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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-212

MAY 26 1995

Pharmacia Inc.
P.O. Box 16529
Columbus, OH 43216-6529

Attention: Frederick Grabb, Ph.D.
Director, Regulatory Affairs

Dear Dr. Grabb:

Please refer to your December 7, 1992 new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Zinecard (dexrazoxane for injection).

We also acknowledge receipt of your resubmission dated August 2, 1994 and the following amendments:

1994 August 22 and 25
September 22 and 27
October 17
December 8 and 29

1995 January 6
February 2 and 3
March 14, 17, 21, 23, and 27
April 12
May 22

This application provides for the prevention of cardiomyopathy associated with doxorubicin administration.

We have completed our review of this application under the policies and procedures set forth under Subpart H, Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses, of 21 CFR Part 314.500. We have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the draft package insert labeling submitted on May 22, 1995 and the final draft carton and container labeling dated April 12, 1995. Accordingly, this application is approved effective as of the date of this letter.

The approval and subsequent marketing of this drug product and related activities are to be in accordance with the substance and procedures reflected in the Accelerated Approval Regulations referenced above.

AP Letter

Products approved under Accelerated Approval Regulations (section 314.510) require further adequate and well-controlled studies to verify and describe clinical benefit. The proposed phase 4 clinical study number

could provide such verification. Clinical benefit could be demonstrated if participants randomized to Zinecard™ had longer time to progression or survival. We acknowledge and concur with your Accelerated Approval postmarketing commitments outlined in your March 24, 1995 letter. Your commitments are as follows:

1. Within six months after the scheduled completion of the study, defined as one year after the end of randomization of the last patient, you will provide the FDA with a study report analyzing at least the major endpoints for effectiveness (time to progression and survival), and safety, along with corresponding data sets as specified by the medical and statistical reviewers. In advance of the completion of this study, you will seek FDA agreement on the specific efficacy and safety analyses to be conducted.

2. Quarterly updates on the progress of the study will be submitted to the FDA that include total number of deaths, patients lost to follow-up, and number of patients discontinuing study medication, as well as the number of any other patients leaving the study and the reasons for leaving.

3. Major amendments to the study design will be submitted to, and discussed with, the FDA prior to enactment.

We also acknowledge your February 15, 1995 letter which commits Pharmacia to steps to resolve several biopharmaceutical concerns; you have agreed to:

1. Characterize the pharmacokinetics of the metabolites of dexrazoxane as well as the relationship between levels of these components in systemic circulation or locally in tissues and the cardioprotective effect of the drug.
2. Evaluate the pharmacokinetics of dexrazoxane in patients with hepatic and renal insufficiency.
3. If adequate information exists, analyze pharmacokinetic data in both genders and submit the results, or design appropriate experiments to assess the pharmacokinetics of dexrazoxane in both males and females.

Further, we acknowledge your February 15, 1995 commitment to address the following microbiology concerns with due diligence:

1. Filters of construction shall not be used for sterile filtration of product until bacterial retention validation data have been submitted and reviewed.
2. Products used to support the should be specifically identified and submitted with complete data packages in order that an adequate review can be completed. However, it would be preferable to submit data on actual bacterial retention validation testing completed by suspending bacteria in the drug product(if possible) and mimicking, to the degree possible, all other process parameters.

The final printed labeling (FPL) must be identical to the enclosed marked up draft labeling. Marketing the product before making, exactly as agreed to, the revisions in the drug product's labeling may render the product misbranded and an unapproved new drug.

Please submit 15 copies of the FPL as soon as available. Seven of the copies should be individually mounted on heavy-weight paper or similar material. For administrative purposes, this FPL submission should be designated " FINAL PRINTED LABELING for approved NDA 20-212". FDA approval of this FPL is not required before it is used.

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

LBL

We acknowledge receipt of your March 28, 1995 initial promotional material which is under review by Drug Marketing, Advertising, and Communications (HFD-240).

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

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.81. To comply with these regulations, all 3-day and 15-day alert reports, periodic adverse experience reports, field alerts, annual reports, supplements, and other submissions should be addressed to NDA 20-212. This includes the quarterly periodic adverse drug experience reports required by this NDA.

Should you have any questions concerning this application, please contact Ms. Maureen Pelosi, Consumer Safety Officer, at 301-594-5768.

Sincerely yours,

Robert Temple

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

Enclosure

NDA 20-212

JUL 13 1992

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Adria Laboratories
P.O. Box 16529
Columbus, Ohio 43216-6529

Attention: Douglas R. Jones
Director, Regulatory Affairs, New Drugs

Dear Mr. Jones:

Please refer to your February 7, 1992 new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Zinecard (dexrazoxane for injection).

We also acknowledge receipt of your amendments dated March 2, 23, April 3, 9, 16, 24, May 1, 8, 11, 12, 14, 22, June 1, 2, 4, 5, 8, 12 and 16, 1992.

We have completed our review of the clinical, pharmacology/ toxicology and primary chemical sections of your NDA and find the information presented is inadequate and the application is not approvable. The deficiencies may be summarized as follows.

CLINICAL

1. There is evidence that dexrazoxane protection is not selective and that it decreases the antitumor effect of doxorubicin. We recommend a meeting with the Agency to discuss plans for the current and future trials.

PHARMACOLOGY

2. The mouse BL/BX7 human mammary tumor study should be repeated to explain the more rapid increase in tumor growth observed in the BL/BX7 xenograft after drug treatment was stopped. Tumor drug levels in this study may also be important in interpreting the results of this study.

CHEMISTRY

3. The melting range for dexrazoxane, claimed on page 03-00002 of the NDA, is not supported by test results for the Reference Standard. State a narrower melting range, based on test data for a Reference Standard of high purity.

NDA letter

4. Submit a full description of the experiments performed to explore whether the drug substance exhibits various crystalline forms (polymorphs), amorphous forms, or solvates. Define the conditions under which phase transformations may occur and characterize the various solid forms.

5. The quality of the IR spectrum for Dexrazoxane Reference Standard Batch No. 89C34A is unacceptable. Provide a larger spectrum, that has adequate contrast and legibility, for structural proof.

6. Submit a full description of the studies establishing the stereochemical configuration and enantiomeric purity of the drug substance.

7. State the proposed batch size ranges for the synthesis of the drug substance at the nd at the ilities.

8. Submit comparative test data for several representative batches of Intermediate II which were prepared at the two different manufacturing facilities.

9. Provide the following information for several typical dexrazoxane drug substance batches.

   a. Individual Related Substance levels including test data for Lots 89C34A and 89C52A and earlier clinical lots with relatively high Related Substance levels.

   b. Actual levels.

   c. Residual levels.

   d. Residual levels.

   e. Moisture (Karl Fisher) levels.

   f. An explanation of which test method can be used for the quantitative assay of Related Substance III.

10. Submit the following information pertaining to Intermediate II.

    a. The specific rotation for pure Intermediate II.

    b. Tighter Specific Rotation specifications for bulk Intermediate II.

    c. Has a stereospecific Assay method been explored for this compound?

    d. To prevent impurity profile variations between drug substance batches, establish narrower Impurity and Potency specifications for the quality control of Intermediate II, which may be sourced by two different manufacturers. A Potency limit without specifications for individual Impurity types and impurity limits is unacceptable.

11. The Dexrazoxane Reference Standard's specifications for Related Substances should be revised by establishing limits for individual Related Compounds.

12. The following comments pertain to HPLC Method

    a. System Suitability should be tested according to USP XXII, <621>, Chromatography. Demonstrate the reproducibility of replicate injections, specify the Relative Standard Deviation and limit peak asymmetry by specifying the Tailing Factor.

    b. Provide an explanation for the use of a

    to prepare the Dexrazoxane Standard Solution and test sample solution. The mobile phase contains only although the drug substance has adequate water solubility (12mg/mL). What is the function of the high level?

    c. The ruggedness of the method is unsatisfactory. The retention time for dexrazoxane varies from in the submitted chromatograms.

d. The submitted methods validation data are inadequate to demonstrate that this method is suitable for the quantitative assay of Related Substances in the test sample for the following reasons.

(1) A stock solution of "a mixture of the related substances in " was used in the validation study. These were, however, not fully characterized standards. The identity, source, proof of structure and molar ratio in the stock solution of the individual related substances were not provided.

(2) Peak positions, peak separation and reproducibility under working conditions to allow the identification and quantitation of individual Related Compounds were not demonstrated.

(3) No adequate chromatograms were provided in support of the claimed 0.2% limit of detection for Related Substances I and II.

13. Provide methods validation for the Assay, Submit the source and acceptance specifications for the Standard and the limit of detection for the test method.

14. Provide methods validation for the Determination of Residual Solvents,

15. The following comments pertain to the regulatory specifications and release testing of the drug substance.

a. Identify the facility responsible for the release testing of the drug substance and establish a mandatory re-test period.

b. Revise the regulatory specifications for the drug substance as follows.

(1) Tighten the Melting Range.

(2) Narrow the limits for Residue on Ignition and Loss on Drying, based on the test data for representative drug substance batches.

(3) Establish limits for Residual and Residual , instead of the proposed Total Residual Solvents specification.

(4) Establish a Moisture (Karl Fisher) limit.

(5) Revise the specifications for Related Substances. Establish appropriate limits for Individual Related Compounds, based on the release and stability data for representative batches of the bulk drug.

(6) Explore the use of a stereospecific Assay method (e.g., chiral HPLC).

16. Provide the following information for each component of the container/closure system used to store and transport the drug.

a. The source, full chemical composition and reference to relevant sections of 21 CFR. Alternatively, permission to review the appropriate Drug Master File may be provided.

b. Acceptance specifications, Sampling Plan and USP test data.

c. The name and address of the facility responsible for the quality control of all container components.

17. The following comments pertain to the stability studies for the drug substance.

a. The test data are inadequate to support the proposed 24-month expiration dating period with controlled room temperature storage.

b. Submit Individual Related Compound stability data for several representative drug substance batches in support of the Individual Related Compound limits for the bulk drug.

c. Conduct additional stress studies to determine the stability profile of dexrazoxane. Investigate the effects of elevated temperature and moisture, acidic and alkaline pH and high oxygen atmosphere. Detect, identify and quantitate degradation products and establish the reaction kinetics, if practicable.

18. The following comments pertain to the manufacture of the drug product.

    a. The drug product should be formulated to 100% target potency of the label claim. The use of the 2% overage is not justified.

    b. Define the commercial batch size range for the manufacture of Zinecard, 250 mg and 500 mg vials.

    c. Investigate the feasibility of the sterilization of Zinecard and submit the test data to the FDA.

    d. Provide the in-process controls for the of the stoppers.

19. Provide the following information pertaining to the In-Process Controls for the manufacture of Zinecard.

    a. The limits for Density, Potency and Bioload for the bulk solution prior to filling and the Sampling Plan for this control operation.

    b. The Fill Volume limits for the 250 mg vial and for the 500 mg vial.

    c. The Sampling Plan for the filling operation; i.e., the frequency of testing and the number of samples taken for production-size batches.

20. The following comments pertain to the regulatory specifications and tests for Zinecard.

    a. Describe the detailed Sampling Plan for production batches.

    b. Provide an Identity Test that is specific to the drug substance. Identification based on the HPLC retention time alone is inadequate.

    c. Develop a stereospecific Assay method.

    d. Provide a full description of the Sterility Test.

e. Provide a full description of the Test, its validation and justification for the proposed EU/mg limit.

f. Provide a full description of the test method for Water Content. In addition, revise the proposed limit for Water. This specification is unacceptable, as only levels were detected in the stability batches.

g. Revise the specifications for Related Substances. Instead of the proposed limit for Total Related Substances, establish appropriate limits for Individual Related Substances based on the release and stability data for representative drug product batches.

h. Provide specifications and an appropriate test method for Heavy Metals.

i. The proposed limit for the Reconstituted Solution Color is unacceptably broad based on the test data in the NDA. Revise the specification, based on data for typical Zinecard batches.

21. The following comments pertain to the primary stability studies for Zinecard.

a. State which samples were stored with and which were stored without the carton.

b. Conduct Particulate Matter testing.

c. Report the Individual Related Compound levels.

d. Submit updated stability data.

e. Explain the sample-to-sample variation in Related Compound levels, within the same lot. For instance, on p. 03-00501, Related Compound levels of 1.3%, 1.8%, 2.4% and 1.4% were reported for Lot #90A12FY, at the 6, 9, 12 and 18 month time stations.

22. The Stability Protocols for commercial batches of Zinecard should be revised in the following manner.

    a. The test samples should be stored at 30°C, the upper limit of the controlled room temperature range.

    b. In addition to Total Related Substance levels, the levels of Individual Related Substances should be monitored.

    c. Testing for Particulates, Stereochemical Purity and Bacterial Endotoxins should be included in the protocols.

    d. After the first three commercial batches, the number of commercial batches in stability testing should depend on the total number of batches produced that year.

    e. The commercial batches of Zinecard should be tested at shorter intervals than proposed in the protocol shown in Table 7.20, namely at the 0, 3, 6, 9, 12, 18, 24, 36 and 48-month time-stations.

23. Have any studies been conducted investigating the compatibility of the drug product with the Sodium DL-Lactate diluent (e.g., diastereomeric complex formation)?

24. The compatibility of the drug product with various metals in the infusion sets (e.g., aluminum versus stainless steel needles) should be studied.

25. Additional test data are necessary to support the stability claims for reconstituted and further diluted solutions of Zinecard. Specifically, the following information is needed:

    a. Test data for several representative Zinecard batches of each strength, demonstrating physical, chemical and microbiological stability after reconstitution and storage as advised in the labeling. The drug product and diluent batches used in these studies should be identified fully (date of manufacture, batch size, age at time of study, etc.). Aged drug and diluent samples should also be used in these studies. The reconstituted solutions of

Zinecard should be stored 30°C and 2°C. The solutions should be shown to meet the full regulatory specifications for the drug product (Potency, Related Compounds, pH, Particulate Matter and Sterility).

b. Corresponding test data for reconstituted and further diluted Zinecard solutions, after storage in IV infusion bags at 30°C and 2°C. The type (i.e., source and chemical composition) of the IV bags used in these studies should be stated.

26. The following comments pertain to the manufacture of the Sodium Lactate Injection, 25 mL and 50 mL vials.

a. Define the commercial batch size range.

b. The composition statement and Batch Formula should be revised, stating the use of Sodium DL-Lactate Solution USP.

c. Adria's regulatory specifications for Sodium DL-Lactate Solution USP should include a test to establish that it is racemic (e.g., optical rotation).

d. State which tests are performed on each batch of Sodium DL-Lactate Solution, USP, Sodium Hydroxide, NF, Hydrochloric Acid, NF, Water for Injection, USP and Nitrogen, NF by ADRIA-SP Inc., and which tests may be accepted based on the manufacturer's Certificate of Analysis.

e. Provide a full description of the Sampling Plan (e.g., Mil. Std. 105D) for the production batches.

27. State who is responsible for performing the USP tests to ascertain the Type I glass status of the vials provided by for the container system for the Sodium Lactate Diluent.

28. Justify the use of

29. The specifications for the Sodium Lactate Injection Diluent should be revised to establish that it contains a racemate.

30. The following comments pertain to the stability data and Stability Protocols for the Sodium Lactate Diluent.

    a. The 9-month stability data are inadequate to support a 24-month expiration dating period. Submit longer-term test data on the stability batches, including Particulate Matter data at 12, 18 and 24 months and Sterility data at 12 and 24 months.

    b. The test samples should be stored at 30°C, the upper limit of the controlled room temperature range.

    c. After the first three commercial batches, the number of commercial batches in stability testing should depend on the total number of batches produced that year.

    d. Testing of all commercial batches should be conducted at the 0, 3, 6, 9, 12, 18 and 24-month test periods.

31. The following comments pertain to the vial label and carton.

    a. Revise the vial label and carton for ZINECARD to state "Each vial contains dexrazoxane hydrochloride equivalent to 250 mg (or 500 mg) dexrazoxane".

    b. The established name should have the required prominence and conspicuousness.

    c. The pH should appear on the vial label and carton.

    d. Include the "pyrogen-free" statement into the vial label and carton.

32. The vial label for the diluent should be revised, expressing the concentration as 0.167 Molar instead of 1/6 Molar (M/6).

33. The following comments pertain to the package insert.

a. Remove the "FOR INTRAVENOUS USE ONLY" statement from line 4 of the package insert. Include the route of administration into the DESCRIPTION section.

b. Revise the DESCRIPTION section to contain the following information as required by 21 CFR 201.57:

(1) The proprietary name and established name of the drug;
(2) the type of dosage form and route of administration;
(3) qualitative and quantitative ingredient information required under 201.100(b);
(4) statement of sterility and apyrogenicity;
(5) pharmacological or therapeutic class;
(6) chemical name and structural formula. In addition, revise the chemical name of the drug by including a hyphen after bis and removing the brackets; and
(7) other important chemical and physical information (physical constants, pH).

c. The DOSAGE AND ADMINISTRATION section should contain information pertaining to the dilution of reconstituted Zinecard solutions.

d. The HOW SUPPLIED section should include the following information:

(1) Statements that the drug product is sterile and pyrogen-free; and
(2) information to facilitate identification of the 250 mg and 500 mg vials of the drug product.

We reserve further comment on the labeling until the application is otherwise approvable.

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 314.120. In the absence of any such action, FDA may withdraw the application.

Should you have any questions, please contact Ellen Cutler at (301) 443-5197.

Sincerely yours,

Gregory Burke, M.D., Ph.D.
Director
Division of Oncology and
Pulmonary Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Pharmacia

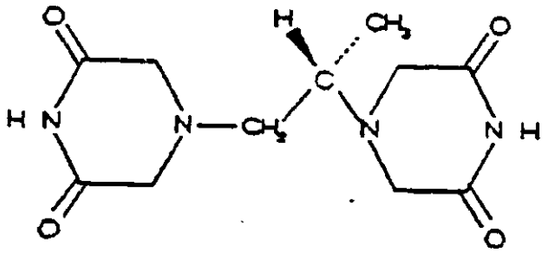
ZINECARD™

(dexrazoxane for injection)

DESCRIPTION

ZINECARD ™ (dexrazoxane for injection) is a sterile, pyrogen-free lyophilizate intended for intravenous administration. It is a cardioprotective agent for use in conjunction with doxorubicin.

Chemically, dexrazoxane is (S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione. The structural formula is as follows:



$C_{11}H_{16}N_4O_4$ M.W. 268.28

Dexrazoxane, a potent intracellular chelating agent is a derivative of EDTA. Dexrazoxane is a whitish crystalline powder which melts at 191° to 197°C, it is sparingly soluble in water and 0.1 N HCl, slightly soluble in ethanol and methanol and practically insoluble in nonpolar organic solvents. The pK_a is 2.1.

Dexrazoxane has an octanol/water partition coefficient of 0.025 and degrades rapidly above a pH of 7.0.

ZINECARD is available in 250 mg and 500 mg single use only vials.

Each **250 mg vial** contains dexrazoxane hydrochloride equivalent to 250 mg dexrazoxane. Hydrochloric Acid, NF is added for pH adjustment. When reconstituted as directed with the 25 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, USP diluent provided, each mL contains: 10 mg dexrazoxane. The pH of the resultant solution is 3.5 to 5.5.

Each **500 mg vial** contains dexrazoxane hydrochloride equivalent to 500 mg dexrazoxane. Hydrochloric Acid, NF is added for pH adjustment. When reconstituted as directed with the 50 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, USP diluent provided, each mL contains: 10 mg dexrazoxane. The pH of the resultant solution is 3.5 to 5.5.

CLINICAL PHARMACOLOGY

Mechanism of Action: The mechanism by which ZINECARD exerts its cardioprotective activity is not fully understood. Dexrazoxane is a cyclic derivative of EDTA that readily penetrates cell membranes. Results of laboratory studies suggest that dexrazoxane is converted intracellularly to a ring-opened chelating agent that interferes with iron-mediated free radical generation thought to be responsible, in part, for anthracycline-induced cardiomyopathy.

Pharmacokinetics: The pharmacokinetics of dexrazoxane have been studied in advanced cancer patients with normal renal and hepatic function. Generally, the pharmacokinetics of dexrazoxane can be adequately described by a two-compartment open model with first-order elimination. Dexrazoxane has been administered as a 15 minute infusion over a dose-range of 60 to 900 mg/m^2 with 60 mg/m^2 of doxorubicin, and at a fixed dose of 500 mg/m^2 with 50 mg/m^2 doxorubicin. The disposition kinetics of dexrazoxane are dose-independent, as shown by linear relationship between the area under plasma concentration-time curves and administered doses ranging from 60 to 900 mg/m^2. The mean peak plasma concentration of dexrazoxane was 36.5 μg/mL at the end of the 15 minute

infusion of a 500 mg/m^2 dose of Zinecard administered 15 to 30 minutes prior to the 50 mg/m^2 doxorubicin dose. The important pharmacokinetic parameters of dexrazoxane are summarized in the following table.

SUMMARY OF MEAN (%CV[a]) DEXRAZOXANE PHARMACOKINETIC PARAMETERS AT A DOSAGE RATIO OF 10:1 OF ZINECARD: DOXORUBICIN

Dose Doxorubicin (mg/m^2)	Dose Zinecard (mg/m^2)	Number of Subjects	Elimination Half-Life (h)	Plasma Clearance (L/h/m^2)	Renal Clearance (L/h/m^2)	[b]Volume of Distribution (L/m^2)
50	500	10	2.5 (16)	7.88 (18)	3.35 (36)	22.4 (22)
60	600	5	2.1 (29)	6.25 (31)	—	22.0 (55)

[a] Coefficient of variation

[b] Steady-state volume of distribution

Following a rapid distributive phase (~ 0.2 to 0.3 hours), dexrazoxane reaches post-distributive equilibrium within two to four hours. The estimated steady-state volume of distribution of dexrazoxane suggests its distribution primarily in the total body water (25 L/m^2). The mean systemic clearance and steady-state volume of distribution of dexrazoxane in two Asian female patients at 500 mg/m^2 dexrazoxane along with 50 mg/m^2 doxorubicin were 15.15 L/h/m^2 and 36.27 L/m^2, respectively, but their elimination half-life and renal clearance of dexrazoxane were similar to those of the ten Caucasian patients from the same study. Qualitative metabolism studies with Zinecard have confirmed the presence of unchanged drug, a diacid-diamide cleavage product, and two monoacid-monoamide ring products in the urine of animals and man. The metabolite levels were not measured in the pharmacokinetic studies.

Urinary excretion plays an important role in the elimination of dexrazoxane. Forty-two percent of the 500 mg/m^2 dose of Zinecard was excreted in the urine.

Protein Binding: *In vitro* studies have shown that Zinecard is not bound to plasma proteins.

Special Populations: The pharmacokinetics of Zinecard have not been evaluated in pediatric populations and in hepatic or renal insufficiency patients.

Drug Interactions: There was no significant change in the pharmacokinetics of doxorubicin (50 mg/m^2) and its predominant metabolite, doxorubicinol, in the presence of dexrazoxane (500 mg/m^2) in a crossover study in cancer patients.

Clinical Studies: The ability of ZINECARD to prevent/reduce the incidence and severity of doxorubicin-induced cardiomyopathy was demonstrated in three prospectively randomized placebo-controlled studies. In these studies, patients were treated with a doxorubicin-containing regimen and either ZINECARD or placebo starting with the first course of chemotherapy. There was no restriction on the cumulative dose of doxorubicin. Cardiac function was assessed by measurement of the left ventricular ejection fraction (LVEF), utilizing resting multigated nuclear medicine (MUGA) scans, and by clinical evaluations. Patients receiving ZINECARD had significantly smaller mean decreases from baseline in LVEF and lower incidences of congestive heart failure than the control group. The difference in decline from baseline in LVEF was evident beginning with a cumulative doxorubicin dose of 150 mg/m^2 and reached statistical significance in patients who received $\geq$400 mg/m^2 of doxorubicin. In addition to evaluating the effect of ZINECARD on cardiac function, the studies also assessed the effect of the addition of ZINECARD on the antitumor efficacy of the chemotherapy regimens. In one study (the largest of three breast cancer studies) patients with advanced breast cancer receiving fluorouracil, doxorubicin and

cyclophosphamide (FAC) with ZINECARD had a lower response rate (48% vs 63%; p=0.007) and a shorter time to progression than patients who received FAC + placebo, although the survival of patients who did or did not receive ZINECARD with FAC was similar.

Two of the randomized breast cancer studies evaluating the efficacy and safety of FAC with either ZINECARD or placebo were amended to allow patients on the placebo arm who had attained a cumulative dose of doxorubicin of 300 mg/m^2 (six courses of FAC) to receive FAC with open-label ZINECARD for each subsequent course. This change in design allowed examination of whether there was a cardioprotective effect of Zinecard even when it was started after substantial exposure to doxorubicin.

Retrospective historical analyses were then performed to compare the likelihood of heart failure in patients to whom ZINECARD was added to the FAC regimen after they had received six (6) courses of FAC (and who then continued treatment with FAC therapy) with the heart failure rate in patients who had received six (6) courses of FAC and continued to receive this regimen without added ZINECARD. These analyses showed that the risk of experiencing a cardiac event (see Table 1 for definition) at a given cumulative dose of doxorubicin above 300 mg/m^2 was substantially greater in the 99 patients who did *not* receive ZINECARD beginning with their seventh course of FAC than in the 102 patients who did receive Zinecard (see Figure 1).

Table 1

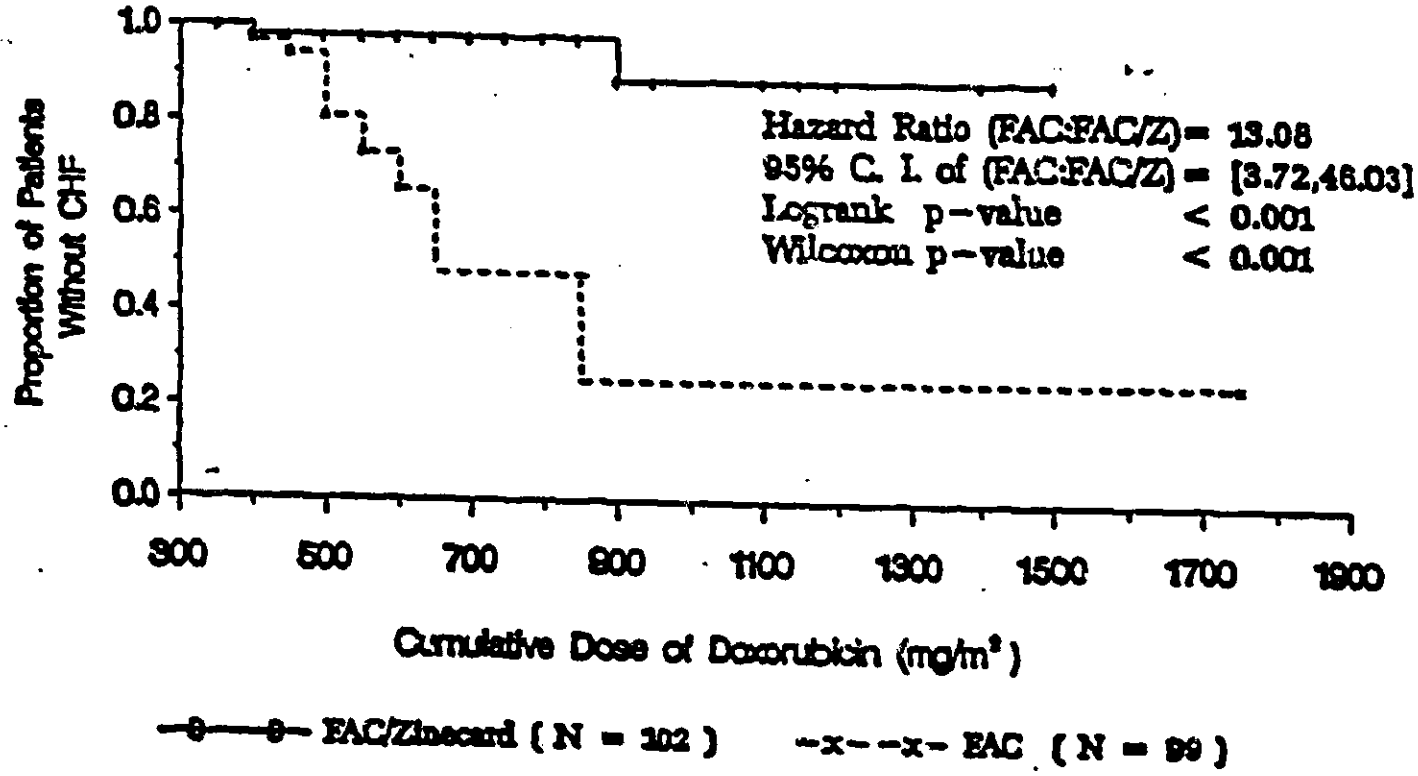
The development of cardiac events is shown by:

1. Development of congestive heart failure, defined as having two or more of the following:
   a. Cardiomegaly by X-ray
   b. Basilar Rales
   c. S_3 Gallop
   d. Paroxysmal nocturnal dyspnea and/or orthopnea and/or significant dyspnea on exertion.
2. Decline from baseline in LVEF by $\geq 10\%$ and to below the lower limit of normal for the institution.
3. Decline in LVEF by $\geq 20\%$ from baseline value.
4. Decline in LVEF to $\geq 5\%$ below lower limit of normal for the institution.

