, including monitoring reports or reports of compliance or noncompliance shall, upon conviction, be punished by a fine of not more than $25,000 per violation.

D. ORC 6111.99 provides that any person who violates Sections 6111.04, 6111.042, 6111.05, or division (A) of Section 6111.07 of the Revised Code shall be fined not more than $25,000 or imprisoned not more than one year, or both.

006 092
168

BVL est. 1938 Ben Venue Laboratories, Inc.

GENERAL COMPLIANCE STATEMENT

Ben Venue Laboratories, Inc. states that it is in compliance with requirements set forth in permits applicable to the production of Amifostine for Injection at its facilities in Bedford, Ohio as well as emission requirements set forth in applicable Federal, State and local statutes and regulations applicable to the production of Amifostine for Injection at its facilities in Bedford, Ohio.

Charles S. J. Insalaco
Vice President of Operations
Ben Venue Laboratories, Inc.

270 Northfield Road. P.O. Box 46568. Bedford, Ohio 44146-0568
Phone: 216-232-3320 Fax: 216-232-2772

006 084
169

Ohio Historic Preservation Office

Ohio Historical Center
1982 Velma Avenue
Columbus, Ohio 43211-2497
614/297-2470
Fax:297-2411

OHIO HISTORICAL SOCIETY
SINCE 1885

November 19, 1992

Charles S. J. Insalaco
Vice President of Operations
Ben Venue Laboratories, Inc.
270 Northfield Road, P.O. Box 46568
Bedford, Ohio 44146

Dear Mr. Insalaco:

Re:New Product Production

This is in response to your correspondence, received on October 22, 1992, concerning the project noted above. My staff has reviewed the information that you provided. Based on their recommendation, it is my opinion that the proposed project will have no effect on properties listed in or eligible for the National Register of Historic Places. No further coordination with this office is necessary unless the scope of the project should change.

Any questions concerning this matter should be addressed to Saul Gleiser D., History/Architecture Reviews Manager, at (614) 297-2470. Thank you for your cooperation.

Sincerely,

Martha J. Raymond

Martha Raymond, Department Head
Technical and Review Services

MJR/SGD:sg

006 086
180

MEMORANDUM

SEP 2 1994

TO: Linda McCollum HFD-150

SUBJECT: Environmental Assessment for NDA 20-221 Ethyol (amifostine) for Injection, 500 mg.

FROM: Christina Good HFD-102

I have reviewed the environmental assessment (EA) for NDA 20-221, Ethyol (amifostine) for Injection, 500 mg. and have found the EA to be inadequate to determine the effects of the drug's manufacture, use and disposal on the environment. Please inform the applicant that the following questions and comments should be addressed in both the confidential and publicly available EA's. The information requested is necessary in order to complete the review.

(1) Under the discussion for Item 4 "Description of the proposed action" it is stated that the drug will be administered to patients throughout the country. Will the drug be administered only in clinics and hospitals ? Will it include small physicians offices ? Estimate the number of hospitals, clinics, etc. likely to use this drug. This can be a general estimate.

(2) The discussion of emission under Item 6 "Introduction of substances into the environment" is too general.

(a) Specifically, the discussion under 6.2.1 (Substances generated during manufacturing of the product) states that there will be no releases of the drug substanceat Ben Venue Labs. However, in paragraph 3 on page 006 014 it lists the processing losses for the drug substance. There needs to be an explanation for each location of loss and the specific controls on each location of emissions. There is usually some emissions--what are the substances emitted, and what are the amounts of emissions before and after control. State what emissions are permitted, state their current emissions, the amount expected to change as a result of manufacture of this drug, and the permit limits. This is particularly relevant for the liquid wastes. Where there are NO emissions there should be a specific explanation about why there are no emissions.

(b) There is no discussion on the company's procedures for spill containment. There are usually specific containment procedures and training for each manufacturing site. These should be discussed in both the confidential and FOI EA's.

(3) For Item 10--Mitigation Measures
This section should be revised to explain how the emissions are being controlled (and to be consistent with a revised item 6). Specifically, the statement "[no] emissions or releases of amifostine will occur during the manufacturing of the drug product" should be revised to reflect the controls and the waste disposal methods used.