[redacted]

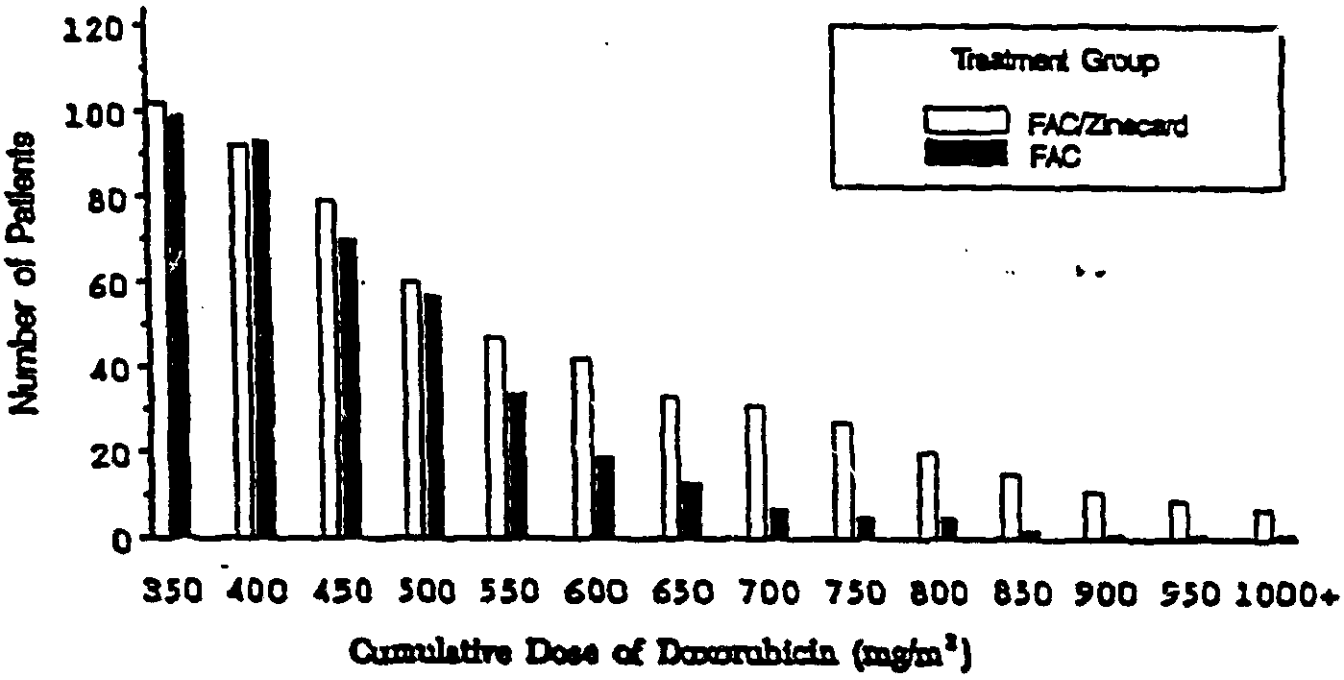
displays the risk of developing congestive heart failure by cumulative dose of doxorubicin in patients who received ZINECARD starting with their seventh course of FAC compared to patients who did not. Patients unprotected by ZINECARD had a 13 times greater risk of developing congestive heart failure. Overall, 3% of patients treated with ZINECARD developed CHF compared with 22% of patients not receiving ZINECARD.

Figure 1
DOX Dose at Congestive Heart Failure (CHF)
FAC vs. FAC/Zinecard Patients
Patients Receiving At Least Seven Courses of Treatment



Because of its cardioprotective effect, ZINECARD permitted a greater percentage of patients to be treated with extended doxorubicin therapy. Figure 2 shows the number of patients still on treatment at increasing cumulative doses.

Figure 2
Cumulative Number of Patients On Treatment
FAC vs. FAC/Zinecard Patients
Patients Receiving at Least Seven Courses of Treatment



In addition to evaluating the cardioprotective efficacy of ZINECARD in this setting, the time to tumor progression and survival of these two groups of patients were also compared. There was a similar time to progression in the two groups and survival was at least as long for the group of patients that received ZINECARD starting with their seventh course, i.e., starting after a cumulative dose of doxorubicin of 300 mg/m^2. These time to progression and survival data should be interpreted with caution, however, because they are based on comparisons of groups entered sequentially in the studies and are not comparisons of prospectively randomized patients.

INDICATIONS AND USAGE

ZINECARD is indicated for reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration in women with metastatic breast cancer who have received a cumulative doxorubicin dose of 300 mg/m^2 and who, in their physician's opinion, would benefit from continuing therapy with doxorubicin. It is not recommended for use with the initiation of doxorubicin therapy (see **WARNINGS**).

CONTRAINDICATIONS

ZINECARD should not be used with chemotherapy regimens that do not contain an anthracycline.

WARNINGS

ZINECARD may add to the myelosuppression caused by chemotherapeutic agents.

There is some evidence that the use of dexrazoxane concurrently with the initiation of fluorouracil, doxorubicin and cyclophosphamide (FAC) therapy interferes with the antitumor efficacy of the regimen, and this use is not recommended. In the largest of three breast cancer trials, patients who received dexrazoxane starting with their first cycle of FAC therapy had a lower response rate (48% vs 63%; $p=0.007$) and shorter time to progression than patients who did not receive dexrazoxane (see Clinical Studies section of **CLINICAL PHARMACOLOGY**). Therefore, ZINECARD should only be used in those patients who have received a cumulative doxorubicin dose of 300 mg/m^2 and are continuing with doxorubicin therapy .

Although clinical studies have shown that patients receiving FAC with ZINECARD may receive a higher cumulative dose of doxorubicin before experiencing cardiac toxicity than patients receiving FAC without ZINECARD, the use of ZINECARD in patients who have already received a cumulative dose of doxorubicin of 300 mg/m^2 without ZINECARD, does not eliminate the potential for anthracycline induced cardiac toxicity. Therefore, cardiac function should be carefully monitored.

Secondary malignancies (primarily acute myeloid leukemia) have been reported in patients treated chronically with oral razoxane. Razoxane is the racemic mixture, of which dexrazoxane is the S(+)-enantiomer. In these patients, the total cumulative dose of razoxane ranged from 26 to 480 grams and the duration of treatment was from 42 to 319 weeks. One case of T-cell lymphoma, a case of B-cell lymphoma and six to eight cases of cutaneous basal cell or squamous cell carcinoma have also been reported in patients treated with razoxane.

PRECAUTIONS

General

Doxorubicin should not be given prior to the intravenous injection of ZINECARD. ZINECARD should be given by slow I.V. push or rapid drip intravenous infusion from a bag. Doxorubicin should be given within 30 minutes after beginning the infusion with ZINECARD. (See **DOSAGE AND ADMINISTRATION**).

As ZINECARD will always be used with cytotoxic drugs, patients should be monitored closely. While the myelosuppressive effects of ZINECARD at the recommended dose are mild, additive effects upon the myelosuppressive activity of chemotherapeutic agents may occur.

Laboratory tests

As ZINECARD may add to the myelosuppressive effects of cytotoxic drugs, frequent complete blood counts are recommended. (See **ADVERSE REACTIONS**).

Drug Interactions

ZINECARD does not influence the pharmacokinetics of doxorubicin.

Carcinogenesis, Mutagenesis, Impairment of Fertility (see **WARNINGS** section for information on human carcinogenicity) - No long-term carcinogenicity studies have been carried out with dexrazoxane in animals. Dexrazoxane was not mutagenic in the Ames test but was found to be clastogenic to human lymphocytes *in vitro* and to mouse bone marrow erythrocytes *in vivo* (micronucleus test).

The possible adverse effects of ZINECARD on the fertility of humans and experimental animals, male or female, have not been adequately studied. Testicular atrophy was seen with dexrazoxane administration at doses as low as 30 mg/kg weekly for 6 weeks in rats (1/3 the human dose on a mg/m^2 basis) and as low as 20 mg/kg weekly for 13 weeks in dogs (approximately equal to the human dose on a mg/m^2 basis).

Pregnancy - *Pregnancy Category C* - Dexrazoxane was maternotoxic at doses of 2 mg/kg (1/40 the human dose on a mg/m^2 basis) and embryotoxic and teratogenic at 8 mg/kg (approximately 1/10 the human dose on a mg/m^2 basis) when given daily to pregnant rats during the period of organogenesis. Teratogenic effects in the rat included imperforate anus, microphthalmia, and anophthalmia. In offspring allowed to develop to maturity, fertility was impaired in the male and female rats treated in utero during organogenesis at 8 mg/kg. In rabbits, doses of

5 mg/kg (approximately 1/10 the human dose on a mg/m^2 basis) daily during the period of organogenesis were maternotoxic and dosages of 20 mg/kg (1/2 the human dose on a mg/m^2 basis) were embryotoxic and teratogenic. Teratogenic effects in the rabbits included several skeletal malformations such as short tail, rib and thoracic malformations, and soft tissue variations including subcutaneous, eye and cardiac hemorrhagic areas, as well as agenesis of the gallbladder and of the intermediate lobe of the lung. There are no adequate and well-controlled studies in pregnant women. ZINECARD should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers - It is not known whether dexrazoxane is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants exposed to dexrazoxane, mothers should be advised to discontinue nursing during dexrazoxane therapy.

Pediatric Use - Safety and effectiveness of dexrazoxane in children have not been established.

ADVERSE REACTIONS

ZINECARD at a dose of 500 mg/m^2 has been administered in combination with FAC in randomized, placebo-controlled, double-blind studies to patients with metastatic breast cancer. The dose of doxorubicin was 50 mg/m^2 in each of the trials. Courses were repeated every three weeks, provided recovery from toxicity had occurred. Table 2 below lists the incidence of adverse experiences for patients receiving FAC with either ZINECARD or placebo in the breast cancer studies. Adverse experiences occurring during courses 1 through 6 are displayed for patients receiving ZINECARD or placebo with FAC beginning with their first course of therapy (column 1 & 3, respectively). Adverse experiences occurring at course 7 and beyond for patients who received placebo with FAC during the first

six courses and who then received either ZINECARD or placebo with FAC are also displayed (column 2 & 4, respectively).

TABLE 2

ADVERSE EXPERIENCE	PERCENTAGE(%) OF BREAST CANCER PATIENTS WITH ADVERSE EXPERIENCE			
	FAC + ZINECARD		FAC + PLACEBO	
	Courses 1-6 N = 413	Courses ≥ 7 N = 102	Courses 1-6 N = 458	Course ≥ 7 N = 99
Alopecia	94	100	97	98
Nausea	77	51	84	60
Vomiting	59	42	72	49
Fatigue/Malaise	61	48	58	55
Anorexia	42	27	47	38
Stomatitis	34	26	41	28
Fever	34	22	29	18
Infection	23	19	18	21
Diarrhea	21	14	24	7
Pain on Injection	12	13	3	0
Sepsis	17	12	14	9
Neurotoxicity	17	10	13	5
Streaking/Erythema	5	4	4	2
Phlebitis	6	3	3	5
Esophagitis	6	3	7	4
Dysphagia	8	0	10	5
Hemorrhage	2	3	2	1
Extravasation	1	3	1	2
Urticaria	2	2	2	0
Recall Skin Reaction	1	1	2	0

The adverse experiences listed above are likely attributable to the FAC regimen with the exception of pain on injection that was observed mainly on the ZINECARD arm.

Myelosuppression

Patients receiving FAC with ZINECARD experienced more severe leucopenia, granulocytopenia and thrombocytopenia at nadir than patients receiving FAC without ZINECARD, but recovery counts were similar for the two groups of patients.

Hepatic and Renal

Some patients receiving FAC + ZINECARD or FAC + placebo experienced marked abnormalities in hepatic or renal function tests, but the frequency and severity of abnormalities in bilirubin, alkaline phosphatase, BUN, and creatinine were similar for patients receiving FAC with or without ZINECARD.

OVERDOSAGE

There have been no instances of drug overdose in the clinical studies sponsored by either Pharmacia Inc. or the National Cancer Institute. The maximum dose administered during the cardioprotective trials was 1000 mg/m^2 every three weeks.

Disposition studies with ZINECARD have not been conducted in cancer patients undergoing dialysis, but retention of a significant dose fraction (>0.4) of the unchanged drug in the plasma pool, minimal tissue partitioning or binding, and availability of greater than 90% of the systemic drug levels in the unbound form suggest that it could be removed using conventional peritoneal or hemodialysis.

There is no known antidote for dexrazoxane. Instances of suspected overdose should be managed with good supportive care until resolution of myelosuppression and related conditions is complete. Management of overdose should include treatment of infections, fluid regulation, and maintenance of nutritional requirements.

DOSAGE AND ADMINISTRATION

The recommended dosage ratio of ZINECARD:DOX is 10:1 (eg, 500 mg/m^2 ZINECARD:50 mg/m^2 DOX). ZINECARD must be reconstituted with 0.167 Molar (M/6) Sodium Lactate Injection, USP, to give a concentration of 10 mg ZINECARD for each mL of sodium lactate. The reconstituted solution should be given by slow I.V. push or rapid drip intravenous infusion from a bag. After completing the infusion of ZINECARD, and prior to a total elapsed time of 30 minutes (from the beginning of the ZINECARD infusion), the intravenous injection of doxorubicin should be given.

Reconstituted ZINECARD, when transferred to an empty infusion bag, is stable for 6 hours from the time of reconstitution when stored at controlled room temperature, 15° to 30°C (59° to 86°F) or under refrigeration, 2° to 8°C (36° to 46°F). DISCARD UNUSED SOLUTIONS.

The reconstituted ZINECARD solution may be diluted with either 0.9% Sodium Chloride Injection, USP or 5.0% Dextrose Injection, USP to a concentration range of 1.3 to 5.0 mg/mL in intravenous infusion bags. The resultant solutions are stable for 6 hours when stored at controlled room temperature, 15° to 30°C (59° to 86°F) or under refrigeration, 2° to 8°C (36° to 46°F). DISCARD UNUSED SOLUTIONS.

Incompatibility

ZINECARD should not be mixed with other drugs.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Handling and Disposal: Caution in the handling and preparation of the reconstituted solution must be exercised and the use of gloves is recommended. If ZINECARD powder or solutions contact the skin or mucosae, immediately wash thoroughly with soap and water.

Procedures normally used for proper handling and disposal of anticancer drugs should be considered for use with ZINECARD. Several guidelines on this subject have been published.[1-7] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

HOW SUPPLIED

ZINECARD™ (dexrazoxane for injection) is available in the following strengths as sterile, pyrogen-free lyophilizates.

NDC 0013-8715-62 — 250 mg single dose vial with a red flip-top seal, packaged in single vial packs.
(This package also contains a 25 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, USP.)

NDC 0013-8725-89 — 500 mg single dose vial with a blue flip-top seal, packaged in single vial packs.
(This package also contains a 50 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, USP.)

Store at controlled room temperature, 15° to 30°C (59° to 86°F). Reconstituted solutions of ZINECARD are stable for 6 hours at controlled room temperature or under refrigeration, 2° to 8°C (36° to 46°F). DISCARD UNUSED SOLUTIONS.

CAUTION: Federal law prohibits dispensing without prescription.

REFERENCES:

1. Recommendations for the Safe Handling of Parenteral Antineoplastic Drugs. NIH Publication No. 83-2621. For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

2. AMA Council Report. Guidelines for Handling Parenteral Antineoplastics JAMA. 1985 March 15.

3. National Study Commission on Cytotoxic Exposure-Recommendations for Handling Cytotoxic Agents. Available from Louis P. Jeffrey, Sc.D., Chairman, National Study Commission on Cytotoxic Exposure, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, Massachusetts 02115.

4. Clinical Oncological Society of Australia. Guidelines and Recommendations for Safe Handling of Antineoplastic Agents. Med J Australia. 1983; 1:426-428.

5. Jones RB. et al. Safe handling of Chemotherapeutic Agents: A report from the Mount Sinai Medical Center. CA - A Cancer Journal for Clinicians. 1983; (Sept/Oct) 258-263.

6. American Society of Hospital Pharmacists Technical Assistance Bulletin on Handling Cytotoxic and Hazardous Drugs. Am J Hosp Pharm. 1990; 47:1033-1049.

7. OSHA Work-Practice Guidelines for Personnel Dealing with Cytotoxic (Antineoplastic) Drugs. Am J Hosp Pharm. 1986; 43:1193-1204.

PHARMACIA INC.
COLUMBUS, OHIO 43216

770000595

May 22, 1995

Pharmacia

Date February 15, 1995

Reference NDA 20-212

NEW CORRESP
NC

DUPLICATE

Charles P. Hoiberg, Ph.D., Acting Director
Center for Drug Evaluation & Research
Division of Oncology Drug Products (HFD-150)
Food and Drug Administration
1451 Rockville Pike
Rockville, MD 20857

RE: ZINECARD NDA 20-212

Dear Dr. Hoiberg:

Maureen Pelosi reviewed with me the status of the Zinecard NDA review on 2/13/95. It was recommended that responses to two issues relating to post approval commitments be submitted in writing.

Pharmacia agrees to generate the data on the filters as described in the attached 2/2/95 fax from FDA. It is also agreed that appropriate experiments will be designed to address as a phase 4 commitment the issues raised by the Biopharmaceutics reviewer in the 2/2/95 fax from FDA (copy of page also attached).

If there are any questions, please contact me at 614/764-8177.

Sincerely,

Robert S. Watson
Associate Director, Regulatory Affairs

Postal address
Pharmacia Inc.
Post Office Box 16529
Columbus, Ohio 43216-6529
USA

Visiting address
7001 Post Road
Dublin, Ohio 43017
USA

Telephone
614-764-8100

Telex
246-620

Telefax
614-764-8102

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

Form Approved: OMB No. 0910-0001
Expiration Date: April 30, 1994
See OMB Statement on Page 3

APPLICATION TO MARKET A NEW DRUG FOR HUMAN USE OR AN ANTIBIOTIC DRUG FOR HUMAN USE

(Title 21, Code of Federal Regulations, 314)

FOR FDA USE ONLY	
DATE RECEIVED	DATE FILED
DIVISION ASSIGNED	NDA/ANDA NO.ASS.

NOTE: No application may be filed unless a completed application form has been received *(21 CFR Part 314)*.

NAME OF APPLICANT	DATE OF SUBMISSION
Pharmacia Inc.	February 15, 1995

ADDRESS *(Number, Street, City, State and Zip Code)*	TELEPHONE NO. *(Include Area Code)*
7001 Post Rd. Dublin, OH 43017 Mailing Address: P.O. Box 16529 Columbus, OH 43216	(614) 764-8177
	NEW DRUG OR ANTIBIOTIC APPLICATION NUMBER *(If previously issued)* 20-212

DRUG PRODUCT

ESTABLISHED NAME *(e.g., USP/USAN)*	PROPRIETARY NAME *(If any)*
Dexrazoxane for Injection	ZINECARD™
CODE NAME *(If any)*	CHEMICAL NAME
ADR-529, ICRF-187, NSC-169780	(S)-4,4'-(1-methyl-1,2-ethanediyl)bis-[2,6-piperazinedione]

DOSAGE FORM	ROUTE OF ADMINISTRATION	STRENGTH(S)
Lyophilized Powder	I.V.	250 mg, 500 mg

PROPOSED INDICATIONS FOR USE

Zinecard for injection is indicated for the prevention of cardiomyopathy associated with doxorubicin administration.

LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS *(21 CFR Part 312)*, NEW DRUG OR ANTIBIOTIC APPLICATIONS *(21 CFR Part 314)*, AND DRUG MASTER FILES *(21 CFR 314.420)* REFERRED TO IN THIS APPLICATION:

INFORMATION ON APPLICATION

TYPE OF APPLICATION *(Check one)*

☒ THIS SUBMISSION IS A FULL APPLICATION *(21 CFR 314.50)* ☐ THIS SUBMISSION IS AN ABBREVIATED APPLICATION (ANDA) *(21 CFR 314.55)*

IF AN ANDA, IDENTIFY THE APPROVED DRUG PRODUCT THAT IS THE BASIS FOR THE SUBMISSION

NAME OF DRUG	HOLDER OF APPROVED APPLICATION

TYPE SUBMISSION *(Check one)*

☐ PRESUBMISSION ☒ AN AMENDMENT TO A PENDING APPLICATION ☐ SUPPLEMENTAL APPLICATION
☐ ORIGINAL APPLICATION ☐ RESUBMISSION

SPECIFIC REGULATION(S) TO SUPPORT CHANGE OF APPLICATION (e.g., Part 314.70(b)(2)(iv)) _____

PROPOSED MARKETING STATUS *(Check one)*

☒ APPLICATION FOR A PRESCRIPTION DRUG PRODUCT *(Rx)* ☐ APPLICATION FOR AN OVER-THE-COUNTER PRODUCT *(OTC)*

CONTENTS OF APPLICATION

This application contains the following items: *(Check all that apply)*

	1. Index
	2. Summary *(21 CFR 314.50(c))*
	3. Chemistry, manufacturing, and control section *(21 CFR 314.50(d)(1))*
	4. a. Samples *(21 CFR 314.50(e)(1))* *(Submit only upon FDA's request)*
	b. Methods Validation Package *(21 CFR 314.50(e)(2)(i))*
	c. Labeling *(21 CFR 314.50(e)(2)(ii))*
	i. draft labeling *(4 copies)*
	ii. final printed labeling *(12 copies)*
	5. Nonclinical pharmacology and toxicology section *(21 CFR 314.50(d)(2))*
	6. Human pharmacokinetics and bioavailability section *(21 CFR 314.50(d)(3))*
	7. Microbiology section *(21 CFR 314.50(d)(4))*
	8. Clinical data section *(21 CFR 314.50(d)(5))*
	9. Safety update report *(21 CFR 314.50(d)(5)(vi)(b))*
	10. Statistical section *(21 CFR 314.50(d)(6))*
	11. Case report tabulations *(21 CFR 314.50(f)(1))*
	12. Case reports forms *(21 CFR 314.50(f)(1))*
	13. Patent information on any patent which claims the drug *(21 U.S.C. 355(b)or(c))*
	14. A patent certification with respect to any patent which claims the drug *(21 U.S.C. 355(b)(2)or(j)(2)(A))*
X	15. OTHER *(Specify)* Responses to FDA request

I agree to update this application with new safety information about the drug that may reasonably affect the statement of contraindications, warnings, precautions, or adverse reactions in the draft labeling. I agree to submit these safety update reports as follows: (1) 4 months after the initial submission, (2) following receipt of an approvable letter and (3) at other times as requested by FDA. If this application is approved, I agree to comply with all laws and regulations that apply to approved applications, including the following:

1. Good manufacturing practice regulations in 21 CFR 210 and 211.
2. Labeling regulations in 21 CFR 201.
3. In the case of a prescription drug product, prescription drug advertising regulations in 21 CFR 202.
4. Regulations on making changes in application in 21 CFR 314.70, 314.71, and 314.72.
5. Regulations on reports in 21 CFR 314.80 and 314.81.
6. Local, state and Federal environmental impact laws.

If this application applies to a drug product that FDA has proposed for scheduling under the controlled substances Act I agree not to market the product until the Drug Enforcement Administration makes a final scheduling decision.

NAME OF RESPONSIBLE OFFICIAL OR AGENT	SIGNATURE OF RESPONSIBLE OFFICIAL OR AGENT	DATE
Robert S. Watson Associate Director, Regulatory Affairs	Robert S. Watson	2/15/95

ADDRESS *(Street, City, State, Zip Code)*		TELEPHONE NO. *(Include Area Code)*
7001 Post Rd. Dublin, OH 43017	Mailing Address: P.O. Box 16529 Columbus, OH 43216	(614) 764-8177

(WARNING: A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

FORM FDA 356h (10/93)

FOOD AND DRUG ADMINISTRATION
OFFICE OF DRUG EVALUATION I

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS

CDER Oncology Group (HFD-150), Parklawn Building
5600 Fishers Lane, Rockville, Maryland 20857

PHONE: (301) 594-5767 FAX: (301) 594-0498

TO: Bob Watson, Pharmacia

(614) 764-8125

FROM: Paul F. Zimmerman, CSO

Total number of pages, including cover sheet: 2

Date: February 2, 1995

COMMENTS: The following concern NDA 20-212 and are comments from the microbiological review of the January 6, 1995 submission.

1. The filters on [redacted] should not be used until bacterial retention validation data for this filter construction is submitted.

In light of the [redacted] used with this product, it will be acceptable to produce product for

distribution using with until the following data can be submitted for review:

2. Products used to support the should be specifically identified and submitted with complete data packages in order that an adequate review can be completed. However, it would be preferable to submit data on actual bacterial retention validation testing completed by suspending bacteria in the drug product (if possible) and mimicking, to the degree possible, all other process parameters.

cc:
Orig NDA
Div file
HFD-150/MPelosi
HFD-150/
HFD-160/

These products also have been shown to protect against doxorubicin-induced inactivation of respiratory enzymes and Calcium ATPase and to inhibit microsomal lipid peroxidation. Therefore, the hydrolytic products appear to contribute significantly in the therapeutic activity of Zinecard. The sponsor should characterize the pharmacokinetics of the metabolites of dexrazoxane as well as the relationship between levels of these components in systemic circulation or locally in tissues and the cardioprotective effect of the drug.

2. From two separate pharmacokinetic studies, it appears that urinary excretion plays an important role in the elimination of dexrazoxane along with hepatic clearance. The sponsor should evaluate the pharmacokinetics of the drug in patients with hepatic and renal insufficiency.

3. The sponsor should assess the pharmacokinetics of dexrazoxane in both males and females. If adequate information is available regarding the pharmacokinetics of dexrazoxane in both gender, the sponsor should analyze the data and submit the results for review and for update of the Package insert.