(4) Item 11--Alternatives to the proposed action

The statement that there will be no release of amifostine during manufacture is not likely. This should be revised to be consistent with the revisions in item 6.

Please do not hesitate to call me (594-6758) if you have any questions concerning these requests.

file:no20221.mem

9/1/94

micro

DEC 16 1994

CONSULTATIVE REVIEW TO HFD-150

DIVISION OF MEDICAL IMAGING, SURGICAL, and DENTAL DRUG PRODUCTS
Microbiologist's Review #3
December 16, 1994

A. 1. NDA: 20-221

APPLICANT: U.S. Bioscience
One Tower Bridge; 100 Front Street
West Conshohocken, Pa 19428

2. PRODUCT NAME: Ethyol (Amifostine for Injection).

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Sterile lyophilized powder for reconstitution, 500 mg/vial, for intravenous infusion. Supplied in single dose 10 mL amber glass vials. Rx.

4. METHOD(S) OF STERILIZATION: Aseptic filtration.

5. PHARMACOLOGICAL CATEGORY: Chemoprotective agent for protection against toxicities associated with platinum and alkylating agent chemotherapy.

B. 1. DATE OF INITIAL SUBMISSION: September 30, 1991
(Review #1)

2. DATE OF AMENDMENT: Amendment #14, August 30, 1993
(Review #2)
Amendment #20, October 24, 1994
Subject of current review (#3)

Amendment dated November 28, 1994

3. SUPPORTING DOCUMENTS:

DMF - Ben Venue Laboratories, Inc., Manufacturer of drug product.

C. REMARKS:

1. The amendment of October 24, 1994 responds to requests for additional information sent in a FAX communication on October 20, 1994.

2. The amendment of November 28, 1994 provides additional information requested in the FAX communication of November 16, 1994.

D. CONCLUSION:

The submission is recommended for approval of sterility assurance of the subject drug.

Vivian Greenman 12/16/94

12/16/94

cc:
NDA 20-221
HFD-160/Consult File
Drafted by: V. Greenman
HFD-150/E. Tolgyesi, S. Koepke, L. McCollum
R/D init. by P.H. Cooney
PC File # ND20221.OA2

SEP 30 1994

CONSULTATIVE REVIEW TO HFD-150

DIVISION OF MEDICAL IMAGING, SURGICAL, and DENTAL DRUG PRODUCTS
Microbiologist's Review #2
September 29, 1994

A. 1. NDA:20-221

APPLICANT: U.S. Bioscience
One Tower Bridge; 100 Front Street
West Conshohcken, Pa 19428

2. PRODUCT NAME: Ethyol (Amifostine for Injection).

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Sterile lyophilized powder for reconstitution, 500 mg/vial, for intravenous infusion. Supplied in single dose 10 mL amber glass vials. Rx.

4. METHOD(S) OF STERILIZATION: Aseptic filtration.

5. PHARMACOLOGICAL CATEGORY: Chemoprotective agent for protection against toxicities associated with platinum and alkylating agent chemotherapy.

B. 1. DATE OF INITIAL SUBMISSION: September 30, 1991
(Review #1)

2. DATE OF AMENDMENT: Amendment #14, August 30, 1993
Subject of this review (#2)

3. SUPPORTING DOCUMENTS:

DMF Ben Venue Laboratories, Inc., Manufacturer of drug product.

C. REMARKS:

1. The current amendment responds to the deficiency letter of May 15, 1992.

2. Since the initial submission of the subject NDA (September 30, 1991), the Ben Venue facility and aseptic processing procedures used at this site have been reviewed for other NDAs. Some of the information and data submitted in the current amendment to the subject NDA are also contained in updates to DMF The updates of February 8, May 29, July 26 and August 20, 1993 have been reviewed for NDA 20-326 by Dr. David Hussong, HFD-160 (Review #1, July 15, 1993 and Review #2,

October 22, 1993). Where specifically relevant, these reviews will be considered in the review of the subject NDA.

3. The Review Chemist should note the comments made under item 6 of this review concerning specifications for holding reconstituted drug product.