DEXRAZOXANE FOR INJECTION NDA
Patent Information

PATENT INFORMATION STATEMENT
FILED PURSUANT TO 21 USC 355(b)(1)

Pursuant to 21 USC 355(b)(1), Applicant states that there are two (2) issued United States patents which claim the drug and compositions containing such drug for which this Application has been submitted. These patents are:

U.S. Patent:	3,941,790
Expires:	March 2, 1993
U.S. Patent:	4,275,063
Expires:	June 23, 1998

Adria Laboratories Division of Erbamont Inc., Applicant herein, is the exclusive licensee under both patents by virtue of a License Agreement dated December 30, 1986 between National Research Development Corporation and Adria Laboratories. Copies of the patents are attached.

Respectfully submitted,

Patricia A. Coburn

Patricia A. Coburn
Director, Intellectual Property

EXCLUSIVITY SUMMARY FOR NDA # 20-212 SUPPL # _____

Trade Name ZINECARD Generic Name DEXRAZOXANE

Applicant Name PHARMACIA HFD # 150

Approval Date If Known _____

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 /✓/ NO /___/

b) Is it an effectiveness supplement?

YES /___/ NO /✓/

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 /✓/ 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 7-90
cc: Original NDA Division File HFD-85 Mary Ann Ward

Exclusivity

d) Did the applicant request exclusivity?

YES /✓/ NO /___/

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

7 orphan drug

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 /✓/

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 /✓/

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 /✓/

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 #(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."

NA

1. Does the application in 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?

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:

_____ _____

_____ _____

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:

_____ _____

_____ _____

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"):

_____ _____

_____ _____

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 /___/ ! NO /___/ Explain: _____

_____

Investigation #2

IND # _____ YES /___/ ! 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

YES /___/ Explain _____ ! NO /___/ Explain _____

_____ _____

_____ _____

Investigation #2

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: _____

_____

Maureen Dillon
Signature
Title: CSO, HFD-150

1/19/95
Date

Robert L. Justice, M.D.
Signature of Office/
Division Director
for C. Hoiberg

3/8/95
Date

cc: Original NDA Division File HFD-85 Mary Ann Ward

NGC)165
96-1

ADRIA LABORATORIES

ADRIA LABORATORIES
Division of Erbamont Inc
P.O. Box 16529
Columbus, OH 43216-6529

January 8, 1992

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Gregory Burke, M.D., Ph.D., Acting Director
Division of Oncology and Pulmonary
Drug Products (HFD-150)
ATTN: DOCUMENT CONTROL ROOM 15B-20
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

Re:

General Correspondence
Serial No. 165

Dear Dr. Burke:

Enclosed is a copy of the letter from the Office of Orphan Products Development stating that Zinecard™ qualifies for orphan designation for the prevention of cardiomyopathy associated with doxorubicin administration. This letter is being submitted to the IND at the request of Ms. Ellen Cutler.

Sincerely yours,

Douglas R. Jones
Director Regulatory Affairs, New Drugs

mk
Enclosure

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Office of Orphan Products Development *(HF-35)*
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

December 17, 1991

Adria Laboratories
Division of Erbamont Inc.
Attention: Mr. Donald R. Jones
Director Regulatory Affairs, New Drugs
P.O. Box 16529
Columbus, OH 43216-6529

Dear Mr. Jones:

Reference is made to your orphan drug application of October 30, 1991 submitted pursuant to section 526 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. §360bb) for the designation of Zinecard™ (dexrazoxane for injection) as an orphan drug (application #91-632).

We have completed the review of this application and have determined that Zinecard™ qualifies for orphan designation for the prevention of cardiomyopathy associated with doxorubicin administration. Please refer to this letter as official notification of designation.

Prior to marketing approval, sponsors of designated orphan products are requested to submit written notification to this Office 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 firm for the statutory period of exclusivity. Also please be advised that if Zinecard™ were approved for an indication broader than the orphan designation, your product might not be entitled to exclusive marketing rights pursuant to Section 527 of the FFDCA (21 U.S.C. §360cc). Therefore, in order to avoid discrepancies between the designated orphan indication and the proposed marketing indication, sponsors of designated orphan products have the option to submit data to amend their orphan designation prior to marketing approval.

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 Zinecard™ as designated. If you need further assistance in the development of your product for marketing, please feel free to contact Dr. C.C. Evans at (301) 443-4718.

Congratulations on obtaining your orphan drug designation.

Sincerely yours,

Marlene E. Haffner, M.D., M.P.H.
Director

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Secretary

Office of the General Counsel
Rockville MD 20857

May 20, 1994

NOTE TO DR. TEMPLE AND DR. BURKE

Re: Memo on dexrazoxane and accelerated approval

The attached memo on whether dexrazoxane could be considered for approval under the agency's accelerated approval procedures incorporates the changes each of you suggested to the draft memo I distributed on March 28, 1994. These few changes are on pp. 1 and 3 of the memo.

Please let me know if there are any additional questions about this matter.

Ann Wion

Attachment

cc: Steve Unger
Catherine Lorraine
Seth Ray
HFD-150 Dw-file
NDA 20-212
IND
HFD-150 Johnson J
HFD-150 Williams G

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the General Counsel
Food and Drug Division

Memorandum

Date May 20, 1994

From Ann Wion (GCF-1)

Subject Dexrazoxane and Accelerated Approval Procedures

To Dr. Robert Temple (HFD-100) and Dr. Gregory Burke (HFD-150)

You have asked for an opinion on whether dexrazoxane injection could be considered for approval under the accelerated approval provisions codified at 21 CFR 314.500-.560. For the reasons discussed in this memorandum, I believe that an NDA for dexrazoxane reasonably could be considered under the accelerated approval regulations.

It is my understanding that Adria Laboratories had submitted an NDA for dexrazoxane (Zinecard; ADR-529) to be used concomitantly with doxorubicin, an approved anti-tumor agent with known cardiotoxicity at certain cumulative dose levels. The proposed usefulness of dexrazoxane was to decrease the cardiotoxicity of the doxorubicin. Subsequent to an advisory committee recommendation against approval of this NDA, CDER issued a not approvable letter in July 1992.

The company and agency have since considered the possibility of revising the proposed indication to limit the dexrazoxane use to patients who have already received a cumulative dose of 300 mg/m^2 of doxorubicin and who would be expected to benefit from continued doxorubicin use based on their initial responses. I understand that there are data demonstrating a cardioprotective effect of dexrazoxane, but that dexrazoxane might diminish the anti-tumor action of doxorubicin. That is, the ultimate clinical effect of using dexrazoxane concomitantly with doxorubicin has not been demonstrated.

<u>Use to treat serious or life-threatening illness and to provide meaningful therapeutic benefit over existing treatments</u>

Whether an application for approval of this revised indication for dexrazoxane in concomitant use with doxorubicin could be considered under the accelerated approval provisions depends both on the nature of the indication and on the nature of the evidence to be submitted. First, products are eligible for these procedures only if they are intended to be used in treating "serious or life-threatening illnesses" and to provide "meaningful therapeutic benefit to patients over existing

Accelerated Approval

treatments" (21 CFR 314.500). In this case, whether the illness is considered congestive heart failure[1] or cancer, it would qualify as serious or life-threatening. Heart failure and cancer were, in fact, listed in the preamble to the accelerated approval proposal as examples of diseases "clearly serious in their full manifestations" (57 FR 13235; April 15, 1992).

Examples given in the codified language regarding meaningful benefit include ability to treat patients intolerant of available therapy and improved patient response over available therapy (314.500). For patients responsive to doxorubicin who are unresponsive to or intolerant of other anti-tumor agents, protection against severe cardiotoxicity and, therefore, ability to continue to use doxorubicin, could be meaningful therapeutic benefit.

Effect on surrogate endpoint or effect on clinical endpoint other than survival or irreversible morbidity

If a drug product falls within the scope of 314.500, then CDER can consider an NDA under accelerated approval procedures if adequate and well-controlled trials establish that the product has (1) an effect on a surrogate endpoint reasonably likely to predict clinical benefit or (2) an effect on a clinical endpoint other than survival or irreversible morbidity (314.510).

As described in the preamble to the final rule, the accelerated approval regulations were intended to apply to NDA's based on clinical endpoints that "leave unanswered major questions about the product's effect on ultimate outcome" (57 FR 58946; Dec. 11, 1992). In this case, if adequate and well-controlled trials demonstrate reduced cardiotoxicity through dexrazoxane use in patients receiving more than a cumulative dose of 300 mg/m^2 of doxorubicin through a clinical endpoint such as ventricular failure, the question of effect on ultimate survival would remain unanswered.

I understand that some data related to mechanism of action and data from animal studies suggest that dexrazoxane does not significantly interfere with the anti-tumor effects of doxorubicin. Nevertheless, in the absence of adequate and well-controlled studies demonstrating the ultimate survival outcomes related to concomitant dexrazoxane and doxorubicin use after 300 mg/m^2 of doxorubicin, the ultimate risk/benefit assessment of the concomitant use would be unknown.

---

[1] The labeling for Adria Laboratories' approved doxorubicin injection product Adriamycin includes a boxed warning about "serious irreversible myocardial toxicity with delayed congestive failure often unresponsive to any cardiac supportive therapy. . . ."

In the preamble to the accelerated approval final rule, the agency gave the example of an effect on weight gain in AIDS patients as a clinical endpoint leaving doubt as to the ultimate value of the effect (57 FR 58949). Similarly, the favorable risk/benefit assessment that demonstration of dexrazoxane's cardioprotective effect when used concomitantly with doxorubicin may support would still have to be confirmed in studies demonstrating the effect of concomitant use on ultimate benefit or irreversible morbidity. That is, there would be "uncertainty as to the relation . . . of the observed clinical benefit to ultimate outcome" (314.510). Consequently, under 314.510, postmarketing studies carried out with due diligence would be required.

An argument that dexrazoxane should not be viewed as eligible for consideration under the accelerated approval procedures could be advanced based on the premise that the drug is only intended as part of combination therapy intended to have an anti-tumor effect. Therefore, unless the combination is shown to have this anti-tumor effect (or at least an effect on a surrogate endpoint for the anti-tumor effect), dexrazoxane should not be considered for approval.

However, it can reasonably be argued that dexrazoxane would be indicated for cardioprotection during a course of doxorubicin therapy and that demonstration of this clinical benefit should suffice for accelerated approval subject to ultimate confirmation and description of the favorable risk/benefit judgment based on survival data. Information on previous approvals of drugs used to mitigate the serious side effects of other drugs would be helpful in clarifying this approach.

cc: Steve Unger (HF-7)
Catherine Lorraine (GCF-1)
Seth Ray (GCF-1)

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

NDA # 20-212 Trade (generic) names ZINECARD (dexrazoxane)

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

NA 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.

NA 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&MC 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: Additional studies are now in planning stage through

Signature of Preparer

3/3/95
Date

cc: Orig NDA 20-212
HFD-150/Div File
NDA Action Package

MEDICAL OFFICER REVIEW #4
(Zinecard)

1. General Information:

1.1 NDA# 20-212

1.1.1 Review: M.O. Review #4

1.1.2 Original NDA Submission: 2-7-92.
-Not Approvable letter: 7-13-92
-Submission of Amendment: 8-2-94
-Date of Review: 12-21-94

1.2 Drug Name

1.2.1 Generic name: dexrazoxane
1.2.2 Proposed trade name: Zinecard
1.2.3 Other names: ICRF-187/ADR-529

1.3 Sponsor: Pharmacia

1.4 Pharmacologic Category: Cardioprotectant

1.5 Proposed indication:
In patients who have received "potentially cardiotoxic doses of doxorubicin" who would benefit from continuing doxorubicin therapy.

1.6 Dosage form and directions for use:
Lyophilized powder for IV injection, reconstituted with M/6 Sodium Lactate Injection, USP to be given IV at a 10:1 ratio prior to doxorubicin.

1.7 Related IND

MOR

2. Summary of 12-12-94 ODAC votes:

On 12-12-94 the Oncologic Drugs Advisory Committee met to consider this application. The following votes were taken:

QUESTION	YES	NO
1. Does DZR provide cardioprotection after 300 mg/m2 dox?	9	0
2. Does cardiac protection outweigh toxicity?	9	0
3. Should Zinecard be approved under accelerated approval mechanism for "women with metastatic breast cancer who have recently received 300 mg/m2 of dox?"	8	1
4. Should the indication be restricted to women with metastatic breast cancer?	7	2

3. Review of Draft Labeling

The following comments pertain to the draft labeling included in the 8-2-94 amendment to the NDA.

Page #	Line #	Comments
5	10	The 2 sentences starting with "These Data..." need to be completely rewritten. The following points need to be emphasized: 'The comparisons are historical and non-randomized. For this reason data on TTP and survival are not reliable. TTP data and survival data should not be included in the table since they are likely to be interpreted as coming from a randomized trial.' Similarly the Applicant should understand and agree that survival and time to progression data from this non-randomized comparison is not to be used in marketing in any form.
8	1	The indication should be change to that voted on by the advisory committee: "women with metastatic breast cancer who have received 300 mg/m2 of doxorubicin."

4. Regulatory Recommendations:

A. Page 3 of this review should be sent by FAX transmission to the applicant as a guide for revision of labeling. The company should re-submit draft labeling which includes these changes.

B. Draft labeling in the 8-2-94 submission should be reviewed by Biopharm and Toxicology reviewers if not already done.

Grant A. Williams, M.D.

Robert L. Justice, M.D. 3/7/95

cc: HFD 150 GWilliams, MPELOSI, CGnecco
Application: NDA 20-212

FEB 2 4 1995

Medical Officer Review of Safety Update

NDA	20-212
Drug:	Zinecard
Submission Date:	2-3-95
Review Date	2-24-95

The safety update contains updates of data from randomized controlled trials reviewed in the original 1992 submission and in the more recent 8-23-94 amendment.

No new findings are noted.

Grant A. Williams, M.D.

cc: NDA 20-212
HFD150: GWilliams, MPelosi

MEDICAL OFFICER REVIEW #3
(Zinecard)

1. General Information:

1.1 NDA# 20-212

1.1.1 Review: M.O. Review #3

1.1.2 Original NDA Submission: 2-7-92.
-Not Approvable letter: 7-13-92
-Submission of Amendment: 8-2-94
-Date of Review: 11-14-94

1.2 Drug Name

1.2.1 Generic name: dexrazoxane

1.2.2 Proposed trade name: Zinecard

1.2.3 Other names: ICRF-187/ADR-529

1.3 Sponsor: Pharmacia

1.4 Pharmacologic Category: Cardioprotectant

1.5 Proposed indication:
In patients who have received "potentially cardiotoxic doses of doxorubicin" who would benefit from continuing doxorubicin therapy.

1.6 Dosage form and directions for use:
Lyophilized powder for IV injection, reconstituted with M/6 Sodium Lactate Injection, USP to be given IV at a 10:1 ratio prior to doxorubicin.

1.7 Related IND

TABLE OF CONTENTS

2. Introductory Comments

The proposed amendment to NDA-20212 for Zinecard (dexrazoxane for injection) for the indication of protecting against Doxorubicin-induced cardiotoxicity, in patients who have already been exposed to a significant dose of Doxorubicin, follows Agency rejection of the original NDA submission in 1992.

This indication was rejected by the Oncologic Drug Advisory Committee on June 19,1992 and by the Agency, in a Not Approvable letter, on July 13 1992. The clinical portion of the letter stated the following reasons for rejecting the application:

> "There is evidence that dexrazoxane protection is not selective and that it decreases the antitumor effect of doxorubicin. We recommend a meeting with the Agency to discuss plans for the current and future trials"

Original NDA findings

In section 4 of this review, an unpublished article written by the NDA review team soon after the 1992 ODAC deliberations details the findings at the time of the Not Approvable decision. The following findings were key:

- DZR was cardioprotective as demonstrated by time to cardiac event analyses; however the clinical impact of this finding on the full population exposed was less clear.
- There was evidence that DZR lessened the antitumor effect of FAC in breast cancer. In the largest study in breast cancer which used the intended 10:1 ratio of DZR to Dox, objective response was 15% lower on the DZR arm (p=0.007) than on the placebo arm. Moreover, time to progression, in a followup analysis of the extended study, was significantly inferior on the DZR arm.
- DZR clearly produced additional myelosuppression, though again the clinical impact of this toxicity was less clear.
- The greatest benefit from DZR appeared in patients who received more than 300 mg/m2 of doxorubicin. However, many women needlessly exposed to DZR progressed prior to receiving this dose. Moreover, the need to treat women with breast cancer beyond 6 courses of FAC was not entirely clear.

History of Crossover of Placebo group to DZR after 300 mg/m2 of doxorubicin

Prior to the original ODAC meeting, after an interim analysis

showed that efficacy of DZR clearly manifested itself after 300 mg/m2 of doxorubicin, all patients on both Placebo and DZR arms were crossed over to DZR after receiving a cumulative dose of 300 mg/m2 of doxorubicin. Additional patients were accrued to both breast cancer studies to gather data on whether late addition of DZR was cardioprotective.

Historical comparison and Accelerated Approval concepts

During later discussions with the Agency the Sponsor introduced the concept of comparing data on delayed addition of DZR to FAC (after 300 mg/m2) with historical data gathered on FAC treatment with placebo alone after 300 mg/m2 of doxorubicin. In analyses presented at a meeting with the Agency the cardioprotective effect demonstrated by such a retrospective comparison seemed convincing; however, given the unconventional nature of the analysis, any conclusions would require detailed review of the data. In addition, it was still unclear whether women with breast cancer benefitted from continuing doxorubicin beyond 300 mg/m2; and the potential for DZR protection of tumor even at this stage was still a concern.

The Agency suggested doing a study which proved that treatment with doxorubicin and DZR in women with breast cancer who had received at least 300 mg/m2 of doxorubicin provided some net benefit versus no further treatment. The concept of approval by the 'Accelerated Approval' mechanism was discussed. After extensive discussions with FDA Legal Counsel, the Accelerated Approval Mechanism was considered viable for this application. The following is from a May 20, 1994 memo from Ann Wion to Dr Temple (HFD-100) and Dr Burke (HFD-150)

> "However, it can reasonably be argued that dexrazoxane would be indicated for cardioprotection during a course of doxorubicin therapy and that demonstration of this clinical benefit should suffice for accelerated approval subject to ultimate confirmation and description of the favorable risk/benefit judgment based on survival data. Information on previous approvals of drugs used to mitigate the serious side effects of other drugs would be helpful in clarifying this approach."

The clinical protocol proposed by the Sponsor and reviewed by the Agency for accelerated approval is discussed in section 8 of this review. Briefly, women with breast cancer who have responded to Doxorubicin therapy and have received 300 mg/m2 of Doxorubicin will be randomized to Doxorubicin plus DZR versus no treatment. Upon progression, the patients in the no-treatment arm will cross over to receive doxorubicin plus DZR. They will be followed for time to progression, survival, and tumor-related symptoms. If benefit is proven for doxorubicin plus DZR, then the drug will remain on the market. If the results of the validating study are negative, then the Accelerated Approval clause provides a

mechanism for removing the drug from the market.

Critical Dates:

As an overview it is helpful to review critical dates in the history of this application:

Original title of Study 88001:

TITLE: ADR-529 as a Cardioprotective in a Phase III Randomized Trial of FAC versus FAC + ADR-529 in the Treatment of Disseminated Carcinoma of the Breast

2-88 Study was begun, 20:1 dose

11-2-88 Dose was modified to 10:1 due to excess of deaths from myelosuppression, amendment #2 outlines these changes.

4-24-89 88006, Second Breast Cancer Study started.

1-14-91 -Accrual cutoff date for NDA.

-Patients on placebo arm were crossed over to DZR after 300mg/m2 of doxorubicin.

3-31-91 -Data cutoff date for NDA.

12-2-92 -End accrual for add-on studies of both 88001 and 88006.

1-14-91 to 12-2-92: **Post NDA accrual:**
353 patients (173 DZR and *180 PLA*

Cutoff dates for randomization agreed upon for comparison in Placebo patients who received at least 7 courses of FAC:

In order to allow a 'clean' comparison of patients who received only placebo with those who crossed over after 300 mg/m2, the dates 1/14/91 and 5/7/90 were selected: all placebo patients randomized after 1/14/91 were crossed over to DZR after mg/m2 of DOX and most patients randomized prior to 5/7/90 were not crossed over to DZR after 300 mg/m2.

Control group: Randomized prior to 5-7-90

Treatment group: Randomized after 1-14-91.

The focus of this NDA is on breast cancer patients who started out on placebo and continued with doxorubicin beyond a dose of

300 mg/M^2 of *doxorubicin*, either with placebo or with DZR. Because of the historical circumstances above, this creates a historical comparison of patients from one arm of the above studies. Patients randomized to the Placebo arm in early years who continued beyond 300 mg/M^2 of doxorubicin constitute the **'PLA/PLA'** historical control; patients randomized to the Placebo arm in later years who continued beyond 300 mg/M^2 of doxorubicin constitute the **'PLA/DZR'** treatment arm. The original trials were started in 2/88 whereas the post-NDA patients started in 1/91; so there is a historical difference of about 3 years between these 2 cohorts.

Patient numbers in analyses:

The attached reviewer diagram prepared from the data base during evaluation of survival provides an overview of the complexity involved in this retrospective analysis. The sponsor proposed to compare placebo patients, who receive at least 7 courses of FAC, who were randomized before 5/7/90 with similar patients randomized after 1/14/91. These latter patients would have received DZR after 6 courses of FAC whereas the former patients would have received only placebo after 6 courses of FAC. As this diagram shows, the 2 randomized arms and 2 proposed randomization time cutoffs produce 6 cells (2 arms versus 3 time periods). The application is emphasizing a comparison of only 2 of these cells. Moreover, about half the patients are lost when one undertakes the primary analysis, a 'course-7 analysis' (ie analysis of patients receiving at least 7 coursed of FAC)..

1. Treatment Scheme:

Treatment Scheme

	Time Period of Randomization			
		1.Early	2.Mid	3.Late
Randomized ARM	1.DZR	DZR/DZR	DZR/DZR	DZR/DZR
	2.PLA	PLA/PLA	PLA/Mixed	PLA/DZR

2. Number of patients in various analyses:

'Course-7' Analyses

	Time Period of Randomization				
		1.Early	2.Mid	3.Late	Total
Randomized ARM	1.DZR	81	56	102	239
	2.PLA	99	56	102	257
	Both Arms	180	112	204	496

'All-Patient' Analyses

	Time Period of Randomization				
		1.Early	2.Mid	3.Late	Total
Randomized ARM	1.DZR	195	121	173	489
	2.PLA	210	129	180	519
	Both Arms	405	250	353	1008

3. Diagram for Visualizing Comparisons:

Comparison Diagram (example DZR vs PLA)

	Period	1	2	3	
A R M	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

(All = all pts., CS=Course 7 pts).

Reviewer approach to Amendment validation

- Since we are basically reducing the total database by 80% from 1008 patients to about 200 patients in the PLA/PLA versus PLA/DZR analysis, the reviewer spent considerable time examining time to event analyses and multivariate analyses on parallel arms, and on more complete arms, to assure that results were robust, and were not merely the result of a particular fragmentation of the data.

- The reviewer also chose to [illegible]is of time to MUGA event rather than time to [illegible]: in this way it could be independently verif[illegible]e highly objective (and verifiable) LVEF changes a[illegible]upport the efficacy of DZR when it is added after 300mg/M of doxorubicin. The clinical findings are certainly important in determining overall clinical significance, and are presented separately by the sponsor in an analysis of CHF. However, since the trials were not blinded on crossover to DZR, it is of interest to note whether the less subjective LVEF findings alone used by the reviewer verify the aggregate endpoint of laboratory and clinical findings used in the sponsor's analysis.

- In addition the reviewer did the time to event analysis of MUGA events using time on the x axis. The sponsor's analyses used cumulative doxorubicin dose for the x axis. The reviewer method gives a different perspective, and also allows use of the data from followup MUGA scans done later but at similar doses of doxorubicin. Verification of the cardiac efficacy findings using somewhat different methodology strengthens my confidence in the reality of underlying efficacy.

3. **Location of Material Reviewed:**

Package Insert . . . . . V 1

Updates of Clinical Studies in Breast Cancer V1-5

PLA vs PLA/DZR Study 88001 . . . . . V6

PLA vs PLA/DZR Study 88006 . . . . . V6

A comparison of risk of CHF in pts treated with FAC who also received DZR or placebo followed by DZR for Breast Cancer. V7

Integrated Summary of Efficacy and Safety of delayed Administration of ADR-529 as a Cardioprotectant... . . . . V7

Electronic patient Data Base: Submitted in 'ACCESS' data base format.

Additional submissions reviewed:
WordPerfect Tables from NDA Submitted 8-25-94

4.0 Review of DATA presented to ODAC in June,1992 (Unpublished Article prepared by NDA review team in 1992)

FDA ONCOLOGY DRUGS ADVISORY COMMITTEE REVIEW OF ZINECARD (Dexrazoxane, ADR-529, ICRF-187)

Grant A. Williams,* John R. Johnson, Gregory Burke.

This article reports the FDA Oncology Drugs Advisory Committee review at its June 19, 1992 meeting of Adria Laboratory's New Drug Application for Zinecard (Dexrazoxane, ADR-529, ICRF-187) for decreasing the incidence and severity of cardiomyopathy associated with the use of doxorubicin. Four randomized clinical studies were conducted in patients with advanced metastatic breast cancer and two in extensive small cell lung cancer, comparing Dexrazoxane (DRZ) to placebo. The DRZ:doxorubicin ratio was initially 20:1, but was decreased to 10:1 after an excess of early deaths in the DRZ patients. The Committee believed that Zinecard decreased cardiomyopathy as measured by left ventricular ejection fraction in patients receiving total doxorubicin doses of 400 mg/M2 or greater. But only a small proportion of the patients appeared to derive major benefit from cardioprotection. For example, in the largest breast cancer study only 33/168 patients in the DRZ group received doxorubicin doses of 400 mg/M2 or greater compared to 65/181 patients in the placebo group. The largest breast cancer study had an objective tumor response rate in the DRZ group of 48% (67/141) and in the placebo group 63% (96/152) (p=0.007). In an extension of this study, time to tumor progression was also decreased in the DRZ group. The Committee recommended that the application for marketing DRZ not be approved because the results of the clinical studies indicate that DRZ may protect the cancer from the effects of the cancer chemotherapy.

On June 19, 1992 the FDA Oncology Drugs Advisory Committee (ODAC) unanimously recommended against approval of Adria Laboratories' Zinecard (Dexrazoxane, ADR-529, ICRF-187) for decreasing the incidence and severity of cardiomyopathy caused by doxorubicin. The ODAC believed that Dexrazoxane (DRZ) demonstrated cardioprotection at high total doses of doxorubicin above 400 mg/M2, but believed the available data failed to show that DRZ did not interfere with the antitumor effect of doxorubicin.

From the Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, Maryland

Data and statistical analyses discussed in this communication were submitted by Adria Laboratories to the FDA with the New Drug Application for DRZ and were discussed in public forum during the June 19, 1992 ODAC meeting (1).

ADRIA Sponsored Studies

The studies conducted by Adria Laboratories were double blind placebo controlled trials of doxorubicin containing chemotherapy regimens for advanced metastatic breast cancer (trials 88001 and 88006) or extensive stage small cell lung carcinoma (trial 88002). Patients were randomized to receive either DRZ or placebo (PLA) IV within 30 minutes prior to the doxorubicin. Initially the ratio of DRZ dose to doxorubicin dose was 20:1; after November 1988 it was 10:1. The regimen for breast cancer was FAC (fluorouracil 500 mg/M^2, doxorubicin 50 mg/M^2 and cyclophosphamide 500 mg/M^2 IV every 3 weeks); that for small cell lung cancer was CAV (cyclophosphamide 750 mg/M^2, doxorubicin 50 mg/M^2, and vincristine 2.0 mg/M^2 IV every 3 weeks). If treatment was delayed past day 22, doses of cyclophosphamide and 5FU were reduced; doses of doxorubicin were not altered. Patients continued therapy until going off-study for toxicity or progression.

The studies evaluated the cardioprotective effect of DRZ when given with doxorubicin, objective tumor response, and toxicity.

Cardiac left ventricular ejection fractions (LVEF) were measured at baseline and were closely monitored at defined intervals beginning at a cumulative doxorubicin dose of 150 mg/M^2. Off-study criteria for cardiac toxicity included clinical congestive heart failure or several predefined decrements in LVEF.

In November 1988 because of an excess of early deaths on studies at the 20:1 ratio, all patients on these studies were switched to the 10:1-ratio and new studies were initiated at the 10:1 ratio.

In November of 1989, based on evidence of cardioprotection with the 10:1 ratio in the 88001 breast cancer trial, a data monitoring committee recommended that all patients in both treatment groups in all studies be given DRZ after a cumulative doxorubicin dose of 350 mg/M^2. Accrual to all of the studies described above was terminated in January of 1991.

Results

Toxicity

10:1 studies

Increased myelosuppression from DRZ was apparent at the 10:1 ratio; the nadir granulocyte count during course 1 in breast cancer trial 88001 was significantly lower in the DRZ group [0.362 versus 0.598 X $10^3/mm^3$, $p<0.001$ by Wilcoxon rank sum (WRS)]. Clinical significance of this difference was not clear. The incidence of sepsis in course 1 was 12% in the DRZ group and 7% in the PLA group, not a statistically significant difference.

20:1 studies

Because of an excess of early deaths on the DRZ arms of these studies (11 deaths in the DRZ groups versus 1 in the PLA groups) patients in these trials receiving DRZ at a 20:1 ratio were switched to the 10:1 ratio in November of 1988. The increase in deaths was thought to be due to increased myelosuppression in the DRZ group, although this could not be verified since study design did not include weekly blood counts. In patients receiving the 20:1 ratio in the breast cancer trial 88001, sepsis during course 1 occurred in 25% in the DRZ group versus 8% in the PLA group [$p<0.05$ by Pearson Chi Square (PCS)] and occurred at some time during therapy in 38% in the DRZ group versus 17% on PLA ($p<0.01$ by PCS).

Cardioprotection

Evidence that DRZ was cardioprotective was based on several studies using both 10:1 and 20:1 ratios of DRZ to doxorubicin. DRZ caused a significant delay in time to going off-study for cardiac toxicity (primarily based on decreases in LVEF). Mean decreases in LVEF compared to baseline were significantly less in the DRZ groups at cumulative doses of doxorubicin of 400 mg/M^2 or greater. In the patients receiving the 10:1 ratio in the 88001 trial, the differences in mean LVEF decrease change from baseline between the two groups were 6.7%, 7.6%, and 10.1% respectively at cumulative doses of 400, 500, and 550 mg/M^2 of doxorubicin. It was noted at the ODAC, however, that relatively few patients appeared to derive major benefit from cardioprotection, primarily because few received these cumulative doxorubicin doses. For example, in the Adria 88001 breast cancer study only 33 of 168 patients in the DRZ group received doxorubicin total doses of 400 mg/M2 or greater compared to 65 of 181 patients in the PLA group. The difference in number of patients with on study congestive heart failure was only 8 in a total of 349 (10 on PLA and 2 on DRZ).

Tumor Response

10:1 studies

Objective tumor response rates from the 10:1 studies are shown in Table 1. In breast cancer study 88001, the largest trial using the 10:1 ratio, the response rate in patients with measurable disease was 15% lower in the DRZ group (48% versus 63%), a difference that was highly statistically significant (p=0.007 by PCS).

This difference appeared to have clinical consequences. Analysis of time to progression in this trial, expanded to include new patients accrued under an amended protocol (n=509 with 313 events), showed that time to progression was also significantly inferior in the DRZ group (hazard ratio P:D of 0.79, p = 0.03 by log rank and p=0.016 by Wilcoxon). Survival in the DRZ and PLA groups was similar with a hazard ratio for P:D of 1.022 with 95% ci (.720, 1.449).

The other breast cancer trial 88006 and the small cell lung cancer trial 88002, each with one third to one half the number of patients with measurable disease as the 88001 breast cancer trial, showed no statistically significant differences in objective tumor response, although there was a strongly unfavorable trend in 88002.

Data on time to off-study for all reasons in the trials using the 10:1 ratio did not suggest that patients in the DRZ group were able to stay on study longer. In the largest study (88001) there was no significant difference between the treatment groups in time to off-study (Hazard ratio P:D 1.11, p=0.38 by LR).

20:1 studies

The objective tumor response rates from the 20:1 studies are shown in Table 2. None of the Adria sponsored studies was conducted using exclusively the 20:1 dose ratio because many of the patients received part of their treatment at the 20:1 ratio and part at the 10:1 ratio.

In breast cancer study 88001 the objective tumor response rate in patients with measurable disease in the DRZ group was 58% and in the PLA group was 54% with the 95% ci on DRZ minus PLA (-14%, 28%). In study 88002 in small cell lung cancer the objective tumor response rate in the DRZ group was 58% and in the PLA group was 68% with the 95% ci on PLA minus DRZ (-36%, 16%).

Results of the New York University breast cancer study 88011 using the 20:1 ratio have been previously reported by Speyer et al.(2) and were also discussed before the ODAC. This is the only

study that used exclusively the 20:1 ratio. The design of this study in advanced metastatic breast cancer was very similar to the Adria sponsored 88001 trial, except that it was not blinded. The study has been interpreted as evidence that DRZ does not affect the antitumor response of doxorubicin chemotherapy, but that conclusion seems premature, given the data described above. When the analysis of objective tumor response is limited to patients with bidimensionally measurable disease, similar to the analyses of tumor response in the Adria sponsored studies, the results are 24/48 (50%) in the DRZ group and 24/41 (59%) in the PLA group with 95% ci on PLA minus DRZ (-30%, 16%). The -30% lower bound of the ci is consistent with a possible response rate for the DRZ group that is unacceptably low. Objective tumor response data from the 20:1 studies may not be relevant to the 10:1 studies because DRZ has antitumor activity as a single agent.

Abbreviations

PCS Pearson Chi Square
WRS Wilcoxon rank sum
DRZ Dexrazoxane
DOX Doxorubicin
ODAC Oncology Drugs Advisory Committee

References

(1) Transcript FDA Oncology Drugs Advisory Committee Meeting, June 19, 1992
(2) Speyer JL, ET AL: ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. J Clin Oncol 10: 117-127, 1992

Table 1

Response Rates in Trials Using 10:1 ratio

Study 88001

Disease	Breast cancer		
	DRZ	PLA	95% ci[1]
# randomized			
total	168	181	
measurable	141	152	
Response	67/141 (48%)	96/152 (63%)	(-27%, -4%)[2]

Study 88006

Disease	Breast cancer		
	DRZ	PLA	95%ci
# randomized			
total	81	104	
measurable	54	69	
Response	41/54 (57%)	36/69 (52%)	(-13%, 23%)

Study 88002

Disease	Small cell lung cancer		
	DRZ	PLA	95% ci
# randomized			
total	73	82	
measurable	67	76	
Response	30/67 (45%)	45/76 (59%)	(-30%, 2%)

[1]95% confidence intervals of DRZ minus PLA.
[2]Difference significant at p=0.007 by Pearson chi square.

Table 2:

Response Rates in Trials Using 20:1 Ratio[1]

Study 88001

Disease	Breast Cancer		
	DRZ	PLA	95% ci[2]
# randomized			
total	67	54	
measurable	67	54	
Response	38/67 (58%)	29/54 (54%)	(-14%, 28%)

Study 88002

Disease	Small Cell Lung Cancer		
	DRZ	PLA	95% ci
# randomized			
total	26	25	
measurable	26	25	
Response	15/26 (58%)	17/25 (68%)	(-36%, 16%)

Study 88011 (NYU)

Disease	Breast Cancer		
	DRZ	PLA	95% ci
# randomized			
total	76	74	
measurable	48	41	
Response	24/48 (50%)	24/41 (59%)	(-30%, 12%)

[1]Most patients in studies 88001 and 88002 received part of their treatment at the 20:1 ratio and part at the 10:1 ratio.
[2]95% confidence intervals of DRZ minus PLA.5.0

5. Protocol from Clinical Study 88001

The following are notes on the protocol and amendments under which studies 88001 and 88006 were performed.

TITLE: ADR-529 as a Cardioprotective in a Phase III Randomized Trial of FAC versus FAC + ADR-529 in the Treatment of Disseminated Carcinoma of the Breast

DATE OF ORIGINAL PROTOCOL: July 28, 1987

DATE OF AMENDMENT NO. 1: November 25, 1987
DATE OF AMENDMENT NO. 2: February 12, 1989
DATE OF AMENDMENT NO. 3: April 12, 1989
DATE OF AMENDMENT NO. 4: January 14, 1991

Historical considerations

Amendment #2 2-12-89
Amendment #3 4-12-89

These are the major amendments defining the protocol as it was applied to the "10 to 1" patients in this study, providing the framework for the data reported in the NDA for these patients.

Amendment #4 1-14-91

This amendment primarily outlined post-NDA handling of accrual, etc. for patients continuing on in new studies.

5.1 Objective:

The 3 objectives have been constant from the original protocol:

-To demonstrate cardioprotective effect of ADR-52..
-To determine if ADR-529 alters response rate to FAC.
-To assess the safety of doxorubicin plus ADR-529.

5.2 Design:

The protocol was for a multicenter randomized double blind placebo controlled trial of FAC (5FU, Adriamycin, and cyclophosphamide) with ADR-529 versus FAC plus placebo. Stratification occurred, after early 1989, on the basis of measurable vs non-measurable and presence of cardiac risk factors:

Cardiac risk factors were to be considered one or more of the following:

-mediastinal radiation
-Age > 65
-History of heart disease (MI, significant arrhythmia, angina).
-Hypertension requiring medication.
-Diabetes mellitus requiring medication.
-Baseline MUGA scan 1-10% above the lower limit of normal for the institution.

5.3 Eligibility:

-women with unresectable or metastatic breast cancer were eligible.

-Initially measurable disease was a requirement, however after the major amendment in early 1989, both measurable and evaluabl disease was allowed (with stratification on this criterion).

-No prior chemotherapy was allowed for metastatic disease.

-No prior anthracycline or anthracene was allowed even as an adjuvant.

-ECOG performance status was required to be 0,1,or 2. PS 2 was excluded at the time of the change to the 10:1 ratio. With the PreNDA amendment (#4), PS 2 was again allowed. So, the bulk of the patients getting 10:1 in the NDA were PS 0 or PS 1.

Reviewer's Comments:
In considering differences in response or efficacy results in various portions of this trial, and in considering generalizability of results or appropriateness of combination via meta-analysis, it is important to remember the various changes occurring with the early 1989 amendment which lowered the ratio of ADR-529 to 10:1, excluded PS 2 patients, included non-measurable disease patients, and changed formulation. With the 4th amendment, the Pre-NDA amendment again PS 2 patients are included.

5.4 Number of patients:

Initially the protocol was to have 72 "response evaluable patients per arm" or 38 patients per arm who had received 500 mg/M2 of doxorubicin, which ever was largest. The DMC recommended on 11-3-90 that accrual need not proceed further due to interim analysis establishing the cardioprotective effect.

The basis of the 38 patient size was to give 80% power to detect a 10% change in LVEF at 500 mg/M2, the primary objective of the trial. The primary dose of interest was to be this dose although 150 mg/M2, 300 mg/M2 and every 100 mg/M2 thereafter were to be evaluated also.

The secondary objective was to compare response rates on the 2 arms. In the patients with measurable disease, 72 patients were required to have a power of 80% to detect a 20% decrease in response rates using a one-tailed test for equality of proportions.

Other comparisons of interest included dropouts for heart failure, dropouts for LVEF decline to at least 20% from baseline, a decline to 10% below normal limit, or decline to at least 5% below normal limit.

Reviewer's Comments:
Note that the original design did not describe an analysis of time to "cardiac event."

The sample size for the ongoing trial described in amendment #4, is based on continuing accrual until 24 cardiac events occur.

5.5 Randomization

Central randomization occurred by phone M-F in Eastern Time Zone. Although the study report states that individual lists were prepared for each center, the sponsor states that they were kept centrally.

5.6 Procedure

Both arms received CAF intravenously every 3 weeks:
Cytoxan 500 mg/M^2 day 1
doxorubicin 50 mg/M^2 day 1
5FU 500 mg/M^2 day 1

According to randomization, patients were to receive either placebo or ADR-529 in a volume of 50 ml/M2 of M/6 sodium lactate by slow IV push prior to, but within 30 minutes of the doxorubicin administration.

As noted in the amendment section, on 1-14-91 after a cumulative 300 mg/M2 of doxorubicin had been given, all patients were to be given ADR-529 with the next doxorubicin dose.

a. Dose modification

Doxorubicin and ADR-529 doses were not to be altered. Cytoxan and 5FU doses were to be decreased by 100 mg/M^2 each for granulocytopenic fever or day 22 granulocyte count below 1500 or platelet count below 90k. Subsequent dose reductions for similar reasons were to be by 50 mg/M^2.

Patients were to continue therapy until cardiac toxicity or disease progression.

b. Criteria for early termination for cardiac toxicity are described in section 8.4:

-congestive heart failure, manifest by at least 2 of the following::

-cardiomegaly by X-ray.
-Basilar rales.
-Cardiac S3 Gallop
-PND and/or orthopnea and/or exertional dyspnea.

-LVEF decline by 0.10 to below normal for institution.

-Decline in LVEF by at lease 0.20 from baseline regardless of relation to institutional norm.

-Decline by 0.05 in LVEF below lower limits of institutional norm.

c. Evaluation schedule:

Assessment for response occurred every 3 courses while patients were receiving study drugs and every 3 months during followup until relapse.

Cardiac toxicity evaluation consisting of MUGA scan and evaluation of cardiac signs and symptoms were to be evaluated after 150 mg/M^2, 300 mg/M^2, 400 mg/M^2, and every 50 mg/M^2 thereafter.

With the amendment changing dose to 10:1 a couple of changes were made. If the patient was going off study only for a MUGA scan change, a repeat MUGA scan was required. The weekly CBC was reintroduced for the first 4 cycles at this time also.

d. Efficacy criteria

Response

Response criteria were fairly standard:

CR
PR 50% decrease in sum of products.
PD any of the following:
-25% increase in SOP for non-responders.
-50% increase in SOP after response
-new lesions.
-"unequivocal progression" of non-measurable disease.

Only lytic bone lesions were considered measurable.

Cardiac efficacy

In section 10.2 under Efficacy Criteria the following is the introductory statement:

"The two treatment arms will be compared with respect to percent decline from baseline in LVEF and incidence of cardiac toxicities at similar cumulative doses of Adriamycin."

It then goes on to list the 5 conditions (one clinical: congestive heart failure and 4 permutations of MUGA scan changes.
Initially the primary endpoint was going to be LVEF decrease of at least 0.10 at 500 mg/M^2.

In table 2-5 of the Integrated Summary the sponsor compares baseline and course-7 demographic characteristics and prognostic factors between the Placebo/placebo (early placebo) and the Placebo/DZR (late placebo) groups. Statistical tests included chi-square and rank sum tests.

The tests done fall in 3 categories (# tests per category in parentheses):

-General Demographic Factors (8)
-Cardiac-Associated Demographic factors(7)
-Tumor-associated demographic factors(12)

The Cardiac-associated factors included:

-prior radiation to mediastinum
-history of heart disease
-hypertension
-diabetes
-LVEF≤ 10% above LLN at baseline
-LVEF≤ 10% above LLN prior to course 7
-Age > 55
-Course of Last LVEF Measurement Prior to Course 7

6.0 Sponsor's New Data and Analyses

6.1 Integrated Summary of Efficacy and Safety of Delayed Administration of ADR529

As noted in earlier sections of this review, the sponsor's analysis of efficacy concentrates on patients who received at least 7 courses of chemotherapy, comparing the early era PLA patients with the later era PLA/DZR patients. This is demonstrated by X and Y in the following diagram:

	Period	1	2	3	
ARM	DZR				
	PLA	X		Y	
		Population			CS

(All = all pts., CS=Course 7 pts).

The numbers of patients in the sponsor's analyses are presented in the following table, taken from the sponsors table on p 21 of the Integrated Summary:

Analysis	PLA/DZR	PLA
Time to Cardiac Event*	91	94
Response rate**	68	87
Other Analyses***	102	99

*Patients receiving at least 7 courses of treatment who received at least one MUGA scan after 7 courses.
**Patients receiving at least 7 courses who had measurable disease at baseline.
***Patients who had at least 7 courses of treatment.

The following table summarizes the findings. There were significant imbalances in 2 of 8 general tests (weight and platelet count), 4 of 12 tumor-related tests, and none of 7 cardiac-associated tests.

Significant Findings from Pharmacia Tables 2-5:

Demographic, Cardiac, and Tumor-related prognostic factors at baseline and prior to Course 7

Category(#tests)	Variable	PLA/DZR	PLA	P VALUE
General(8)				
	Weight(kg)			
	median	69.4	64	0.017
	Platelet count			
	median	285	328	0.001
Tumor (12)				
	No. Disease Sites			
	mean	2.9	3.7	0.004
	Dominant Disease Site			0.02
	Visceral(%)	68	76	
	Bone(%)	25	10	
	Soft tissue (%)	9	14	
	Measurability Status			
	No measurable Dz (%)	33	12	0.001
	Response at Last Dz measurement Prior to Course Seven (%)	51	66	0.035
Cardiac (7)				
	LVEF ≤ 10% Above LLN at Baseline			
	Yes (%)	18	24	0.25
	LVEF ≤ 10% Above LLN at prior to Course 7			
	Yes (%)	51	38	0.085

The findings suggest that the 'early' and 'late' placebo populations indeed are not identical; however no major differences in cardiac-associated findings are suggested. These differences in tumor-related factors suggest that any historical comparison of tumor-associated endpoints such as time to progression, may not be reliable and should certainly include multivariate analyses of prognostic factors.

Sponsor analyses of time to Cardiac Event

The sponsor's retrospective analysis of time to Cardiac Event of the early(randomized prior to May 7, 1990) Placebo/Placebo and the late(randomized after January 14, 1991) Placebo/DZR group is shown in attached Table 6 and Figure 1 from the submission. With about the same number of patients in each group(91 vs 94), more than twice as many cardiac events were noted in the Placebo/Placebo group. The hazard ratio of 3.5 ($p<0.001$) and adjusted hazard ratio of 3.3 ($p<0.001$) leave little doubt that cardiac events were much more common on the PLA/PLA arm. (Sponsor's adjusted analysis included Age, mediastinal radiation, history of heart disease, hypertension, diabetes, and low-normal baseline ejection fraction.)

TABLE 6

TIME TO CARDIAC EVENT AFTER COURSE SIX - DECEMBER 31, 1993

PLACEBO VS. PLACEBO/DZR PATIENTS[a]
PATIENTS RECEIVING AT LEAST ONE MUGA SCAN AFTER
SEVEN COURSES OF TREATMENT

STUDY NO. 088001 AND 088006

VARIABLE	PLA/DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	91	94		
No. Events (%)	25 (27%)	59 (63%)		
Median Event Time (mg/M^2 of DOX[c])	—[d]	550		
Hazard Ratio (P:P/D)	3.508		LR	$\chi^2 = 31.72$ P < 0.001
95% C.I. of (P:P/D)	(2.153, 5.716)		GW	$\chi^2 = 19.88$ P < 0.001
Adjusted Hazard Ratio (P:P/D)[e]	3.289		WCS	$\chi^2 = 20.52$ P < 0.001
95% C.I. of Adjusted (P:P/D)[e]	(1.965, 5.505)			

[a] Placebo patients were enrolled in the Placebo group before May 7, 1990. Placebo/DZR patients were enrolled in the Placebo group after January 14, 1991, and crossed over to open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank; WCS = Wald chi-squared.

[c] DOX = Doxorubicin.

[d] The median is inestimable.

[e] Adjusted for age (>65 vs. ≤65), prior radiation to mediastinum, history of heart disease, hypertension, diabetes, and the last LVEF measurement prior to course seven within 10% above the lower limit of normal.

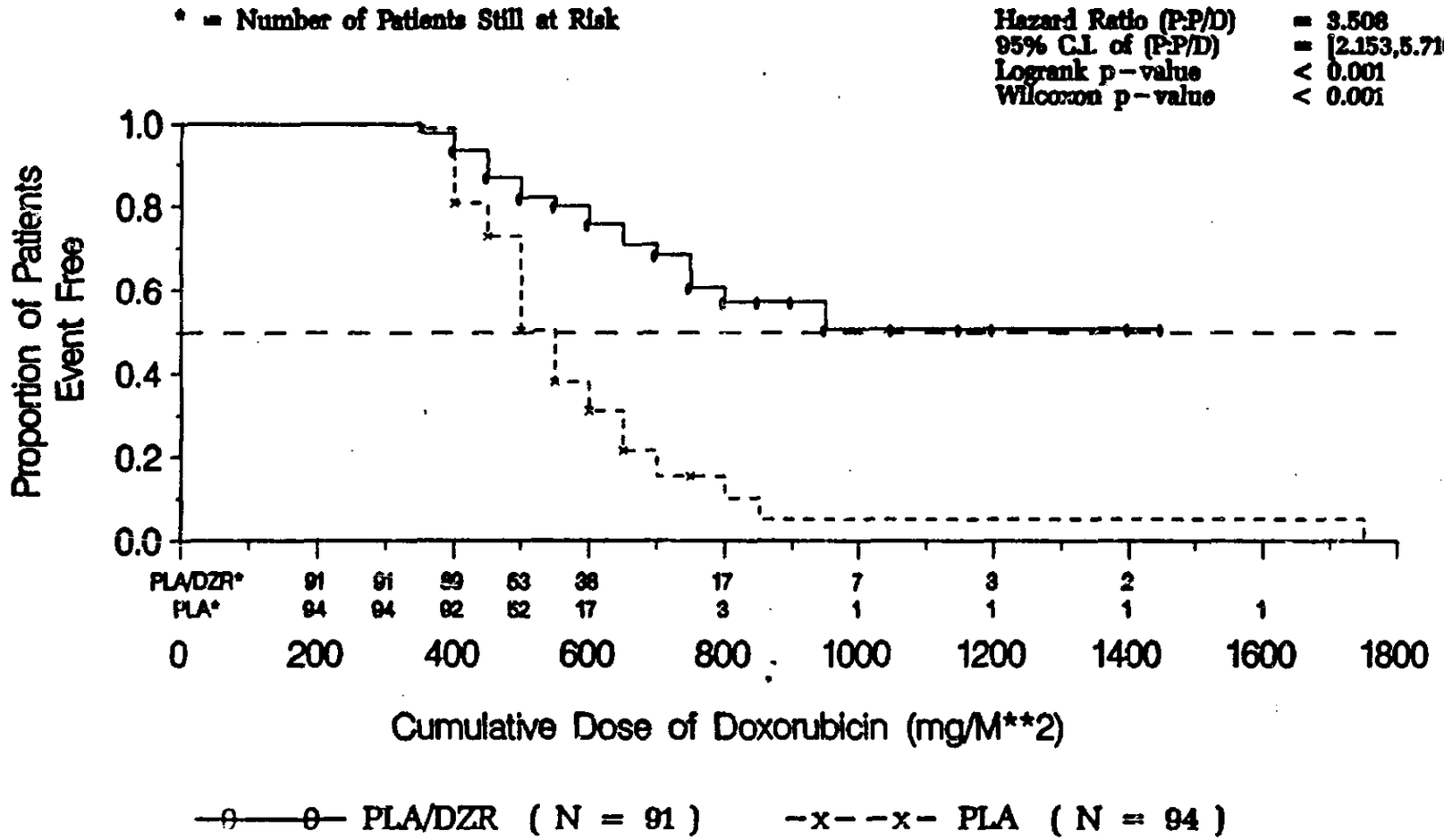
Figure 1

Time to Cardiac Event After Course Six – December 31, 1993

Placebo vs. Placebo/DZR Patients

Patients Receiving At Least One MUGA Scan After Seven Courses of Treatment

Study No. 088001 and 088006



It is interesting to compare the reviewer analysis, done with only MUGA events and using 'time since course 7' instead of 'total dose of Doxorubicin:'

Sponsor's Analysis of time to 'Cardiac Event' in Placebo/Placebo group versus Placebo/DZR group

VARIABLE	PLA/DZR	PLA	STATISTICAL TEST	
			Statistic	Result
No. Patients	91	94	[redacted]	[redacted]
No. Events	25	59	[redacted]	[redacted]
Median Event Time (mg/M2 of Dox)	NA	550	LR	P < 0.001
Hazard Ratio	3.51		GW	P < 0.001
95% c.i. of HR	(2.15, 5.72)			

Reviewer's Analysis of time to 'MUGA Event' in Placebo/Placebo group versus Placebo/DZR group

VARIABLE	PLA/DZR	PLA	STATISTICAL TEST	
			Statistic	Result
No. Patients	88	93	[redacted]	[redacted]
No. Events	29	58	[redacted]	[redacted]
Median Event Time (Days since course 7)	268 days	117 days	LR	P < 0.001
Hazard Ratio	1.97			
95% c.i. of HR	1.74, 2.51			

Although the hazard ratios are different, the number of patients, number of events, and the overall findings are consistent between the Reviewer and Sponsor analyses.

The comparison of cardiac events in early versus late randomization periods of the DZR arms of the studies is presented in the attached table 13 and figure 7. There are so few cardiac events in these arms that the hazard ratio (early:late) of 0.76 has very wide confidence intervals (0.31-1.91), clearly not significant (LR P=0.56).

A more extensive listing of reviewer analyses and figures is presented in the appendix. This includes a comparison of all periods of randomization, and a comparison of the DZR and PLA arms during the various periods. Again, these analyses were done using time to MUGA event without including the more subjective (but certainly very relevant) clinical findings.

The results of the Reviewer multivariate analysis using the MUGA event endpoint are presented in the following table:

Comparison Groups	Hazard ratio	95% ci	Adjusted[a] Hazard ratio	95% ci	Adjusted Statistic[b]
PLA:DZR (all patients)	2.57	{1.93-3.43}	2.55	{1.91-3.40}	P<0.001
Early:Late (DZR arm)	0.95	{0.62-1.45}	0.92	{0.60-1.41}	P= 0.69
Early:Late (PLA arm)	1.97	{1.55-2.51}	1.94	{1.53-2.47}	P<0.001

[a]Ad usted for 'Card 1'(presence of any several cardiac risk factors), age, and baseline LVEF <10% above Lower limit of Normal.
[b]Wald chi square.

TABLE 13

TIME TO CARDIAC EVENT AFTER COURSE SIX - DECEMBER 31, 1993
DZR VS. DZR/DZR PATIENTS[a]
PATIENTS RECEIVING AT LEAST ONE MUGA SCAN AFTER
SEVEN COURSES OF TREATMENT

STUDY NO. 088001 AND 088006

VARIABLE	DZR/DZR	DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	92	76		
No. Events (%)	11 (12%)	8 (11%)		
Median Event Time (mg/M^2 of DOX[c])	—[d]	—[d]		
Hazard Ratio (D:D/D)	0.765		LR	$\chi^2 = 0.34$ P = 0.56
95% C.I. of (D:D/D)	(0.307, 1.906)		GW	$\chi^2 = 1.12$ P = 0.29
Adjusted Hazard Ratio (D:D/D)[e]	0.488		WCS	$\chi^2 = 1.83$ P = 0.18
95% C.I. of Adjusted (D:D/D)[e]	(0.173, 1.381)			

[a] DZR patients were enrolled in the DZR group before May 7, 1990. DZR/DZR patients were enrolled in the DZR group after January 14, 1991, and began receiving open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank; WCS = Wald chi-squared.