D. CONCLUSION:

The submission is not recommended for approval on the basis of sterility assurance. Specific comments are provided in "E. Review Notes" and "Microbiologist's Draft of Letter to Applicant".

Vivian Greenman 9/29/94
Vivian Greenman
Dir 9/30/94

cc:
NDA 20-221
HFD-160/Consult File
Drafted by: V. Greenman
- HFD-150/J. Blumenstein, S. Koepke, L. McCollum
R/D init. by P.H. Cooney
PC File # ND20221.OA1

1.1

CONSULTATIVE REVIEW TO HFD-150

DIVISION OF MEDICAL IMAGING, SURGICAL, and DENTAL DRUG PRODUCTS
Microbiologist's Review # 1
April 30, 1992

A. 1. NDA:20-221

APPLICANT: U.S. Bioscience
One Tower Bridge; 100 Front Street
West Conshohcken, Pa 19428

2. PRODUCT NAME: Ethyol (Amifostine for Injection).

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Sterile lyophilized powder for reconstitution, 500 mg/vial, for intravenous infusion. Supplied in single dose 10 mL amber glass vials. Rx.

4. METHOD(S) OF STERILIZATION: Aseptic filtration.

5. PHARMACOLOGICAL CATEGORY: Chemoprotective agent for protection against toxicities associated with platinum and alkylating agent chemotherapy.

B. 1. DATE OF INITIAL SUBMISSION: September 30, 1991

2. DATE OF AMENDMENT: N/A

3. SUPPORTING DOCUMENTS:

DMF Ben Venue Laboratories, Inc., manufacturer of drug product.

C. REMARKS:

1. The Review Chemist has requested an evaluation of those sections of the application that pertain to the microbiological aspects of manufacturing procedures and sterility assurance.

2. The subject drug currently has Orphan Drug status for use as a chemoprotective agent in the treatment of ovarian carcinoma with cisplatin (# 89-83-427-6) or cyclophosphamide (# 89-83-428-6) and in the treatment of metastatic melanoma (89-83-429-6). Orphan Drug status was given on May 30, 1990.

MAY 7

D. CONCLUSIONS:

The submissions are not recommended for approval on the basis of sterility assurance.

Vivian Greenman 5/1/92
Vivian Greenman
PHC 5/1/92
VMC 5/5/92

cc:

NDA
HFD-160/Consult File
Drafted by: V. Greenman
HFD-150/J. Blumenstein, K. Downs
R/D init. by P.H. Cooney
PC File # NDA20221

NDA 20-221

U.S. Bioscience, Inc.
One tower Bridge; 100 Front Street
West Conshohocken, Pennsylvania 19428

Attention: Barbara Scheffler
Senior Vice President, Clinical Operations
and Regulatory Affairs

Dear Ms. Scheffler:

Please refer to your new drug application (NDA) submitted pursuant to section 505(b) of the Federal Food, Drug, and Cosmetic Act for Ethyol® (amifostine) Injection 500mg/vial.

We are continuing our review and are requesting the following biopharmaceutical information which Dr. Mallikaarjun cited at our January 17, 1992 meeting. Dr. Mallikaarjun has requested that you submit the information by February 3, 1992.

1. Provide information regarding the 910mg/m² pharmacokinetic study that you have started, including:

   a. subject demography,

   b. formulation and dosing schedule,

   c. blood/urine sampling schedule,

   d. assay methodology, and

   e. methodology for pharmacokinetic analysis.

2. Provide the following information for the interaction study with dexamethasone and metoclopramide.

   a. Subject demography.

   b. Study design.

   c. Individual and mean plasma concentration-time data and profiles.

   d. Analytical validation including:
      (1) method validation, and
      (2) study validation.

3. Provide the following information for the 740mg/m^2 study (Dr. Shaw, Univ. of Pennsylvania).

   a. Subject demography.

   b. Study design.

   c. Individual and mean plasma concentration-time data and profiles.

   d. Analytical validation including:
      (1) method validation, and
      (2) study validation.

   e. Pharmacokinetic parameters (individual and mean).

4. Provide information on binding (percent) in human plasma/blood.

The following information should be submitted as soon as possible.