[c] DOX = Doxorubicin.

[d] The median is inestimable.

[e] Adjusted for age (>65 vs. ≤65), prior radiation to mediastinum, history of heart disease, hypertension, diabetes, and the last LVEF measurement prior to course seven within 10% above the lower limit of normal.

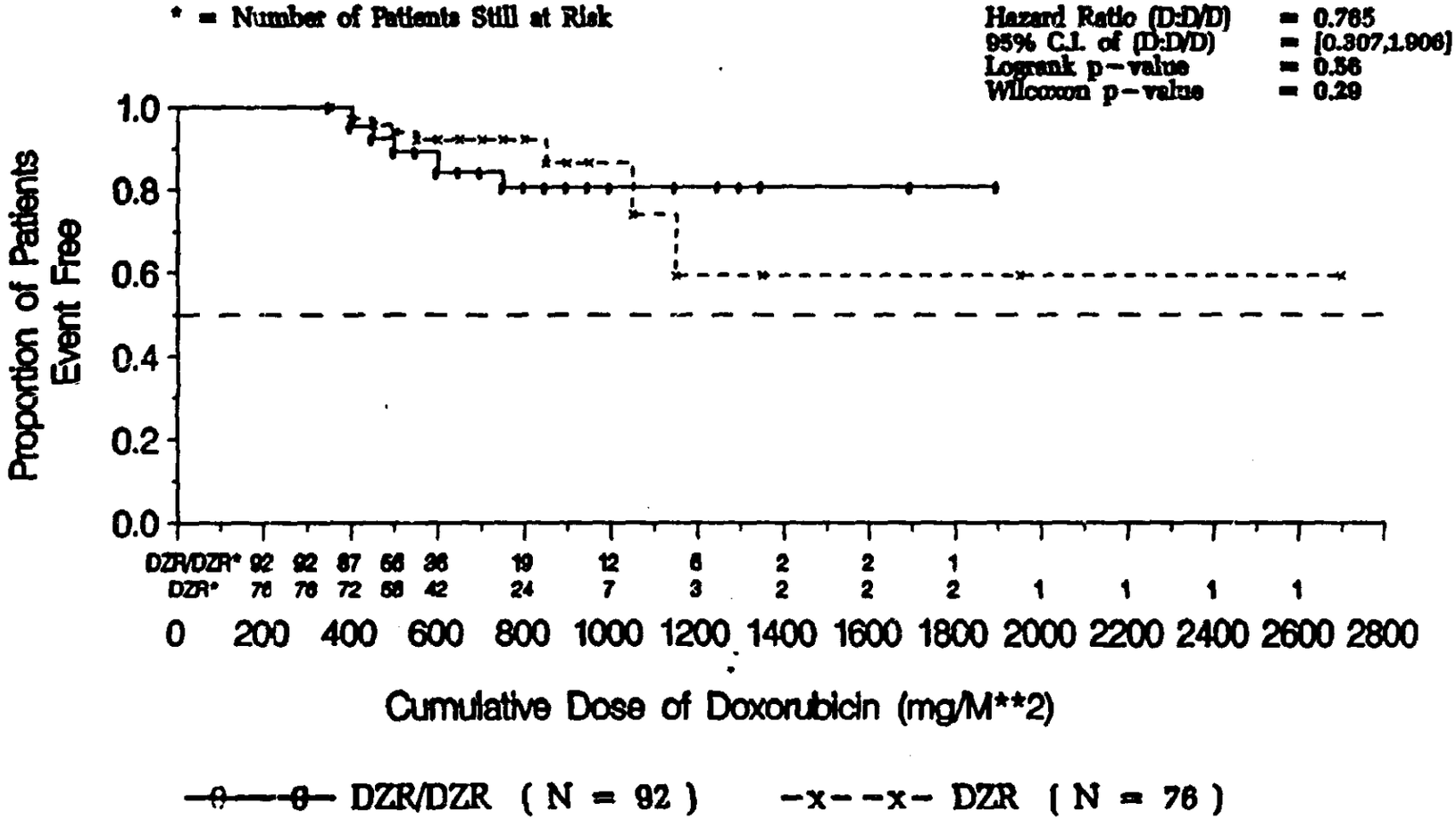
Figure 7

Time to Cardiac Event After Course Six – December 31, 1993

DZR vs. DZR/DZR Patients

Patients Receiving At Least One Muga Scan After Seven Courses of Treatment

Study No. 088001 and 088006



Sponsor's analysis of Tumor response:

As the sponsor notes, response occurred prior to course 7 an 80% of responders so that DZR given after course 6 is unlikely to affect response rates.

The following is extracted from the Sponsor's table 7:

RESPONSE RATES IN PATIENTS RECEIVING AT LEAST 7 COURSES OF DOXORUBICIN			
VARIABLE	**PLA/DZR**	**PLA**	**STATISTICS**
Patients with Measurable Dz			
No. Patients	68	87	
Response	52(76%)	74(85%)	P = 0.17

The sponsor's analysis of time to progression in patients receiving at least 7 courses of doxorubicin is summarized in attached table 8 and Figure 2. With about 100 patients in each arm, 60% to 70% on each arm had a progression event. The hazard ratio was near unity (0.94) with confidence intervals extending from 0.66 to 1.33 (P/P:P/D) and P = 0.72 by LR. When adjusted for prognostic factors (prior chemotherapy, last recorded response, number of disease sites, and disease measurability) the Hazard ratio leaned in favor of placebo at 0.77, but confidence intervals were wide (0.53 to 1.13) and the adjusted statistic was not significant (p=0.18).

The following Reviewer analyses utilized the individual sponsor data for date of progression. Details are slightly different from the sponsor's analyses due to different ways of selecting the group analyzed (see Reviewer methods in Appendix), but findings were similar. The reviewer analysis also looked at the DZR arm over the same early and late eras. The time to progression was actually longer in the Late DZR group, although this was not significant with prognostic factor adjustment. Additional reviewer anlyses of time to progression in various groups, including 'mid era' and all patient analyses are found in appendix C.

TABLE 8

TIME TO DISEASE PROGRESSION FROM THE SEVENTH COURSE
DECEMBER 31, 1993
PLACEBO VS. PLACEBO/DZR PATIENTS[a]
PATIENTS RECEIVING AT LEAST SEVEN COURSES OF TREATMENT
STUDY NO. 088001 AND 088006

VARIABLE	PLA/DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients[c]	102	99		
No. Events (%)	61 (60%)	68 (69%)		
Median Failure Time from the Seventh Course (Days)	265	250		
Hazard Ratio (P:P/D)	0.937		LR	$\chi^2 = 0.13$ P = 0.72
95% C.I. of (P:P/D)	(0.657, 1.337)		GW	$\chi^2 = 0.31$ P = 0.58
Adjusted Hazard Ratio (P:P/D)[d]	0.774		WCS	$\chi^2 = 1.77$ P = 0.18
95% C.I. of Adjusted (P:P/D)[d]	(0.531, 1.129)			

[a] Placebo patients were enrolled in the Placebo group before May 7, 1990. Placebo/DZR patients were enrolled in the Placebo group after January 14, 1991, and crossed over to open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank; WCS = Wald chi-squared.

[c] Five patients in the PLA arm progressed before the seventh course and eight patients in the PLA/DZR arm had not been assessed after the seventh course. These patients are censored at day one.

[d] Adjusted for prior chemotherapy at baseline, the last recorded disease response prior to course seven, and the number of disease sites and disease measurability at course six.

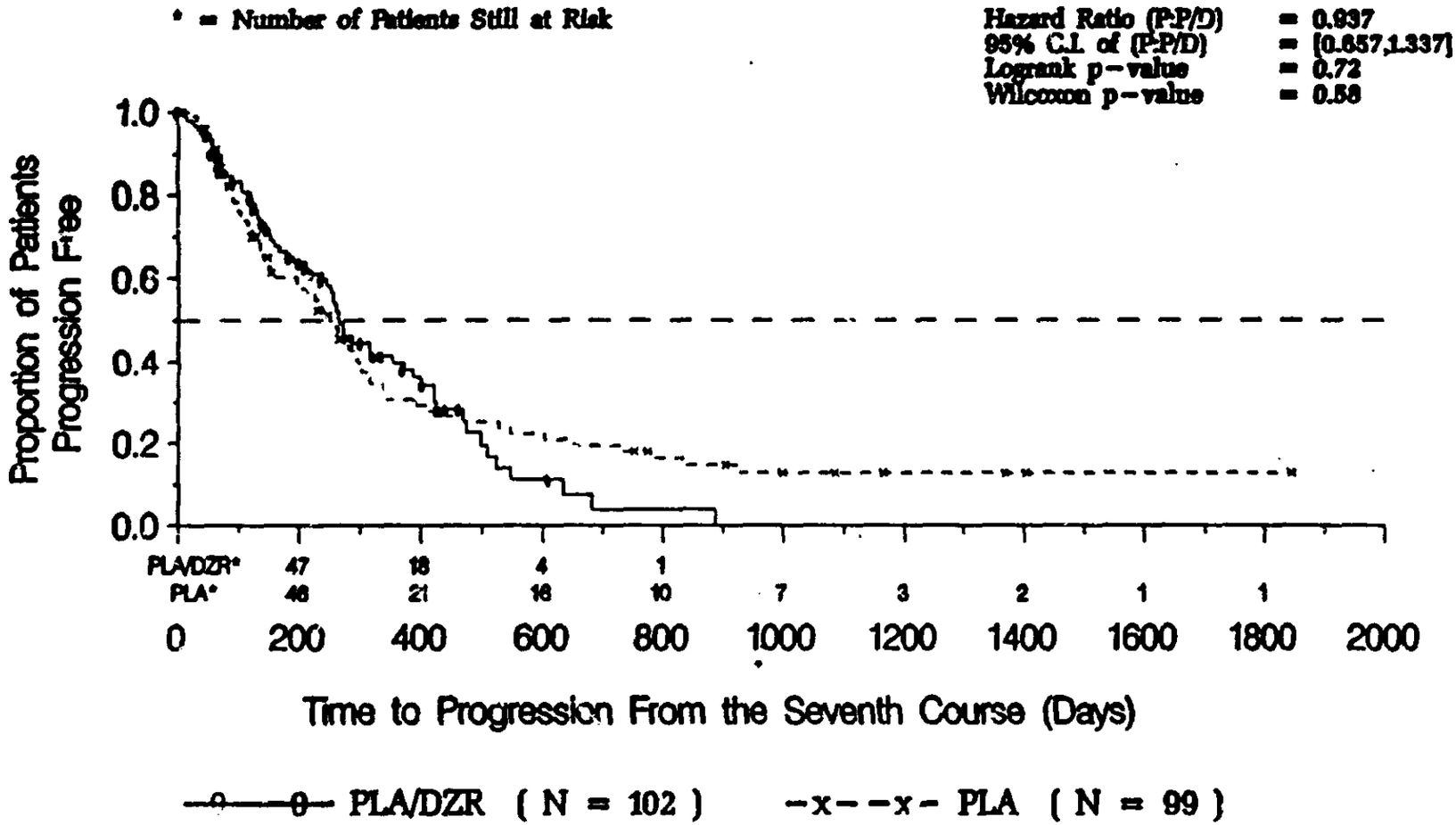
Figure 2

Time to Disease Progression From the Seventh Course – December 31, 1993

Placebo vs. Placebo/DZR Patients

Patients Receiving At Least Seven Courses of Treatment

Study No. 088001 and 088006



08-01919

TABLE 15

TIME TO DISEASE PROGRESSION FROM THE SEVENTH COURSE
DECEMBER 31, 1993
DZR VS. DZR/DZR PATIENTS[a]
PATIENTS RECEIVING AT LEAST SEVEN COURSES OF TREATMENT

STUDY NO. 088001 AND 088006

VARIABLE	DZR/DZR	DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients[c]	102	81		
No. Events (%)	72 (71%)	70 (86%)		
Median Failure Time from the Seventh Course (Days)	185	279		
Hazard Ratio (D:D/D)	0.722		LR	$\chi^2 = 3.57$ P = 0.059
95% C.I. of (D:D/D)	(0.514, 1.014)		GW	$\chi^2 = 3.04$ P = 0.081
Adjusted Hazard Ratio (D:D/D)[d]	0.710		WCS	$\chi^2 = 3.07$ P = 0.080
95% C.I. of Adjusted (D:D/D)[d]	(0.484, 1.042)			

[a] DZR patients were enrolled in the DZR group before May 7, 1990. DZR/DZR patients were enrolled in the DZR group after January 14, 1991, and began receiving open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank; WCS = Wald Chi-squared.

[c] One patient in the DZR arm and five patients in the DZR/DZR arm progressed before the seventh course, while one patient in the DZR/DZR arm had not been assessed after the seventh course. These patients are censored at day one.

[d] Adjusted for prior chemotherapy at baseline, the last recorded disease response prior to course seven, and the number of disease sites and disease measurability at course six.

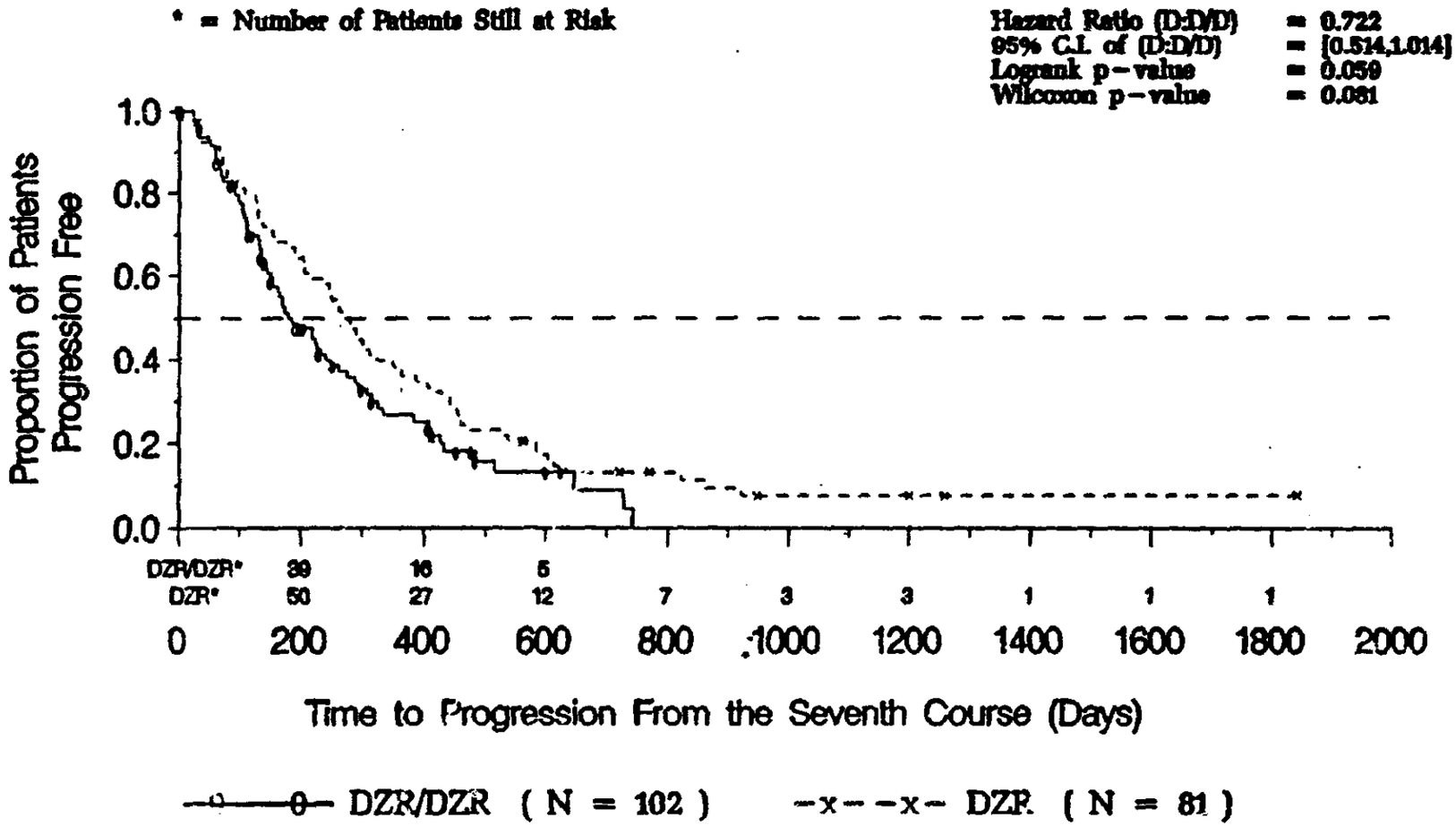
Figure 8

Time to Disease Progression From the Seventh Course – December 31, 1993

DZR vs. DZR/DZR Patients

Patients Receiving At Least Seven Courses of Treatment

Study No. 088001 and 088006



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08-01925

Reviewer Proportional-Hazards/Multivariate analysis of TTP of Placebo/DZR (Late) group versus Placebo/Placebo (early) group in Course-7 patients.

VARIABLE	PLA/DZR (late)	PLA (early)	STATISTICAL TEST	
			Statistic	Result
No. Patients	95	93		
No. Events	61	68		
Median Event Time (from Rand. Time)	405	376	WCS	P = 0.36
Hazard Ratio(L:E)	0.92			
95% c.i. of HR	(0.78, 1.1)			
Adjusted Hazard Ratio*	0.99			
95% c.i. of Adjusted HR	(0.8-1.2)		WCS	P = 0.91

*Adjusted for #lesions, Response at course 6, Tumor measurability, and prior chemotherapy.

Reviewer Proportional-Hazards/Multivariate analysis of TTP of Late DZR/DZR group versus Early DZR/DZR group in Course-7 patients.

VARIABLE	DZR/DZR (late)	DZR/DZR (early)	STATISTICAL TEST	
			Statistic	Result
No. Patients	92	79		
No. Events	71	69		
Median Event Time (from Rand. Time)	329	411	WCS	P = 0.03
Hazard Ratio(L:E)	1.2			
95% c.i. of HR	(1.01-1.42)			
Adjusted Hazard Ratio*	1.17			
95% c.i. of Adjusted HR	(0.97-1.4)		WCS	P = 0.11

*Adjusted for #lesions, Response at course 6, Tumor measurability, and prior chemotherapy.

The sponsor's tabes 10 & 17 and figures 4 & 10 (attached) present the survival analyses of early versus late periods in either the Placebo arm or the DZR arm in patients receiving at least 7 courses of Doxorubicin. The result is somewhat surprising; survival is superior in the late era versus the early era in the Placebo arm (HR 2.2; 95% ci 1.45-3.33) but not in the DZR arm (HR 0.94; 95% ci 0.53-1.13).

The reviewer analysis of survival is presented in appendix D. The purpose of this analysis was to assure that the sponsor's findings were not due to a chance finding from fragmentation of the data due to exclusion of patients by category (such as 'course-7' instead of all-patients analyses) or by time (exclusion of mid-era patients). Review of the tables and graphs suggests that the findings cannot be explained away by such considerations.

The unexpectedly good survival of the PLA/DZR group was also responsible for a nearly-significant superior survival of the PLA arm in the late period(PLA/DZR) versus the opposite randomized arm (DZR/DZR) during the same period (HR 0.76; ci 0.57-1.02, P=0.07; see Appendix D, p iv). The survival trend over time in the DZR:DZR/DZR arm was actually slightly in the opposite direction (HR L:E 1.18) than in the PLA:PLA/DZR arm (HR 0.67). The findings were not significantly altered by either the sponsor's multivariate analysis (prior chemotherapy, response at course 7, number of disease sites, or disease measurability)nor the reviewer's multivariate analysis (age, performance status, and number of tumor sites).

In summary, the non-randomized comparison of survival between the early placebo patients and the late placebo patients(who crossed over to DZR) demonstrates a statistically superior survival for the PLA/DZR group.

TABLE 10

SURVIVAL FROM THE SEVENTH COURSE - DECEMBER 31, 1993
PLACEBO VS. PLACEBO/DZR PATIENTS[a]
PATIENTS RECEIVING AT LEAST SEVEN COURSES OF TREATMENT

STUDY NO. 088001 AND 088006

VARIABLE	PLA/DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	102	99		
No. Dead (%)	33 (32%)	77 (78%)		
Median Survival Time from the Seventh Course (Days)	882	460		
Hazard Ratio (P:P/D)	2.197		LR	$\chi^2 = 14.53$ $P < 0.001$
95% C.I. of (P:P/D)	(1.451, 3.328)		GW	$\chi^2 = 14.81$ $P < 0.001$
Adjusted Hazard Ratio (P:P/D)[c]	2.016		WCS	$\chi^2 = 10.06$ $P = 0.002$
95% C.I. of Adjusted (P:P/D)[c]	(1.307, 3.108)			

[a] Placebo patients were enrolled in the Placebo group before May 7, 1990. Placebo/DZR patients were enrolled in the Placebo group after January 14, 1991, and crossed over to open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank; WCS = Wald chi-squared.

[c] Adjusted for prior chemotherapy at baseline, the last recorded disease response prior to course seven, and the number of disease sites and disease measurability at course six.

Figure 4
Survival From the Seventh Course – December 31, 1993
Placebo vs. Placebo/DZR Patients
Patients Receiving At Least Seven Courses of Treatment
Study No. 088001 and 088006

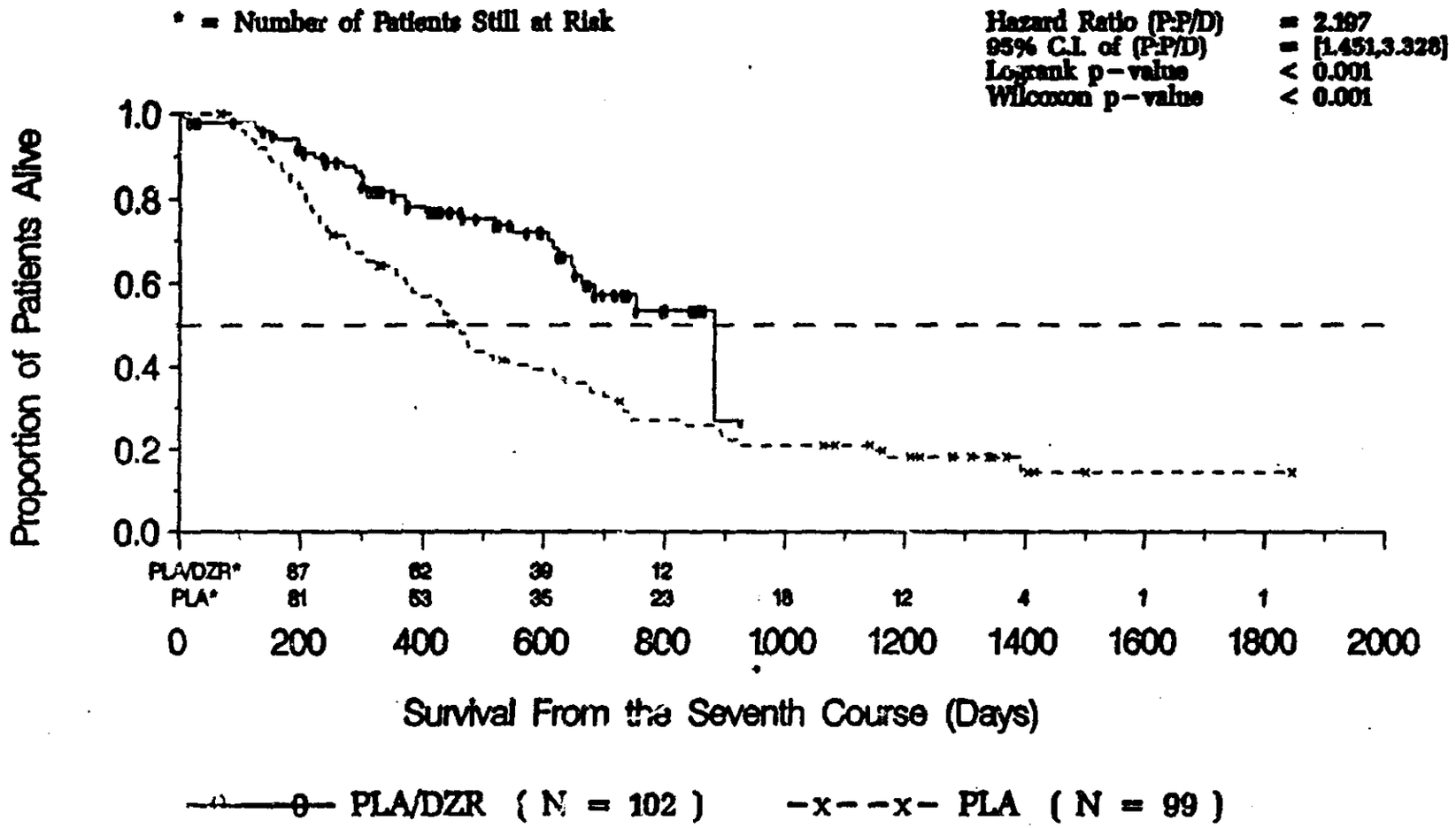


TABLE 17

SURVIVAL FROM THE SEVENTH COURSE - DECEMBER 31, 1993

DZR VS. DZR/DZR PATIENTS[a]

PATIENTS RECEIVING AT LEAST SEVEN COURSES OF TREATMENT

STUDY NO. 088001 AND 088006

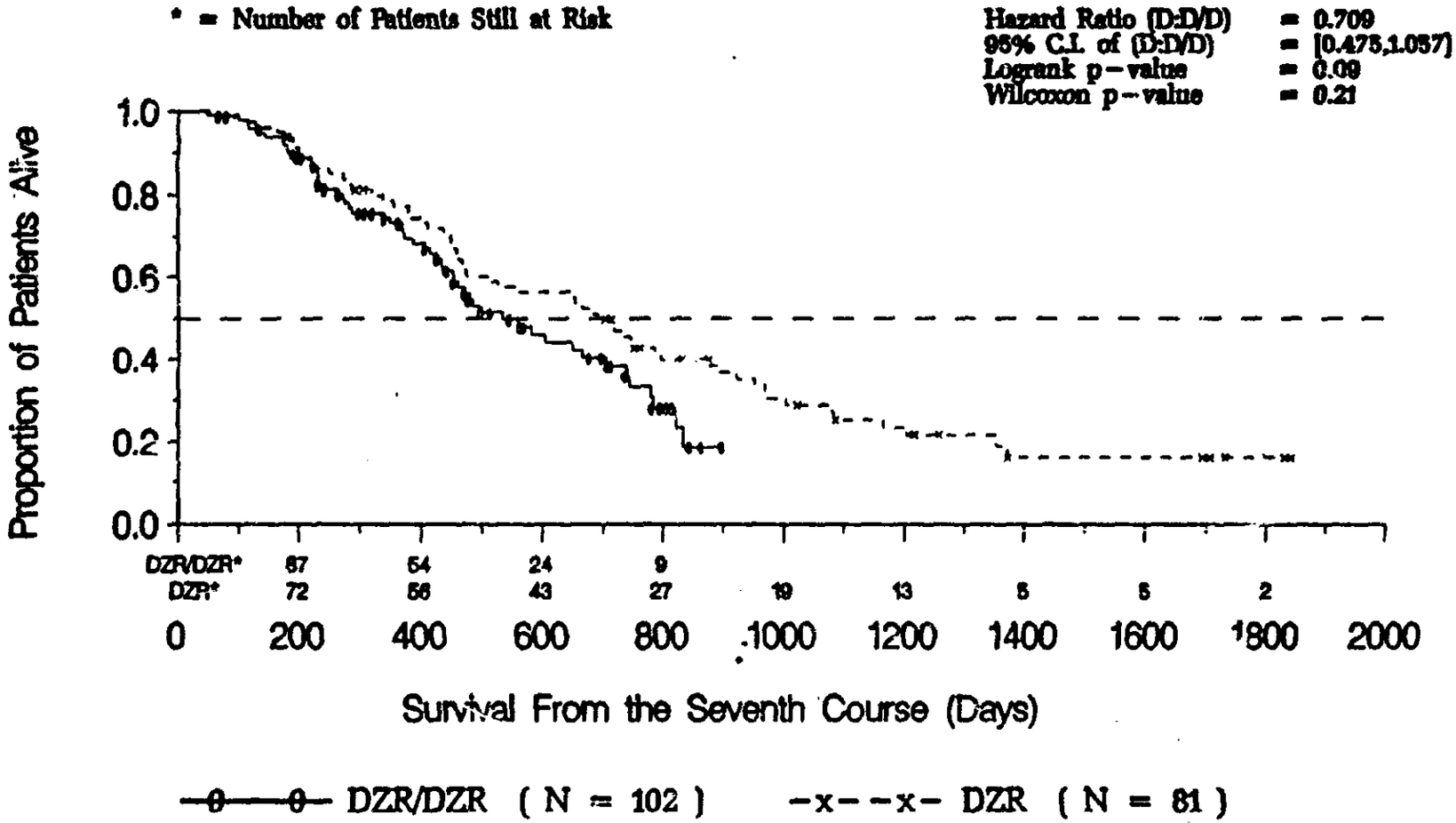
VARIABLE	DZR/DZR	DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	102	81		
No. Dead (%)	54 (53%)	59 (73%)		
Median Survival Time from the Seventh Course (Days)	534	692		
Hazard Ratio (D:D/D)	0.709		LR	$\chi^2 = 2.87$ P = 0.090
95% C.I. of (D:D/D)	(0.475, 1.057)		GW	$\chi^2 = 1.59$ P = 0.21
Adjusted Hazard Ratio (D:D/D)[c]	0.773		WCS	$\chi^2 = 1.30$ P = 0.25
95% C.I. of Adjusted (D:D/D)[c]	(0.498, 1.202)			

[a] DZR patients were enrolled in the DZR group before May 7, 1990. DZR/DZR patients were enrolled in the DZR group after January 14, 1991, and began receiving open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank; WCS = Wald chi-squared.