1. Protocols for interaction studies between Ethyol® and other concomitantly administered antineoplastic agents.

We look forward to your response so that we may continue review of your application.

Sincerely yours,

Kathleen Downs

Kathleen Downs

Information Request #4

TABLE 19

Incidence of Adverse Experiences Associated With Amifostine by Dose

Adverse Experience[a]	Per Patient[b]		Per Infusion[b]	
	n	%	n	%
Amifostine + Chemo				
≥ 1100mg/m²	(n =19)		(n = 34)	
Vomiting/Nausea	18	94.7	26	76.5
Flushing/Feeling of Warmth	14	73.7	20	58.8
Sneezing	10	52.6	14	41.2
Dizziness/Lightheadedness	4	21.1	4	11.8
Sleepiness/Somnolence	5	26.3	8	23.5
Hiccups	0	-	0	-
Chills	0	-	0	-
Hypocalcemia	0	-	0	-
Allergy/Rash	0	-	0	-
740 - 1099 mg/m²	(n = 689)		(n = 2346)	
Vomiting/Nausea	568	82.4	1500	63.9
Flushing/Feeling of Warmth	282	40.9	511	21.8
Sneezing	215	31.2	418	17.8
Dizziness/Lightheadedness	96	13.9	115	4.9
Sleepiness/Somnolence	55	8.0	66	2.8
Hiccups	33	4.8	42	1.8
Chills	19	2.8	24	1.0
Hypocalcemia[c]	10	1.3	10	0.4
Allergy/Rash	0	-	0	-
< 740 mg/m²	(n = 28)		(n = 247)	
Vomiting/Nausea	17	60.7	160	64.8
Flushing/Feeling of Warmth	4	14.3	22	8.9
Sneezing	7	25.0	40	16.2
Dizziness/Lightheadedness	0	-	5	2.0
Sleepiness/Somnolence	4	14.3	10	4.0
Hiccups	0	-	1	0.4
Chills	0	-	1	0.4
Hypocalcemia	0	-	0	-
Allergy/Rash	0	-	0	-

TABLE 19 -Continued-

Incidence of Adverse Experiences Associated With Amifostine by Dose

Adverse Experience[a]	Per Patient[b]		Per Infusion[b]	
	n	%	n	%
Amifostine + RT				
340 mg/m² [f]	(n = 106)		(n = 1540)	
Vomiting/Nausea[c]	98	92.5	794	51.6
Sneezing	60	56.6	383	24.9
Sleepiness/Somnolence	33	31.1	188	12.2
Dizziness/Lightheadedness	15	14.2	17	1.1
Flushing/Feeling of Warmth	19	17.9	27	1.8
Hiccups	3	2.8	4	0.3
Chills	2	1.9	2	0.1
Hypocalcemia	1	0.9	1	<0.1
Allergy/Rash	4	3.8	4	0.3
740 mg/m²	(n = 8)		(n = 58)	
Vomiting/Nausea	4	50.0	15	25.9
Flushing/Feeling of Warmth	0	-	0	-
Sneezing	0	-	0	-
Dizziness/Lightheadedness	0	-	0	-
Sleepiness/Somnolence	0	-	0	-
Hiccups	0	-	0	-
Chills	1	12.5	2	3.4
Hypocalcemia	0	-	0	-
Allergy/Rash	0	-	0	-

[a] Occurring on the day of amifostine administration.

[b] Per patient reflects dose per protocol; per infusion reflects actual dose at time event was reported. Total number of patients treated = 580; AEs for 55 patients who received both chemotherapy and radiotherapy are included under amifostine + Chemo if AE occurred on the day of chemotherapy and under amifostine + RT if AE occurred on day of radiotherapy.