[c] Adjusted for prior chemotherapy at baseline, the last recorded disease response prior to course seven, and the number of disease sites and disease measurability at course six.

Figure 10
Survival From the Seventh Course – December 31, 1993
DZR vs. DZR/DZR Patients
Patients Receiving At Least Seven Courses of Treatment
Study No. 088001 and 088006



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08-01927

In table 11 and figure 5 (attached) the sponsor presents an analysis of number of courses of chemotherapy received by the PLA/PLA versus the PLA/DZR groups(in patients receiving at least 7 courses). The tail of the curve begins to favor the PLA/DZR after about 10 courses. The non-randomized comparison is significant (HR 1.71; 95% ci 1.27-2.29; LR P=0.002), and the comparison of the tails, if accurate, represents the relative numbers of patients who remain on study with late addition of DZR. Reasons for going offstudy would be primarily for progression or for cardiac event.

The sponsor's Figure 6 presents this analysis in terms of time rather than number of courses; whereas the median duration from course 7 appears similar on the 2 groups (123 versus 91 days), the twenty fifth percentile of the 2 arms appears separated by about 100 days.

Time Trends

The sponsor's table 18 (attached) presents a time-trend analysis within each of the course-7 arms for the various endpoints. A significant time trend was noted in the PLA arm for Cardiac Event (HR 0.86; 95% ci 0.77-0.97; P = 0.01). As noted in the footnote to the table, this indicates that the risk of a cardiac event was less by a factor of 0.86 if a patient was randomized 100 days later than another patient. This trend suggests that part of the observed difference in cardiac efficacy seen between PLA and PLA/DZR arms could be from a shift in unrecognized prognostic factors for cardiac toxicity over time instead of being entirely from addition of DZR.

TABLE 11

NUMBER OF COURSES - DECEMBER 31, 1993
PLACEBO VS. PLACEBO/DZR PATIENTS[a]
PATIENTS RECEIVING AT LEAST SEVEN COURSES OF TREATMENT

STUDY NO. 088001 AND 088006

VARIABLE	PLA/DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	102	99		
No. Off Study (%)	100 (98%)	99 (100%)		
Median Number of Courses	10	10		
Hazard Ratio (P:P/D)	1.499		LR	$\chi^2 = 9.55$ P = 0.002
95% C.I. of (P:P/D)	(1.121, 2.004)		GW	$\chi^2 = 4.58$ P = 0.032

[a] Placebo patients were enrolled in the Placebo group before May 7, 1990. Placebo/DZR patients were enrolled in the Placebo group after January 14, 1991, and crossed over to open label DZR after six courses of treatment.

[b] GW = Generalized Wilcoxon; LR = Logrank.

Figure 5
Number of Courses – December 31, 1993
Placebo vs. Placebo/DZR Patients
Patients Receiving At Least Seven Courses of Treatment
Study No. 088001 and 088006

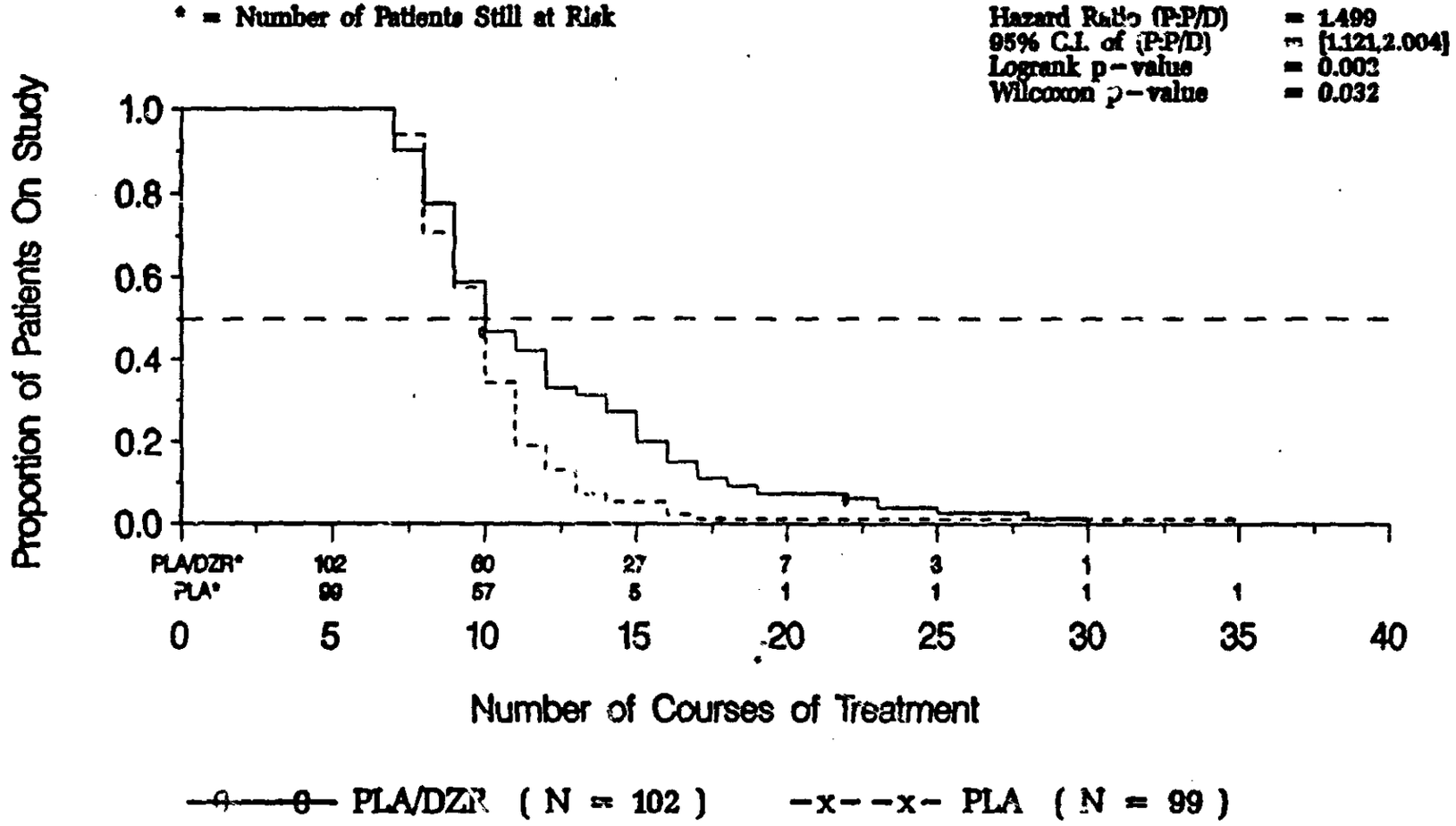


Figure 6
Duration on Study From the Seventh Course – December 31, 1993
Placebo vs. Placebo/DZR Patients
Patients Receiving At Least Seven Courses of Treatment
Study No. 088001 and 088006

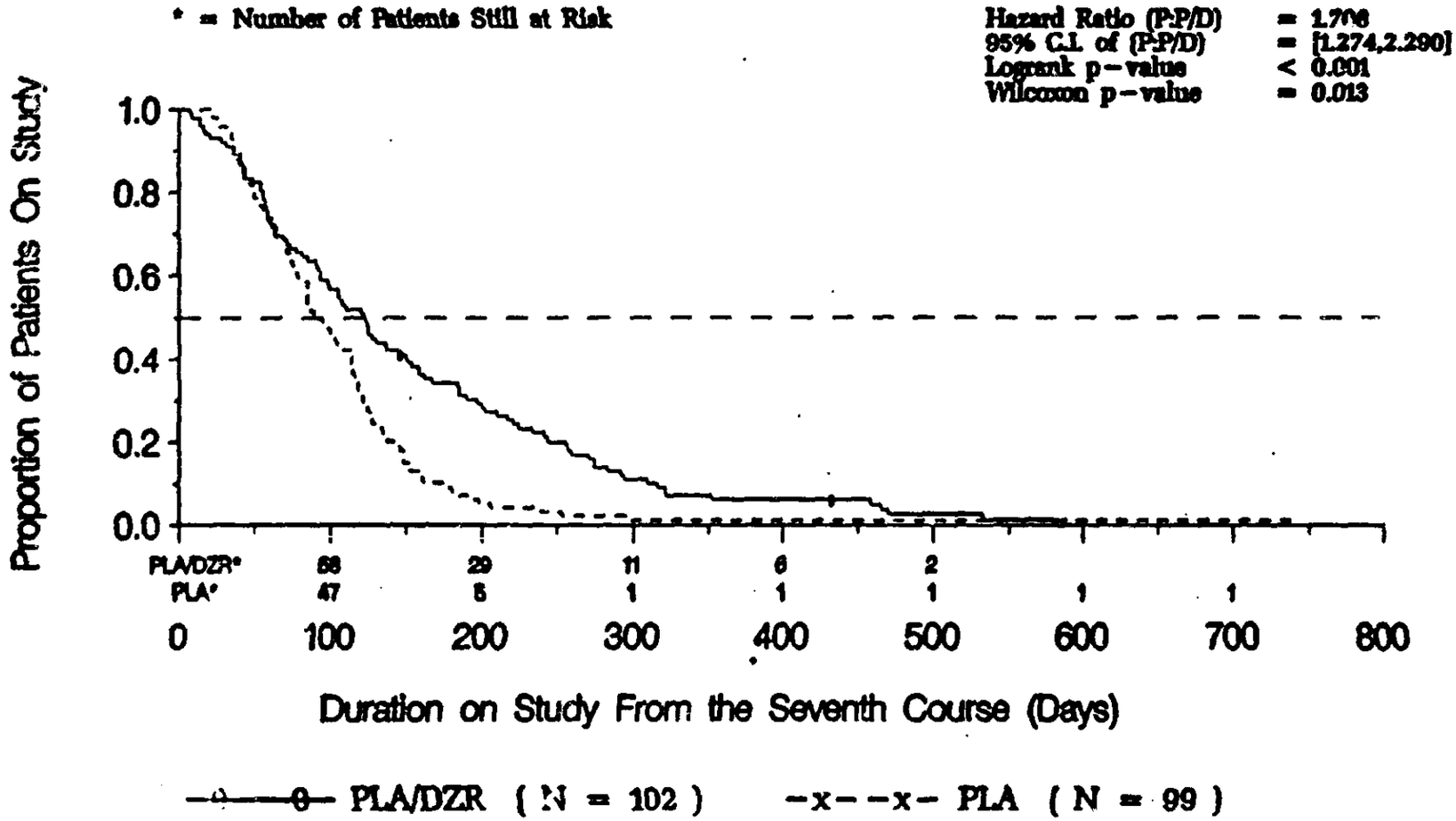


TABLE 18

THE EFFECT OF RANDOMIZATION DATE ON EFFICACY OUTCOMES IN PLACEBO PATIENTS AND IN PLACEBO/DZR PATIENTS[a]

STUDY NO. 088001 AND 088006

GROUP	NO. PATIENTS[b]	HAZARD RATIO[c]	95% C.I.[c]	STATISTICAL TEST	
				Statistic[d]	Result
Time to Cardiac Events After Course Six					
PLA/DZR	91	1.036	(0.837, 1.284)	WCS	$\chi^2 = 0.11$ P = 0.74
PLA	94	0.863	(0.769, 0.968)	WCS	$\chi^2 = 6.31$ P = 0.012
Time to Disease Progression from the Seventh Course					
PLA/DZR	102	1.107	(0.949, 1.291)	WCS	$\chi^2 = 1.68$ P = 0.20
PLA	99	0.947	(0.858, 1.046)	WCS	$\chi^2 = 1.16$ P = 0.28

[a] Placebo patients were enrolled in the Placebo group before May 7, 1990. Placebo/DZR patients were enrolled in the Placebo group after January 14, 1991 and crossed over to open label DZR after six courses of treatment.

[b] Patients receiving at least seven courses of treatment with at least one MUGA scan, if applicable.

[c] In units of 100 days. For example, a hazard ratio of 1.036 indicates that a patient randomized 100 days later in the PLA/DZR group has a risk of 1.036 of having a cardiac event compared with a patient randomized 100 days earlier in the PLA/DZR group. A hazard ratio of 0.863 indicates that a patient randomized 100 days later in the PLA group has a risk of 0.863 of having a cardiac event compared with a patient randomized 100 days earlier in the PLA group.

[d] WCS = Wald chi-squared.

TABLE 18 (Cont.)

THE EFFECT OF RANDOMIZATION DATE ON EFFICACY OUTCOMES IN PLACEBO PATIENTS AND IN PLACEBO/DZR PATIENTS[a]

STUDY NO. 088001 AND 088006

GROUP	NO. PATIENTS[b]	HAZARD RATIO[c]	95% C.L.[c]	STATISTICAL TEST	
				Statistic[d]	Result
Time to On Study Disease Progression from the Seventh Course					
PLA/DZR	102	1.078	(0.906, 1.284)	WCS	$\chi^2 = 0.72$ P = 0.40
PLA	99	0.952	(0.809, 1.119)	WCS	$\chi^2 = 0.36$ P = 0.55
Survival from the Seventh Course					
PLA/DZR	102	1.011	(0.795, 1.287)	WCS	$\chi^2 = 0.01$ P = 0.93
PLA	99	0.946	(0.862, 1.039)	WCS	$\chi^2 = 1.36$ P = 0.24

[a] Placebo patients were enrolled in the Placebo group before May 7, 1990. Placebo/DZR patients were enrolled in the Placebo group after January 14, 1991 and crossed over to open label DZR after six courses of treatment.

[b] Patients receiving at least seven courses of treatment with at least one MUGA scan, if applicable.

[c] In units of 100 days. For example, a hazard ratio of 1.036 indicates that a patient randomized 100 days later in the PLA/DZR group has a risk of 1.036 of having a cardiac event compared with a patient randomized 100 days earlier in the PLA/DZR group. A hazard ratio of 0.863 indicates that a patient randomized 100 days later in the PLA group has a risk of 0.863 of having a cardiac event compared with a patient randomized 100 days earlier in the PLA group.

[d] WCS = Wald chi-squared.

Sponsor's summary of Efficacy:

The following table reproduced from page 31 of the Sponsor's Integrated Summary presents the sponsor's summary. In this table Capital Letters indicate a leaning (ns = non-significant) or finding(s = significant) toward the groups randomized later (PLA/DZR or DZR/DZR). The point of this table is that the statistically significant findings of increased survival, number of courses, duration onstudy, and time to cardiac event noted between early (PLA) and late (PLA/DZR) placebo groups are not duplicated in the early (DZR) and late (DZR/DZR) groups.

	PLA vs. PLA/DZR	DZR vs. DZR/DZR
Time to Cardiac Event	S/S	ns/ns
Response	ns	ns
Time to Disease Progression	ns/ns	b/b
Time to On Study Disease Progression	NS/NS	ns/ns
Survival	S/S	ns/ns
Number of Courses	S	-
Duration On Study	S	-

S or s = Significant ($p \leq 0.05$).
B or b = Borderline significance ($0.05 < p \leq 0.10$).
NS or ns = Not significant ($p > 0.10$).

Unadjusted analysis/Adjusted analysis.

CAPITAL LETTERS favor the PLA/DZR or DZR/DZR group.
small letters favor the PLA or DZR group.

The sponsor presents an analysis of safety on pp. 31-40 of the integrated summary along with tables on pp 74-134. The comparison is between the early and late groups on the placebo arm (ie PLA versus PLA/DZR). Each of these groups is compared for toxicity during similar treatment periods, ie courses 1-6 for each group is compared and courses >7 are compared. This non-randomized comparison in small numbers of patients does not reveal any toxicities unique to late addition of DZR; a much better estimate of DZR toxicity can be found in the original NDA with many more patients compared in a randomized fashion.

Despite these caveats the following are a few of the findings:

Finding	PLA/DZR		PLA	
Pain on injection	13%	vs	0%	p=0.001
Congestive heart failure	1%	vs	10%	
Dysphagia	0%		5%	

Severe leukopenia and thrombocytopenia were more common on the PLA/DZR arm in later courses, but data was not collected equally on both arms due to protocol amendments. No differences in chemistries were noted.

6.2 Sponsor analysis of Congestive Heart Failure

The sponsor analysis of CHF is located on p 08-012747 of the NDA, in volume 7 of the Amendment:

Title: **A COMPARISON OF THE RISK OF CONGESTIVE HEART FAILURE IN PATIENTS TREATED WITH FAC WHO ALSO RECEIVED DZR, OR PLACEBO, OR PLACEBO FOLLOWED BY DZR FOR THE TREATMENT OF ADVANCED CARCINOMA OF THE BREAST.**

The sponsor undertook a retrospective analysis of cases of suspected congestive heart failure in the database. Cases were suspected of having congestive heart failure, and hence referred to a blinded referee cardiologist for evaluation when:

- the investigator reported that CHF was present

OR

- at least 2 of four of the following sets of criteria were present:

  -cardiomegaly by X-ray
  -basilar rales
  -S3 gallop
  -PND, and/or orthopnea, and/or dyspnea on exertion.

Records of such patients suspected of having CHF by these criteria were reviewed by a blinded cardiologist **"based on the patient's clinical manifestations and the LVEF levels"** and assigned a score on the following 5-point scale:

1= Total lack of suspicion of CHF.
2= Vague suspicion not based on clinical or lab findings.
3= Mild clinical or mild lab evidence.
4= Mild clinical and some lab evidence
5= Clinical and laboratory evidence of high probability.

A score of 3 or above was considered CHF, and the time of this score was noted by the referee and was used in time to event analyses.

Reviewer comments:

To become eligible for the analysis the investigator had to indicate either a suspicion of CHF or document signs of CHF. Thereafter, the minimal evidence needed by the referee for declaring CHF was mild clinical or mild lab (presumably a decrease in LVEF) evidence of CHF. One can imagine scenarios in which CHF classification could be based primarily upon LVEF measurement; hence some of the cases may not represent clinically symptomatic events.

The sponsor identified 3 groups of patients:

DZR/DZR: *422 patients receiving a 10:1 DZR/DOX ratio starting with the amendment of 11-2-88.*

PLA: *210 patient randomized before May 7, 1990 only 2 of which ever received any DZR.*

PLA/DZR 180 patients randomized to placebo after January 14, 1991 all of whom received DZR after a cumulative dose of 300 mg/M^2 of doxorubicin.

The analyses of time to CHF as demonstrated by Kaplan-Meier plots are shown in the Sponsor's table 1 and Figure 1(attached). The sponsor notes that of the 210 patients in the PLA group, 24 (11%) had CHF due to Adriamycin. The risk was 5% at a cumulative dose of 400 mg/M^2 of doxorubicin, 21% at 500 mg/M^2 of doxorubicin, and about 50% at 650 mg/M^2 of doxorubicin. By contrast, in the DZR patients only 3/422 (1%) had CHF and only 4/180 (2%) had CHF in the PLA/DZR arm.

The sponsor notes that the difference between the PLA group and the PLA/DZR group is significant ($p<0.001$ by LR, $p=0.003$ by GWC) but that the difference between the PLA/DZR and DZR groups is not significant ($p=0.14$ by LR, $p=0.48$ by GWC).

The sponsor concludes:

> "These data demonstrate the efficacy of DZR in reducing the risk of CHF if high cumulative doses of doxorubicin are planned as part of a treatment regimen. Delaying the introduction of DZR until a cumulative dose of 300 mg/M^2 of doxorubicin has been administered does not significantly reduce this type of cardioprotection."

Reviewer comments:

This analysis is somewhat different from that presented in the integrated summary of efficacy and safety:

> *-it has more emphasis on clinical findings, at least in the screening stage. Cases with isolated changes in LVEF would not have been sent for further evaluation. As a result there are fewer events.*
>
> *-The comparison group which received DZR includes the middle era patients in addition to early and late groups.*
>
> *-DZR comparison group excludes patients with 20:1 ratio therapy while they are included in some analyses in the integrated summary.*

TABLE 1

THE ESTIMATED CUMULATIVE PERCENTAGES[a] OF PATIENTS WITH CHF DUE TO ADRIAMYCIN[●]

Study 088001 and 088006

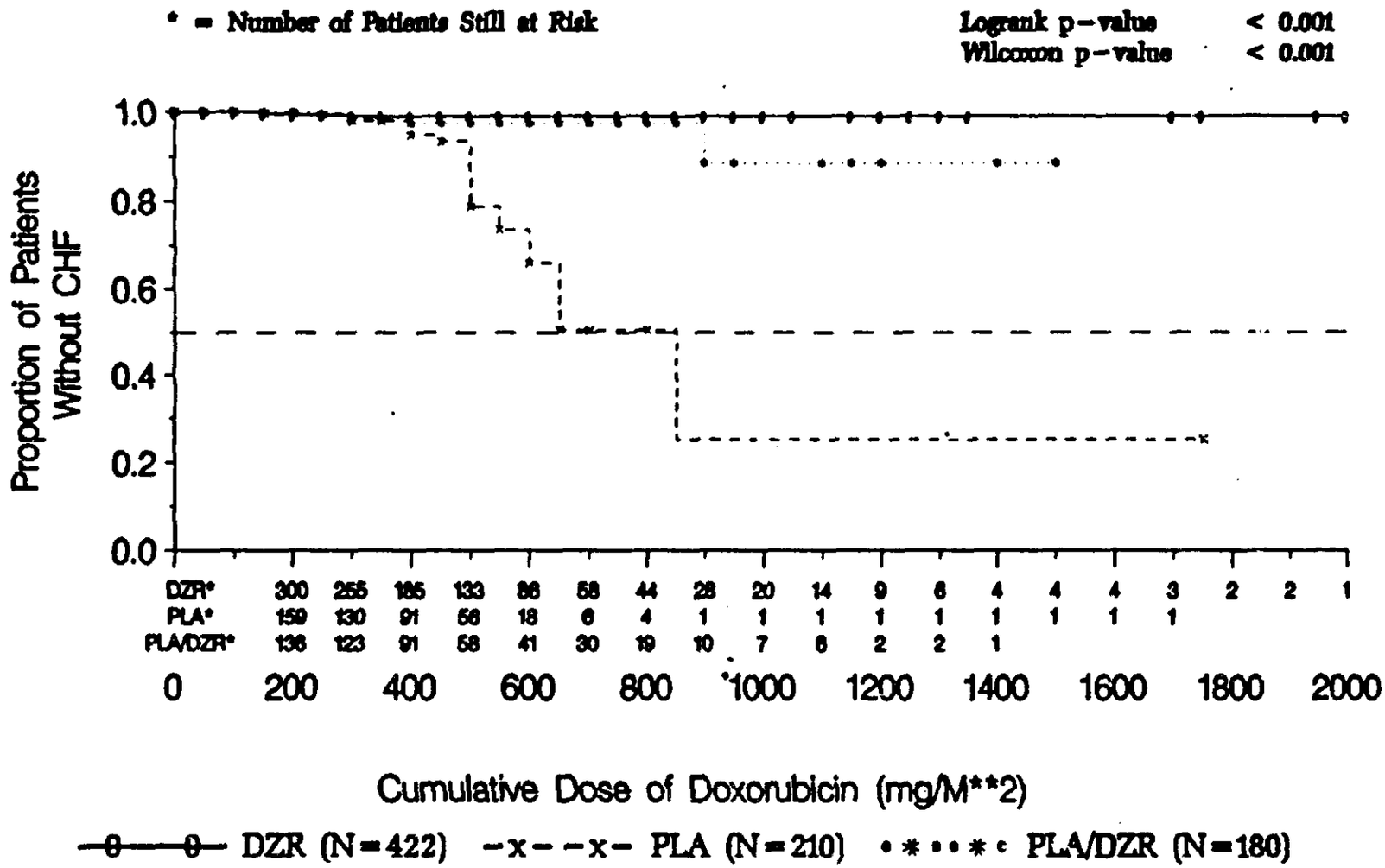
	DZR[b]		PLA[b]		PLA/DZR[b]	
Patients	422		210		180	
CHF Events	3		24		4	
Cumulative Dose of Doxorubicin (mg/M^2)	%	SE[c]	%	SE[c]	%	SE[c]
200	0.6	0.4	0.5	0.5	0.0	0.0
300	1.0	0.6	2.1	1.2	1.6	1.1
400	1.0	0.6	5.2	2.2	2.6	1.5
450	1.0	0.6	6.6	2.5	2.6	1.5
500	1.0	0.6	21.4	5.0	2.6	1.5
550	1.0	0.6	26.4	5.8	2.6	1.5
600	1.0	0.6	34.2	7.3	2.6	1.5
650	1.0	0.6	49.4	9.5	2.6	1.5
700	1.0	0.6	49.4	9.5	2.6	1.5
750	1.0	0.6	49.4	9.5	2.6	1.5
800	1.0	0.6	49.4	9.5	2.6	1.5
850	1.0	0.6	74.5	18.5	2.6	1.5
900	1.0	0.6	74.5	18.5	11.5	8.6

[a]Estimated using Kaplan-Meier plots.

[b]DZR patients are patients randomized to the DZR arm after November 2, 1988. PLA patients are patients randomized to the placebo arm prior to May 7, 1990. PLA/DZR patients are patients randomized to the placebo arm after January 14, 1991 who crossed over to open-label DZR after a cumulative doxorubicin dose of 300 mg/M^2.

[c]Standard error of estimated cumulative percentages.