[c] Discontinued amifostine therapy because of specific AE

[d] Six patients did not receive antiemetic therapy

[e] Fifty one patients did not receive antiemetic therapy

[f] Administered four times per week for five weeks

[g] Reported at any time during the study

TABLE 22

Summary of All Hypotensive Episodes in Chemotherapy Patients Treated with Amifostine (740 to 910 mg/m²)[a]

Number of patients with ≥1 hypotensive episode		320/652 (49%)
Number of hypotensive episodes		687/2402 (28%)
Patients who terminated infusion	98/320 (31%)	
Patients with temporary interruption of infusion	126/320(39%)	
Patients with no interruption of infusion	96/320 (30%)	

[a] Includes four patients who received 1100 mg/m² of amifostine and 4 patients who received 340 mg/m² of amifostine

TABLE 23

Summary of All Hypotensive Episodes in Radiation Therapy Patients Treated with Amifostine (340 or 740 mg/m²)

Number of patients with ≥1 hypotensive episode		13 (11%)
Number of hypotensive episodes		29 (2%)
Patients who terminated infusion	0 (--)	
Patients with temporary interruption of infusion	1 (8%)	
Patients with no interruption of infusion	12 (92%)	

TABLE 26

IND Safety Reports

IND Serial Number	Pt. No./ Initials	Age/Sex	Adverse Event	Study
		66/F	Hypotension	WR-2721-1
		45/F	Tremors/shaking	Wr-2721-1
		69/M	Metabolic acidosis	WR-2721-6
		59/F	Death - aspiration pneumonia[b]	WR-2721-1
		42/M	Death - sepsis	WR-2721-5
		65/M	Transient oliguric	WR-2721-7
		75/F	Mitomycin pneumonitis	WR-2721-2
		54/M	Mitomycin pneumonitis	WR-2721-2
		52/F	Hearing loss	WR-2721-11
		31/F	Carpo-pedal spasm and positive Chvostek's sign	WR-2721-16
		63/F	Acute tubular necrosis	WR-2721-1
		63/F	Hypothermia	WR-2721-A504
		67/M	Loss of consciousness	WR-2721-18
		54/F	Loss of consciousness	WR-2721-1
		56/F	Myalgia, Paraspinal pain	WR-2721-032
		27/F	Tingling, burning pain in extremities	WR-2721-045
		47/M	Death - Respiratory obstruction	WR-2721-A502

[a] Date filed; Amendment number not assigned
[b] Occurred 4 months after last dose of amifostine

US BIOSCIENCE

Christine Smith

Director, Regulatory Affairs

AUG 0 3 1994

Dr. Gregory Burke, Director
Division of Oncology and Pulmonary
Drug Products (HFD-150)
FOOD AND DRUG ADMINISTRATION
Parklawn Building
5600 Fishers Lane
Rockville, MD 20857

RE: IND #31,157; ETHYOL® FOR INJECTION
IND SAFETY REPORT
SERIAL NO. 182

Dear Dr. Burke:

Reference is made to U.S. Bioscience protocol WR-0032: A Phase II/III Randomized Study of Cisplatin and Vinblastine Plus or Minus Ethyol in Patients With Stage IV Non-Small Cell Lung Cancer, which was submitted to the Division on April 14, 1993. Provided here is an IND Safety Report submitted in accordance with 21 CFR § 312.32 for a patient who participated in the above cited study.

This report describes patient a 56-year old female diagnosed with Stage IV lung cancer, who experienced involuntary tremors and rigors and was admitted to the hospital with severe myalgia and "paraspinal" pain after receiving the third cycle of the protocol regimen. Attached is a description of this adverse event based on our current information. The investigator's judgement was that this event was possibly related to Ethyol. Any additional information that becomes available will be submitted as a follow-up report. All participating investigators are being informed of this event.

The submission of this information is without prejudice and does not constitute an acknowledgement of the validity of the information contained herein or an admission that the drug caused or contributed to an adverse event.

Thank you for your attention.

Sincerely,

Christine Smith

Christine Smith

Enclosures

cc: Dr. R. Capizzi
Dr. E. Mitchell
Dr. W. Oster
Dr. R. Reynolds
Ms. B. Scheffler

[illegible]

IND SAFETY REPORT
ETHYOL IND

Description of Episode:

This patient is a 56-year old female diagnosed with Stage IV lung cancer. On July 7, 1994, three minutes following the third cycle of Ethyol (910 mg/m^2), cisplatin (60 mg/m^2) and vinblastine (5 mg/m^2), the patient experienced involuntary tremors and rigors which lasted for approximately nine minutes and resolved without treatment. Three minutes later a second episode occurred lasting approximately four minutes, also resolving without further treatment. Three days following these episodes (7/10/94) the patient was evaluated for severe myalgia and was hospitalized the following day (four days after Ethyol administration) to evaluate diffuse muscle aches, in particular "paraspinal" pain in thoracic region, as well as to receive IV hydration and antiemetics. Additionally, during this time, the patient was receiving Diflucan for the treatment of oral thrush. The patient was discharged on 7/12/94 and was feeling "somewhat better."

Medical Evaluation of Episode:

The investigator felt that this adverse event was not likely related to disease, and was possibly related to the Ethyol treatment. The patient is continuing her treatment with Ethyol and follow-up information will be available with regard to the above referenced experience.

U.S. Bioscience will notify investigators of this event and will continue to monitor any additional information that may be obtained concerning this episode.

Previous IND Safety Reports of Similar Adverse Experiences:

Reference is made to the IND Safety Report, which was submitted to the Division on August 4, 1988, describing a 45-year old female (patient who experienced tremors/shaking while participating in Ethyol protocol WR-1. This patient recovered from the incident and continued her Ethyol treatment. She showed tumor response and her overall tolerance of the regimen was good.

IND SAFETY REPORT
ETHYOL IND

Description of Episode:

This patient is a 27-year old female diagnosed with breast cancer who experienced an adverse event similar to Raynaud's syndrome. The patient experienced tingling and burning in her fingers, toes and heals, with a "purplish-black tone" to her skin color and pain with the intensity described as "10 out of 10 when touching something." On December 30, 1994 , the patient received amifostine (910 mg/m^2), followed by doxorubicin (75 mg/m^2, i.v.) and cyclophosphamide (600 mg/m^2). The patient also received antiemetics, Vistaril (25 mg, p.o.) on this date and Zofran (8mg q6h, p.o.) on December 31, 1994. On January 6, 1995, the patient received G-CSF (300 mg q.d.) and on January 8, 1995, Floxin (400 mg b.i.d.). On January 13, 1995 the patient began complaining of tingling and burning of her fingers, toes and heels. The condition worsened to pain of "10 out of 10 when touching something" on January 30, 1995. The patient also stated she had a "blackish-purplish hue or tone" to her skin color. Her extremities remained warm. On February 8, 1995 the patient stated her condition remained similar to her report on January 30, 1995, however she then began to experience mild blistering on her feet for 5 days and her hands were looking "blacker" for at least one day. The patient stated her oncologist in Phoenix did not know the cause (contacted on February 8, 1995). She will be evaluated on February 10, 1995 by Dr. Stopeck. The information from Dr. Stopeck's evaluation is pending.

Medical Evaluation of Episode:

The investigator felt that this adverse event was not likely related to disease, and was possibly related to the amifostine treatment. When further information regarding to the above referenced experience becomes available a follow-up report will be completed.

Previous IND Safety Reports of Similar Adverse Experiences:

This is the first IND safety report on the above referenced adverse event. Raynaud's phenomenon is characterized by transient episodes of vasoconstriction accompanied by changes in color of the affected digits. Exposure to cold or emotional stress are common precipitating factors. Raynaud's phenomenon has been observed following bleomycin, alone and in combination with vinca alkaloids and cisplatin. Raynaud's phenomenon has also been observed in women after augmentation mammoplasty with silicone implants. Raynaud's phenomenon has not been observed in breast cancer patients receiving doxorubicin and cyclophosphamide. Further, there are no reports in the literature associating amifostine with Raynaud's phenomenon.

It should be noted that in May, 1992, the patient had a mastectomy, and sometime there after, received silicone implants. It is not known whether there is any connection between her implants and the presentation of Raynaud's phenomenon.