Figure 1
Congestive Heart Failure Due to Adriamycin® – December 31, 1993
DZR vs. Placebo vs. Placebo/DZR Patients
Study No. 088001 and 088006



While this analysis does provide additional evidence of the efficacy of DZR when it is added to FAC after 300 mg/M^2 of doxorubicin, it does not give a clear measurement of symptomatic impact of therapy.

6.3 Sponsor's summary and conclusions:

The following points were noted in the sponsor's discussion and conclusions on pp 40-47 of the integrated summary:

- The risk of having a cardiac event at any cumulative dose of doxorubicin is 3.5 times as great for patients who did not receive DZR after course 6. Even the lower limits of the 95% confidence interval is over 2. While 63% of the patients who received placebo after course six had a cardiac event, only 27% of the patients receiving DZR after course 6 had a cardiac event.

- Only one of the 6 cardiac risk factors (baseline LVEF < 10% below LLN) was imbalanced; the hazard ratio was still greater than 3 with adjustment for prognostic factors.

- Response rates of nearly 80% in each group is expected, since patients remaining onstudy for 6 courses did not include non-responders who progressed prior to this time.

- According to the sponsor's analysis, patients without DZR progressed at a rate less than that of those who had addition of DZR (HR = 0.8) after adjustment of prognostic factors, but the difference was not significant, and the 95% confidence interval of the hazard ratio extended above 1.1.

- The risk of dying at any time after course 7 was 2.2 times as great for patients not receiving DZR after course 7. This difference was maintained when corrected for disease-related prognostic factors.

- The sponsor did a stratified analysis of survival according to disease-related prognostic factors, and found that the hazard ratio favored the PLA/DZR arm over the PLA/PLA arm in each of the strata (+/- prior chemotherapy, # of disease sites, +/- Response at course 7, +/- Measurable disease, and LVEF within 10% of LLN). The trend was especially strong within the stratum with a low baseline ejection fraction (HR 4.27; 95%ci 2.1 to 8.8; p<0.001).

- Patients receiving DZR at course 7 remained onstudy longer.

- Concurrent comparisons in the DZR arm of the study were undertaken. Changes in the placebo arm over time were not mirrored in the DZR arm. A significantly improved survival and time to cardiac event were seen on the PLA arm with time

but not on the DZR arm with time. The time to progression analysis which favored the earlier DZR group to the later DZR group was borderline statistically significant; the same analysis in the Placebo arm over time only favored the earlier group after prognostic factor adjustment, and the difference was not of borderline significance. These findings strengthen the contention that the cardiac event and survival findings were due to DZR instead of changes in the population over time.

- The sponsor notes that 32 of 81 patients in the early DZR group received the 20:1 ratio of DZR to Doxorubicin. When these 32 patients were excluded from the DZR:DZR/DZR analyses, the results were virtually unchanged as shown in the sponsors table reproduced from page 45 of the summary:

D/D/D

Variable	Number (Events)		Hazard Ratio P:P/D	(95% C.I.)	Adjusted Hazard Ratio P:P/D	(95% C.I.)	P-Value
	DZR/DZR	DZR					
Time to Cardiac Event	92 (11)	45 (7)	1.14	(0.44, 2.95)	0.88	(0.32, 2.42)	0.78* 0.81+
Time to Disease Progression	102 (72)	49 (42)	0.80	(0.54, 1.17)	0.76	(0.50, 1.16)	0.25* 0.20+
Time to On Study Disease Progression	102 (47)	49 (22)	0.84	(0.51, 1.40)	0.70	(0.41, 1.21)	0.51* 0.20+
Survival	102 (54)	49 (34)	0.71	(0.45, 1.13)	0.74	(0.44, 1.21)	0.15* 0.23+

*Unadjusted hazard ratio p-value based on the logrank test.
+Adjusted hazard ratio p-value based on the Wald chi-squared test.

- The sponsor also did a formal time trend test/ comparing outcome to date of randomization, within each of the groups. was done in both PLA/PLA and PLA/DZR groups for important endpoints.

"There was a statistically significant relationship between time enrolled in the study and the risk of a cardiac event for patients who crossed over to DZR after course 6 that favored the patients who were enrolled later. However, while not statistically significant, the time trend favored the placebo patients who did not received DZR after Course 6 who were enrolled earlier. The overall effect of these results also suggest that a time trend is not responsible for the longer time to cardiac event and survival for patients receiving a delayed dose of DZR."

Reviewer comments:
I believe the sponsor is in error on this summary point. As noted earlier in the sponsor's analysis, the significant time trend difference (HR L:E = 0.86, p = 0.01) favoring late to early enrollment was noted in the PLA/PLA group instead of the PLA/DZR group. The finding in the PLA/DZR group was slightly in the opposite direction (1.04, p=0.74).

- *The major differences noted in safety and laboratory parameters between the 2 groups compared after course 6 were more pain on injection and lower nadir counts with late DZR, and more investigator reports of congestive heart failure in the late placebo group.*

Sponsor's Conclusion:

The following is the sponsor's conclusion from page 47 of the Integrated Summary:

"Patients in studies 088001 and 088006 assigned to the placebo treatment who did not cross over to open label DZR after Course 6 were compared to patients in these two studies who were assigned to the placebo treatment and crossed over to open label DZR after Course 6. The risk of having a cardiac event was not as great for patients who received DZR and these patients also received more courses of treatment, remained on study and survived longer. The time to disease progression and response were comparable in the two groups. Placebo patients who did not cross over to DZR after Course 6 were enrolled in the study earlier than placebo patients who crossed over starting with course 7, but the results favoring the patients who crossed over do not appear to be merely the result of a time trend. As a result of crossing over to DZR after Course 6, these patients had lower nadir white blood cell and platelet counts, but their recovery counts were similar to those patients who continued to take placebo."

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8. Comments on proposed labeling:

Review of the labeling reveals 2 significant issues that need consideration:

Indication

The proposed indication section follows:

ZINECARD is indicated for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin in patients who have received potentially cardiotoxic doses of doxorubicin and who, in their physician's opinion, would benefit from continuing therapy with doxorubicin."

Issues for discussion include:

- Should the approval be limited to breast cancer? If not, what limitations should apply?

- Might not any dose of doxorubicin be considered potentially cardiotoxic and, therefore, would this wording imply that DZR is indicated for any use of doxorubicin? Should the indication recommend a minimum of 300 mg/M^2 of doxorubicin?

Adverse reactions and Safety findings in Clinical Trials Section

These sections of the labeling refer primarily to the retrospective comparison of PLA/PLA with PLA/DZR in courses after course 6. This results in a safety analysis with about 100 patients in each arm with little power and a suboptimal design to document the toxicity of DZR compared to PLA when given with FAC.

- The randomized comparison of early courses of DZR versus Placebo produced a database of about 1000 patients. The full analysis comparing toxicity of the 10:1 patients to concurrently treated Placebo patients should be included in the labeling. This is appropriate, since it is likely that patients receiving their first course of DZR at course 7 are likely to experience at least as much toxicity as patients in original NDA analyses exposed to DZR at course 1.

Conclusions:

The application should be discussed before the Oncology Drug Advisory Committee. The following are points which the committee might address.

1. Does the committee believe that the methodology and analyses presented demonstrate that DZR provides Cardioprotection when given initially after a cumulative dose of 300 mg/M^2 of doxorubicin?

2. Does the clinical significance of the cardioprotection outweigh the toxicity of DZR, primarily myelosuppression, demonstrated in the initial application?

3. Does the probability that doxorubicin is beneficial in advanced breast cancer after 300 mg/M^2 justify approval under the Accelerated Approval mechanism, given the protocol described in section 7 of this review? (This protocol randomizes 200 patients with breast cancer who have recently received 300 mg/M^2 of doxorubicin to either Doxorubicin plus DZR or to observation alone until progression.)

4. Is the proposed indication too broad?(Should a minimum prior dose of doxorubicin be specified; should the indication be limited to breast cancer?)

11-14-94
Grant A. Williams, MD

Robert L. Justice, M.D. 3/7/95

NDA 20-212
HFD-150 G Williams, M Pelosi, Div. File
HFD-340

Appendix A

1. Treatment Scheme:

Treatment Scheme

Randomized ARM	Time Period of Randomisation			
		1.Early	2.Mid	3.Late
	1.DZR	DZR/DZR	DZR/DZR	DZR/DZR
	2.PLA	PLA/PLA	PLA/Mixed	PLA/DZR

2. Number of patients in various analyises:

'Course-7' Analyses

Randomized ARM	Time Period of Randomisation				
		1.Early	2.Mid	3.Late	Total
	1.DZR	81	56	102	239
	2.PLA	99	56	102	257
	Both Arms	180	112	204	496

'All-Patient' Analyses

Randomized ARM	Time Period of Randomisation				
		1.Early	2.Mid	3.Late	Total
	1.DZR	195	121	173	489
	2.PLA	210	129	180	519
	Both Arms	405	250	353	1008

3. Diagram for Visualizing Comparisons:

Comparison Diagram (example DZR vs PLA)

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

(All = all pts., CS=Course 7 pts).

Patient Population:	All Patients
Groups compared:	PLA vs DZR
Comparison within:	All Periods

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

Reviewer analysis of survival of DZR group versus PLA group in All patients.

VARIABLE	DZR	PLA	STATISTICAL TEST	
			Statistic	Result
No. Patients	489	519		
No. Events	340	339		
Median Event Time (from Rand. Time)	557 d	568	LR	P = 0.26
Hazard Ratio (PLA:DZR)	0.92		GW	P = 0.27
95% c.i. of HR	0.79	1.07		
Adjusted Hazard Ratio*	0.96			
95% c.i. of Adjusted HR	0.82	1.11	WCS	P = 0.56

* Adjusted for age, performance status, and number of tumor sites.

^ GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	Course 7 Patients
Groups compared:	DZR arm vs PLA arm
Comparison within:	All periods

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			CS

Reviewer analysis of survival by randomized Arm in 'Course 7' patients

VARIABLE	DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	239	257		
No. Events	151	148		
Median Event Time (from Rand. Time)	706	748	LR	P = 0.58
Hazard Ratio	0.94		GW	P = 0.98
95% c.i. of HR	0.75	1.18		
Adjusted Hazard Ratio[a]	1			
95% c.i. of Adjusted HR	0.8	1.25	WCS	P = .99

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	DZR and PLA arms
Comparison within:	Late Period of Randomization(3)

	Period	1	2	3	
ARM	DZR			X	
	PLA			Y	
		Population			All

Reviewer analysis of survival of DZR group versus PLA group in Late Period

VARIABLE	DZR arm (DZR/DZR)	PLA arm (PLA/DZR)	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	173	180		
No. Events	100	82		
Median Event Time (from Rand. Time)	585	748	LR	P = .07
Hazard Ratio (PLA:DZR)	0.76		GW	P = .28
95% c.i. of HR	0.57	1.02		
Adjusted Hazard Ratio [a]	0.82			
95% c.i. of Adjusted HR	0.61	1.1	WCS	P = .18

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	Periods 1,2,3
Comparison within:	Combined Arms

	Period	1	2	3	
ARM	DZR	X	Y	Z	
	PLA	X	Y	Z	
		Population			All

Reviewer analysis of survival versus time period in All patients.

VARIABLE	Early Period	Middle Period	Late Period	STATISTICAL TEST	
				Statistic[b]	Result
No. Patients	405	250	353		
No. Events	318	179	182		
Median Event Time (from Rand. Time)	536	548	631	LR	0.05
				GW	0.04

GW = Generalized Wilcoxon. LR = Logrank

Patient Population:	Course 7 Patients
Groups compared:	Periods 1,2,3
Comparison within:	Combined Arms

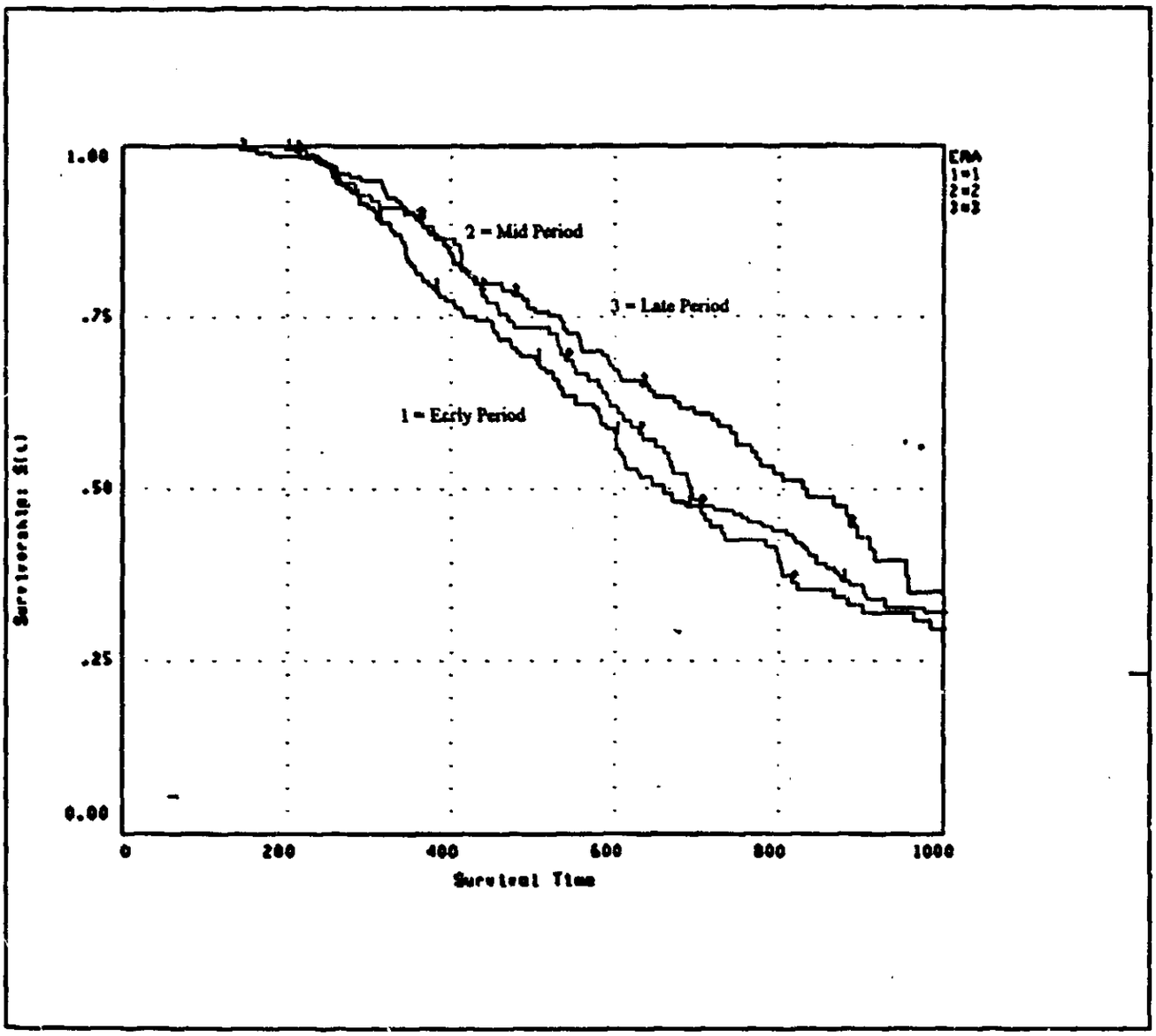
	Period	1	2	3	
ARM	DZR	X	Y	Z	
	PLA	X	Y	Z	
		Population			CS

Reviewer analysis of survival versus period of randomization in Course 7 patients

VARIABLE	Early Period	Middle Period	Late Period	STATISTICAL TEST	
				Statistic[b]	Result
No. Patients	180	112	204		
No. Events	136	76	87		
Median Event Time (from Rand. Time)	659	691	829	LR	P = 0.23
				GW	P = 0.11

GW = Generalized Wilcoxon. LR = Logrank

Survival by Period of Randomization in Course-7 patients (Arms Combined)



Patient Population:	All Patients
Groups compared:	Randomization periods 1,2,3.
Comparison within:	DZR arm

	Period	1	2	3	
ARM	DZR	X	Y	Z	
	PLA				
		Population			All

Reviewer analysis of survival versus time period in patients randomized to DZR arm.

VARIABLE	Early Period	Middle Period	Late Period	STATISTICAL TEST	
				Statistic[b]	Result
No. Patients	195	121	173		
No. Events	153	87	100		
Median Event Time (from Rand. Time)	529	535	585	LR	P = 0.36
				GW	P = 0.75

Patient Population:	All Patients
Groups compared:	Randomization periods 1,2,3.
Comparison within:	Placebo arm

	Period	1	2	3	
ARM	DZR				
	PLA	X	Y	Z	
		Population			All

Reviewer analysis of survival versus time period in patients randomized to Placebo arm.

VARIABLE	Early Period	Middle Period	Late Period	STATISTICAL TEST	
				Statistic[b]	Result
No. Patients	210	129	180		
No. Events	165	92	82		
Median Event Time (from Rand. Time)	536	556	748	LR	P = 0.02
				GW	P = 0.08

Patient Population:	All Patients
Groups compared:	Randomization Period 1 Pts. vs. Randomization period 3
Comparison within:	Combined Arms

	Period	1	2	3	
ARM	DZR	X		Y	
	PLA	X		Y	
		Population			All

Reviewer analysis of survival in Period 1 versus Period 3 patients.

VARIABLE	Period 1 (early)	Period 3 (late)	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	405	353		
No. Events	318	182		
Median Event Time (from Rand. Time)	536	631	LR	P = 0.03
Hazard Ratio (late:early)	0.9		GW	P = 0.02
95% c.i. of HR	0.82	0.98		
Adjusted Hazard Ratio[a]	0.91			
95% c.i. of Adjusted HR	0.83	1	WCS	P = 0.05

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	Randomization Period 1 vs. Period 3
Comparison within:	DZR Arm

	Period	1	2	3	
ARM	DZR	X		Y	
	PLA				
		Population			All

Reviewer analysis of survival in patients randomized to DZR in Period 1 versus Period 3.

VARIABLE	Period 1 (early) DZR/DZR	Period 3 (late) DZR/DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	195	173		
No. Events	153	100		
Median Event Time (from Rand. Time)	529	585	LR	P = 0.62
Hazard Ratio (late:early)	0.97		GW	P = 0.26
95% c.i. of HR	0.85	1.1		
Adjusted Hazard Ratio[a]	0.97			
95% c.i. of Adjusted HR	0.89	1.07	WCS	P = 0.67

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	Randomization Period 1. vs. Randomization period 3
Comparison within:	Placebo

	Period	1	2	3	
ARM	DZR				
	PLA	X		Y	
		Population			All

Reviewer analysis of survival in patients randomized to Placebo in Period 1 versus Period 3.

VARIABLE	Period 1 (early) PLA/PLA	Period 3 (late) PLA/DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	210	180		
No. Events	165	82		
Median Event Time (from Rand. Time)	536	748	LR	0.007
Hazard Ratio (late:early)	0.83		GW	0.025
95% c.i. of HR	0.72	0.94		
Adjusted Hazard Ratio[a]	0.86			
95% c.i. of Adjusted HR	0.75	0.98	WCS	P = 0.005

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	Course 7 patients
Groups compared:	Randomization Period 1 vs. Randomization Period 3
Comparison within:	DZR Arm

	Period	1	2	3	
ARM	DZR	X		Y	
	PLA				
		Population			CS

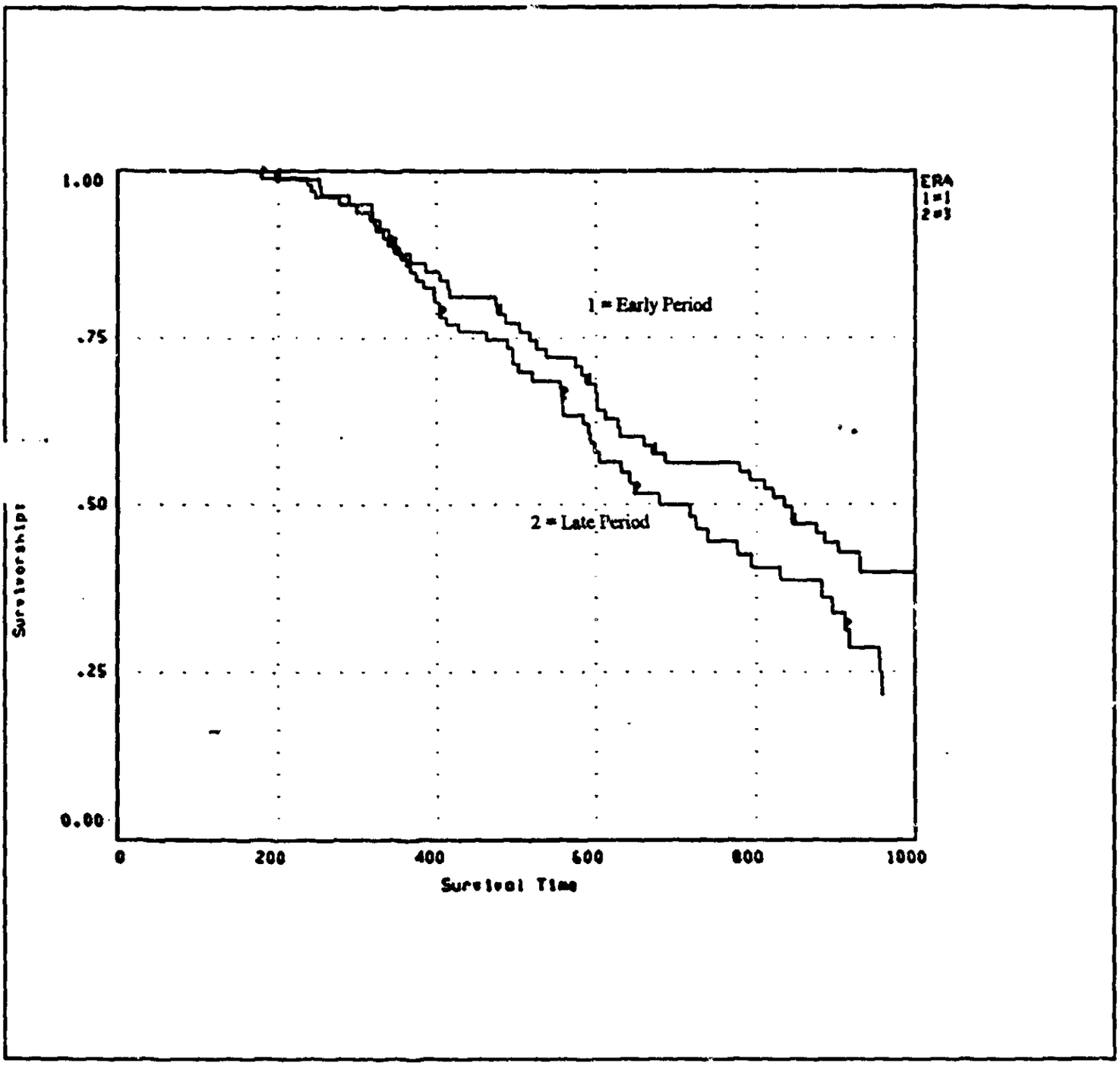
Reviewer analysis of survival in Course-7 patients randomized to DZR in Period 1 versus Period 3.

VARIABLE	Period 1 (early) DZR/DZR	Period 3 (late) DZR/DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	81	102		
No. Events	59	54		
Median Event Time (from Rand. Time)	832	680	LR	0.12
Hazard Ratio (late:early)	1.18		GW	0.22
95% c.i. of HR	0.97	1.44		
Adjusted Hazard Ratio[a]	1.19			
95% c.i. of Adjusted HR	0.98	1.46	WCS	0.085

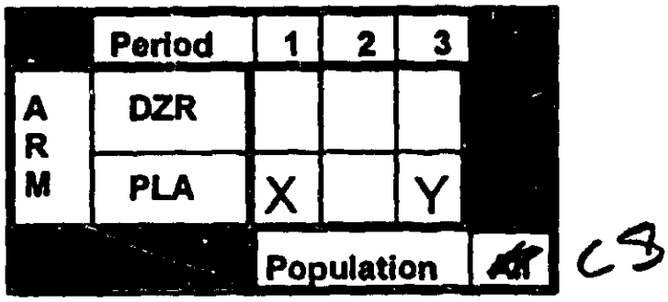
[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Survival of DZR Arm, Course-7 patients, early period vs late period of Randomisation.



Patient Population:	**Course 7 patients**
Groups compared:	**Randomization Period 1 Pts. versus Randomization period 3**
Comparison within:	**PLA Arm**



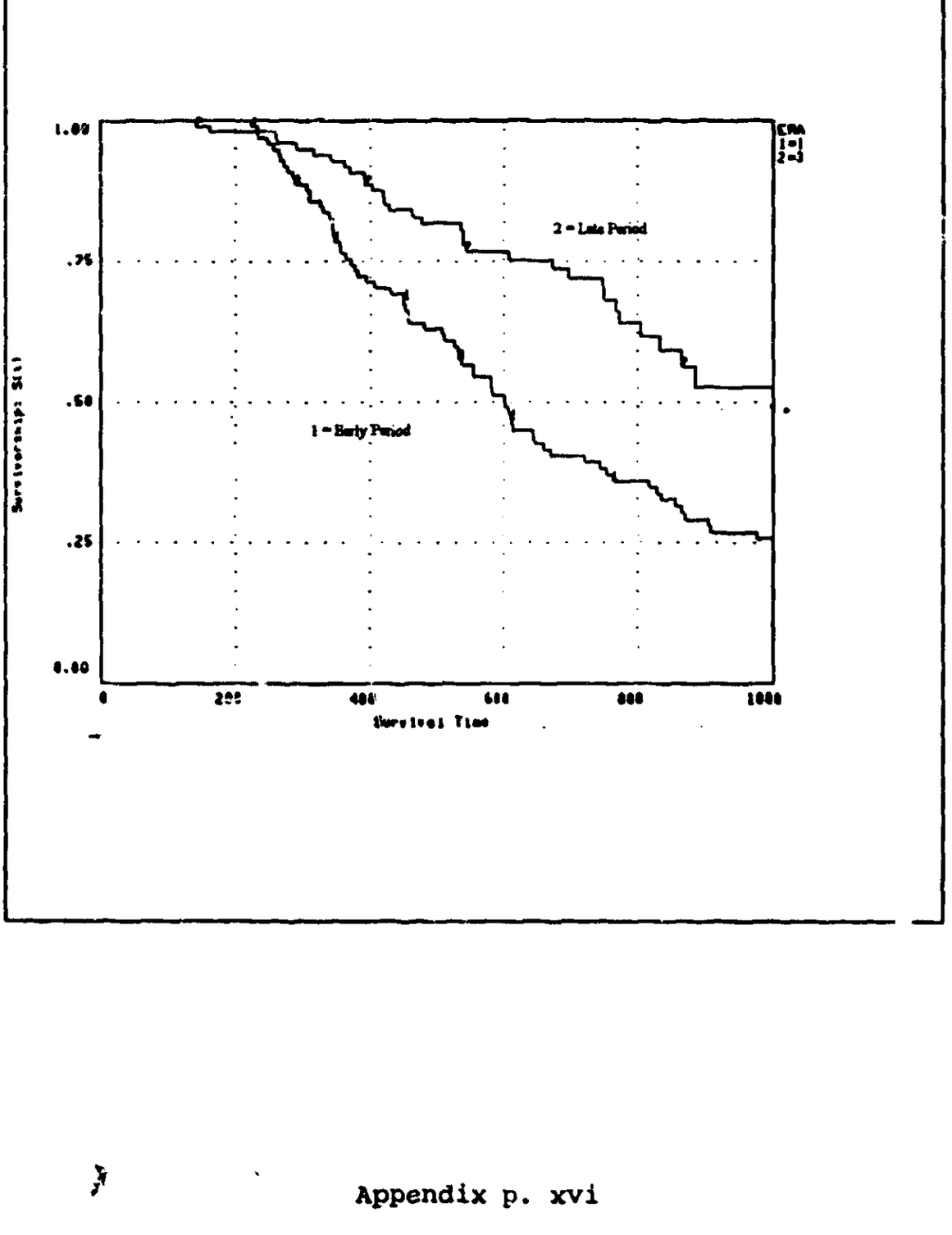
Reviewer analysis of survival in Course-7 patients randomized to Placebo in Period 1 versus Period 3.