Medical Summary for Patient (Protocol 0230)

This is a report of a 27-year-old female patient with breast cancer who experienced an adverse event similar to Raynaud's phenomenon following a treatment regimen consisting of Ethyol, doxorubicin and cyclophosphamide. To date, there is no purported connection between Raynaud's phenomenon and Ethyol, doxorubicin or cyclophosphamide (alone or in combination). On December 30, 1994, the patient received Ethyol (910 mg/m^2, i.v.) followed by doxorubicin (75 mg/m^2, i.v.) and cyclophosphamide (600 mg/m^2, i.v.). The patient also received the antiemetics, Vistaril (25 mg, p.o.) on this date and Zofran (8 mg q6h, p.o.) on December 31, 1994. On January 6, 1995, the patient received G-CSF (300 mg q.d.) and on January 8, 1995, the oral antibiotic, Floxin (400 mg b.i.d.). On January 13, 1995, she noted tingling and burning of fingers, toes and heels. The condition worsened to pain of "10 out of 10" when touching something (per patient on January 30, 1995). The patient also stated that she now has a "blackish-purplish hue or tone" to her skin color. Her extremities remain warm. On February 8, 1995, the patient stated the condition remained similar to her report on January 30, 1995; however, she now had mild blistering on her feet for at least 5 days and her hands were "looking blacker" for at least 1 day. The patient states her oncologist in Phoenix did not know the cause (contacted on February 8, 1995). The patient will be evaluated by Dr. Stopack on February 10, 1995.

Raynaud's phenomenon is characterized by transient episodes of vasoconstriction accompanied by changes in color of the affected digits. Exposure to cold or emotional stress are common precipitating factors. Raynaud's phenomenon has been observed following bleomycin, alone and in combination with vinca alkaloids and cisplatin (Doll and Yarbro, *Semin Oncol* 19:580-596, 1992). Raynaud's phenomenon has also been observed in women after augmentation mammoplasty with silicone implants (Varga, et al. *Ann Intern Med* 111:377-383, 1989; *Lappe, Medical Hypotheses* 41:348-352, 1993). Raynaud's phenomenon has not been observed in breast cancer patients receiving doxorubicin and cyclophosphamide. Further, there are no reports in the literature (Medline and Embase databases) associating Ethyol (amifostine) with Raynaud's phenomenon.

It is interesting to note that in May, 1992, the above patient had a mastectomy, and sometime after, had received silicone implants. It is not known whether there is any connection between her implants and the presentation of Raynaud's phenomenon.

Robert L Capizzi, MD
2/14/95

Christine Smith

Director, Regulatory Affairs

JUL 1 0 1995

Division of Oncology and Pulmonary Drug Products (HFD-150)
FOOD AND DRUG ADMINISTRATION
1451 Rockville Pike; Woodmont II
Rockville, MD 20852

RE: IND ETHYOL® FOR INJECTION
IND SAFETY REPORT
SERIAL NO. 226

Dear Sir or Madam:

Reference is made to U.S. Bioscience protocol WR-A502: "*A Phase I/II Dose Escalation Study of Cisplatin with or without Ethyol (Amifostine) in Patients with Head and Neck Cancer*", which was submitted to the Division on October 19, 1993 Provided here is an IND Safety Report submitted in accordance with 21 CFR § 312.32 for a patient who participated in the above cited study.

This report describes patient a 47-year old male diagnosed with cancer of the right tonsil and who was to be treated with amifostine (740 mg/m^2) and cisplatin (70 mg/m^2) weekly for 6 weeks. Immediately following the second weekly administration of amifostine, the patient experienced headache, dizziness, and coughing. The patient became unconscious and died shortly thereafter.

Additional information has been requested along with the results of a post mortem evaluation. This will be submitted to the agency as soon as they are available. This is the first incidence of a death following treatment with amifostine.

The submission of this information is without prejudice and does not constitute an acknowledgement of the validity of the information contained herein or an admission that the drug caused or contributed to an adverse event.

Thank you for your attention.

Sincerely,

Christine Smith

Christine Smith

cc: Dr. R. Capizzi
Dr. M. Kurman
Dr. W. Oster
Ms. B. Scheffler

\regiwramends\227.WR

One Tower Bridge
100 Front Street • West Conshohocken, PA 19428

US BIOSCIENCE

cc: Desk Copy
Dr. R. DeLap

Christine Smith

Director, Regulatory Affairs

August 1, 1995

Division of Oncology and Pulmonary Drug Products
FOOD AND DRUG ADMINISTRATION HFD-150
1451 Rockville Pike
Rockville, MD 20852

RE: IND ETHYOL ® FOR INJECTION
IND SAFETY REPORT: FOLLOW-UP TO A WRITTEN REPORT
SERIAL NO. 229

Dear Sir or Madam:

Reference is made to U.S. Bioscience protocol WR-A5C2: "*A Phase I/II Dose Escalation Study of Cisplatin with or without Ethyol (amifostine) in Patients with Head and Neck Cancer*", which was submitted to the Division on October 19, 1993 (Serial No. 167). Reference is also made to our submission dated July 10, 1995 (Serial No. 226) which provided initial IND Safety Report information on the death of a patient participating in the above mentioned study.

Provided here is follow-up information for Patient who died after receiving the second weekly administration of amifostine. This patient was a 47 year old male with recurrent, extensive squamous cell carcinoma of the tonsil and palpable bilateral lymph node metastases which caused intermittent respiratory obstruction. Discussion with the investigator and post mortem results indicate that the direct cause of death was asphyxia due to mechanical obstruction by the tumor and the resulting secretions. There were no reports of emesis during this event. The contribution of Ethyol-related hypotension in possibly worsening the patient's condition cannot be excluded. The Do Not Resuscitate instructions of the patient also influenced the outcome. The attached documentation provides additional detail. Additional information relating to this patient will be provided to the agency as it becomes available.

The submission of this information is without prejudice and does not constitute an acknowledgement of the validity of the information contained herein or an admission that the drug caused or contributed to an adverse event.

Thank you for your attention.

Sincerely,

Christine Smith

Christine Smith

cc: Dr. R. Capizzi
Dr. M. Kurman
Ms. B. Scheffler

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IND Safety Report

Ethyol (amifostine) IND No.

1. **Description of Episode**

Patient _____

The patient was a 47 year old male with recurrent, extensive squamous cell carcinoma of the tonsil (primary tumor mass was 5 cm in diameter) and palpable bilateral jugular lymph node metastases. The large tumor masses and oral secretions caused intermittent respiratory obstruction for which the patient would initiate suctioning. The patient initiated protocol therapy with Ethyol and cisplatin on June 27, 1995. Performance status prior to study entry was 2.

Antiemetic pretreatment with dexamethasone and Zofran were administered per protocol. The first infusion of Ethyol administered (740 mg/m^2) resulted in hypotension, the lowest value being 70/50. The infusion was interrupted after 5 minutes and the blood pressure recovered to threshold levels after 12 minutes. The infusion was not re-started. The patient went on to receive his full cisplatin dose as scheduled.

Because of the hypotension seen during the first administration of Ethyol, the second infusion, administered on July 4, 1995, should have been at a reduced dose per protocol requirements. However, there was a protocol violation, the dose reduction was overlooked, and the patient was programmed to again receive the 740 mg/m^2 dose.

On July 4, 1995, antiemetic pretreatment with dexamethasone and Zofran was administered per protocol. Nine minutes into the 15 minute Ethyol infusion, the patient developed hypotension (70/50), and the infusion was stopped. At this same time, the patient started having difficulty in breathing and the patient produced a lot of secretion which he found difficult to remove. The patient complained of headache and dizziness and lost consciousness. The patient was suctioned and became semi-conscious, at which time the pulse was palpated at 60 beats per minute. The patient expired a few minutes later. No fluids were administered. The patient had requested not to be resuscitated at admission to the study.

2. **Medical Evaluation of Episode**

The direct cause of death is reported as asphyxia due to mechanical obstruction by the tumor and the resulting secretions. The post mortem findings revealed a large tumor mass and lymphadenopathy as well as pulmonary oedema which is consistent with the reported cause of death. There were no reports of emesis during this event or evidence of aspiration pneumonia.

This event appears to be directly related to the respiratory obstruction from the tumor mass and secretions. The contribution of the hypotension in possibly worsening the patient's condition cannot be excluded. The Do Not Resuscitate instructions of the patient also influenced the outcome.

3. **Previous IND Safety Reports Related to this Episode**

This is the first report of a death following treatment with amifostine.

END

DD

J.H.M. Research & Development, Inc., 5776 Second Street, N.E., Washington, D.C. 20011