VARIABLE	Period 1 (early) PLA/PLA	Period 3 (late) PLA/DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	99	102		
No. Events	77	33		
Median Event Time (from Rand. Time)	601	897	LR	0.001
Hazard Ratio (late:early)	0.67		GW	0.002
95% c.i. of HR	0.83	0.55		
Adjusted Hazard Ratio[a]	0.71			
95% c.i. of Adjusted HR	0.87	0.57	WCS	P = 0.001

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Survival in Placebo arm, course-7 patients; Period 1(PLA/PLA) vs Period 3 (PLA/DZR).



Appendix B: Reviewer Analysis of Time To MUGA Event

Review of Cardiac Endpoint criteria:

Cardiac risk factors identified in protocol:
-mediastinal RT
-Age over 65
-Hx heart disease (MI, significant arrythmia)
-HTN requiring medication
-Diabetes requiring medication
-Baseline MUGA 1-10% above lower limit of normal for institution.

One of the stratification factors was presence or absence of cardiac risk factors.

Pertinent Inclusion criteria:
-No previous chemotherapy for metastatic disease.
-No prior anthracycline or anthracene.

Criteria for early termination:

- chf with at least 2 of the following:
  - -cardiomegaly by xray.
  - -basilar rales.
  - -S3 Gallop.
  - -PND or orthopnea or exertional dyspnea.
- LVEF decrease by 0.10 to below normal
- Decline by at least 0.20.
- Decline to less than 0.05 below lower limits of institution.

Note primary endpoint was to be decline by 0.10 in LVEF. This was later changed to time to cardiac event.

Evaluation of cardiotoxicity was to be done at 150, 300, 400, and every 50 mg/m2.

With the change to 10:1 ratio, a repeat muga scan was required for the patient to go offstudy.

Reviewer method for determining time to MUGA event.

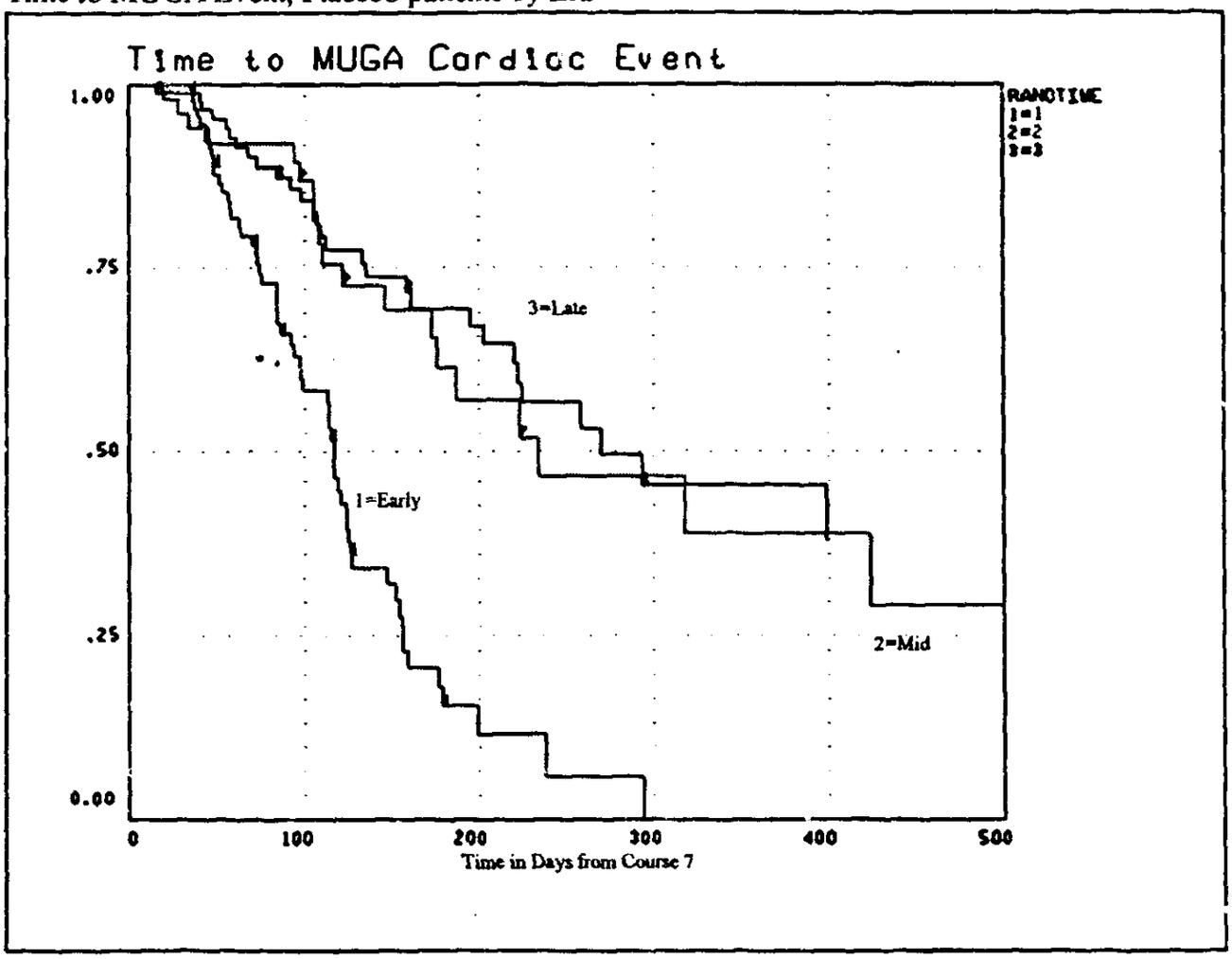
The following independent reviewer analysis of this endpoint from the primary data involved the following:

- Failure was confined to change in cardiac ejection fraction over time and did not include clinical symptoms and signs.
- Repeat ejection fraction verification was not required.
- The X axis of analyses was expressed in time rather than in dose of doxorubicin.

Time to MUGA Cardiac event, PLACEBO ARM by Era of Randomization

Study Era	Total # Patients	# MUGA Events	# Censored	Median Time to Event (from Dose 7)	LogRank test (p value)
1-Early	93	58	35	117	0
2-Mid	52	20	32	227	
3-Late	88	29	59	268	

Time to MUGA Event, Placebo patients by Era



This is suggestive but not absolute proof: One still could theorize that adverse prognostic factors were responsible for late era poor performance on placebo arm. Further, the lack of such a trend in the DZR arm could be from obliteration of toxicity in all DZR patients lifting them to an insensitive portion of the dose-toxicity curve.

To test this, a multivariate analysis was done in the full group of patients and was then applied to early and late cohorts. Several different groupings of the sponsor's list of covariates were tested from inclusive to exclusive. The most inclusive grouping correlated best with time to event and was used in the multivariate analysis with age and baseline LVEF.

Comparison Groups	Hazard ratio	95% ci	Adjusted[a] Hazard ratio	95% ci	Adjusted Statistic[b]
PLA:DZR (all patients)	2.57	{1.93-3.43}	2.55	{1.91-3.40}	P<0.001
Early:Late (DZR arm)	0.95	{0.62-1.45}	0.92	{0.60-1.41}	P= 0.69
Early:Late (PLA arm)	1.97	{1.55-2.51}	1.94	{1.53-2.47}	P<0.001

[a]Ad usted for 'Card 1'(presence of any several cardiac risk factors), age, and baseline LVEV <10% above Lower limit of Normal.
[b]Wald chi square.

Appendix C: Reviewer Analysis of Time To Progression

Methods for Reviewer TTP analyses:

1008 patients included 994 d atients in dosing table. Progression date was obtaine 762 patients from the REV_RESP table as the minimum date for which these patients had a listing of PD. The other patients were censored at the last visit listed in either the t_meas or t_n_meas tables. Progression time was measured from date of Randomization.

Using this method, 958 patients had at least one followup visit in the tumor evaluation tables and form the data base for the time to progression graphs and analyses.

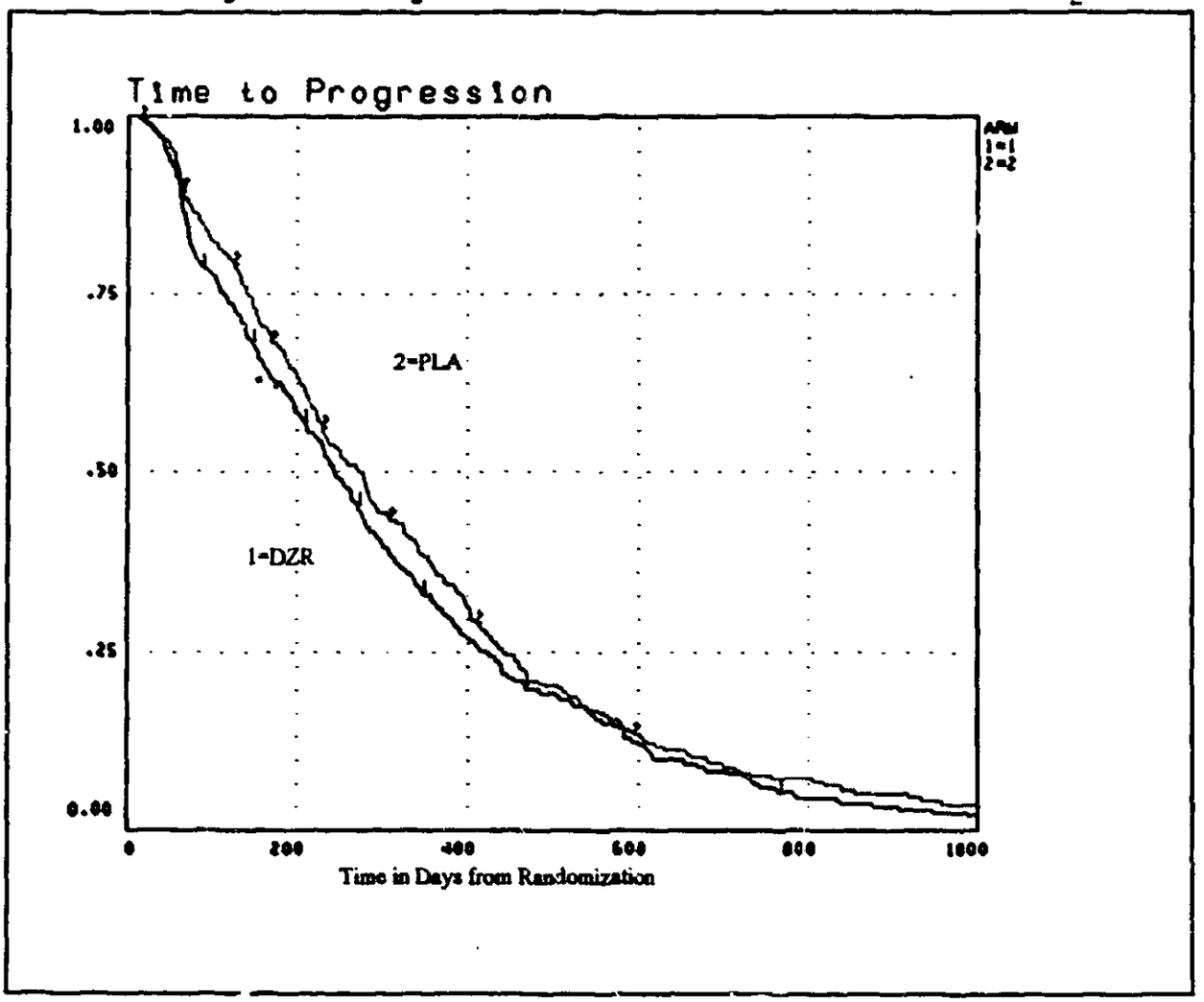
466 patients form the subgroup of patients with who received at least 7 courses.

Comparison of time to progression (measured from date of randomization) is shown for all 958 patients in the following 2 analyses, first done by Randomized arm, then by study era of randomization.

Study Arm	Total # Patients	# Progressed	# Censored	Median Time to Progression	Statistical Test*
1-DZR	458	379	79	244	LR: 0.11 GW: 0.06
2-PLACEBO	500	381	119	272	

*P value for Log Rank (LR) and Gehans-Wilcoxon tests as generated by NCSS Statistical Package.

Time to Progression by arm in all Randomized Patients

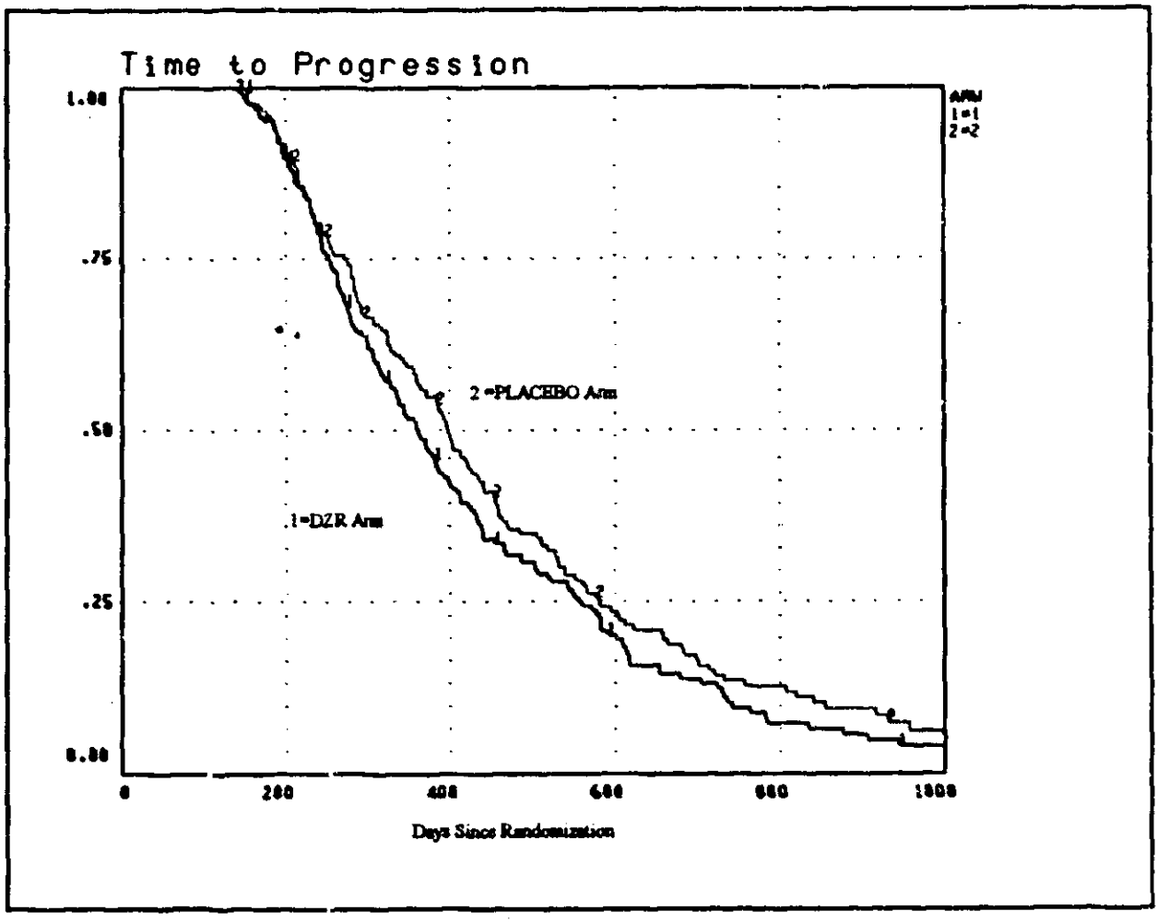


Time to Progression by Study Arm in all Patients who received at least 7 courses of Doxorubicin.

Study Arm	Total # Patients	# Progressed	# Censored	Median Time to Progression	Statistical Test*
1 -DZR	226	188	38	361	LR:0.19 GW:0.21
2-PLACEBO	240	174	66	397	

*P value for Log Rank (LR) and Gehans-Wilcoxon tests as generated by NCSS Statistical Package.

TTP by Study Arm in All Patients receiving at least 7 Courses of Doxorubicin

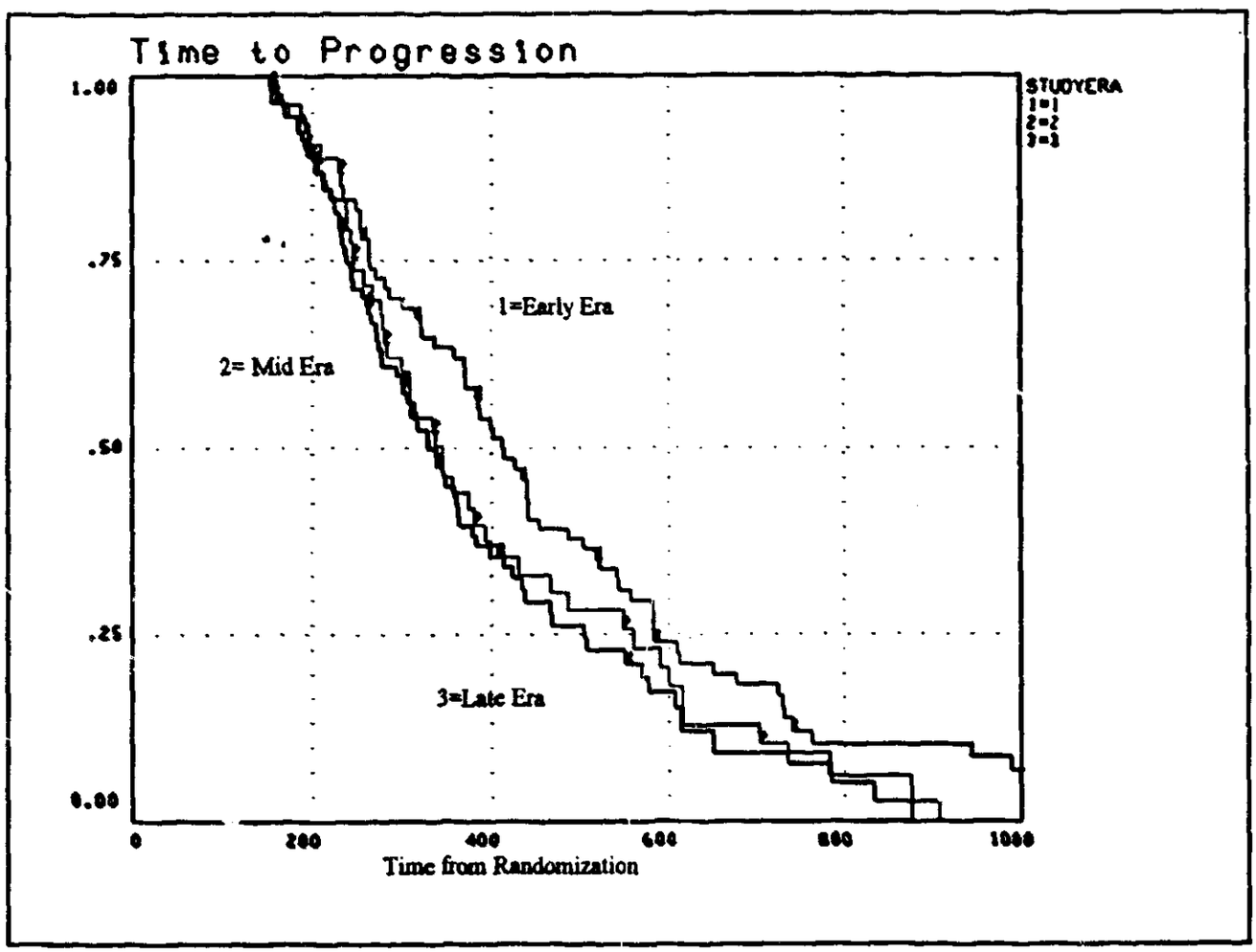


Time to Progression, DZR ARM by Era of Randomization, Patients with at least 7 courses of Doxorubicin.*

Study Era	Total # Patients	# Progressing	#Censored	Median TTP*	Statistical Test**
1-Early	79	69	10	411	LR = 0.07
2-Mid	55	47	8	337	GW = 0.14
3-Late	92	71	21	329	

*TTP measured from time of randomization..
**P value for Log Rank (LR) and Gehana-Wilcoxon tests as generated by NCSS Statistical Package.

Time to Progression, DZR ARM by Era of Randomization, Patients with at least 7 courses of Doxorubicin.

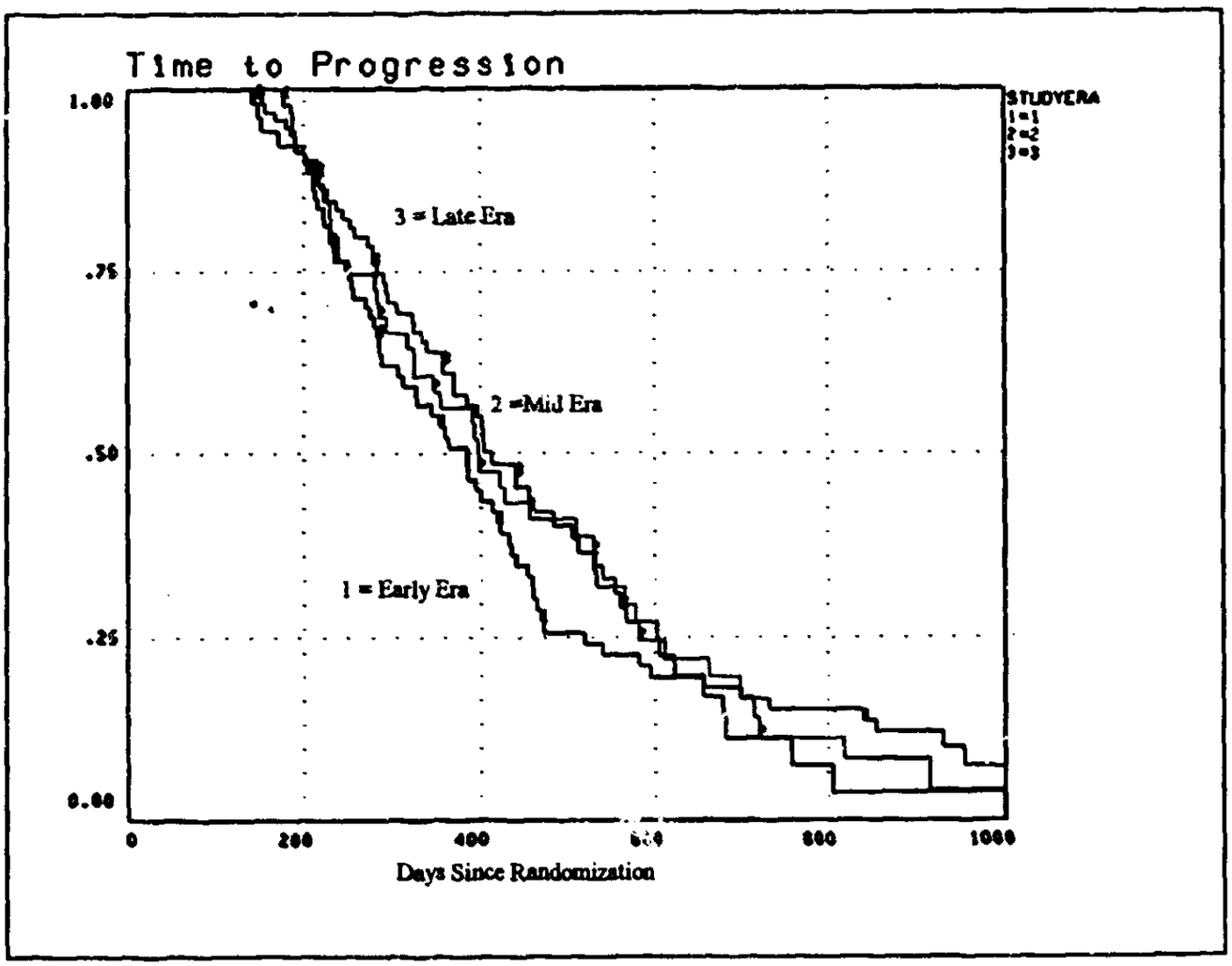


Time to Progression, PLACEBO ARM by Era of Randomization, Patients with at least 7 courses of Doxorubicin.*

Study Era	Total # Patients	# Progressing	#Censored	Median TTP	Statistical Test**
1-Early	93	68	25	376	LR = 0.94
2-Mid	52	44	8	396	GW = 0.53
3-Late	95	61	34	405	

*TTP measured from time of Randomization.
**P value for Log Rank (LR) and Gehanne-Wilcoxon tests as generated by NCSS Statistical Package.

Time to Progression, PLACEBO ARM by Era of Randomization, Patients with at least 7 courses of Doxorubicin.



Appendix D: Reviewer Analysis of Survival:

1. **Numbers of patients in different Survival Analyses:**

'Course-7' Analyses

Randomized ARM	Time Period of Randomization				
		1.Early	2.Mid	3.Late	Total
	1.DZR	81	56	102	239
	2.PLA	99	56	102	257
	Both Arms	180	112	204	496

'All-Patient' Analyses

Randomized ARM	Time Period of Randomization				
		1.Early	2.Mid	3.Late	Total
	1.DZR	195	121	173	489
	2.PLA	210	129	180	519
	Both Arms	405	250	353	1008

2. **Diagram for Visualizing Comparisons:**

Comparison Diagram (example DZR vs PLA)

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

(All = all pts., CS=Course 7 pts).

Patient Population:	All Patients
Groups compared:	PLA vs DZR
Comparison within:	All Periods

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

Reviewer analysis of survival of DZR group versus PLA group in All patients.

VARIABLE	DZR	PLA	STATISTICAL TEST	
			Statistic	Result
No. Patients	489	519		
No. Events	340	339		
Median Event Time (from Rand. Time)	557 d	568	LR	P = 0.26
Hazard Ratio (PLA:DZR)	0.92		GW	P = 0.27
95% c.i. of HR	0.79	1.07		
Adjusted Hazard Ratio*	0.96			
95% c.i. of Adjusted HR	0.82	1.11	WCS	P = 0.56

* Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	Course 7 Patients
Groups compared:	DZR arm vs PLA arm
Comparison within:	All periods

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			CS

Reviewer analysis of survival by randomized Arm in 'Course 7' patients

VARIABLE	DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	239	257		
No. Events	151	148		
Median Event Time (from Rand. Time)	706	748	LR	P = 0.58
Hazard Ratio	0.94		GW	P = 0.98
95% c.i. of HR	0.75	1.18		
Adjusted Hazard Ratio[a]	1			
95% c.i. of Adjusted HR	0.8	1.25	WCS	P = .99

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	DZR and PLA arms
Comparison within:	Late Period of Randomization(3)

	Period	1	2	3	
ARM	DZR			X	
	PLA			Y	
		Population			All

Reviewer analysis of survival of DZR group versus PLA group in Late Period

VARIABLE	DZR arm (DZR/DZR)	PLA arm (PLA/DZR)	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	173	180		
No. Events	100	82		
Median Event Time (from Rand. Time)	585	748	LR	P = .07
Hazard Ratio (PLA:DZR)	0.76		GW	P = .28
95% c.i. of HR	0.57	1.02		
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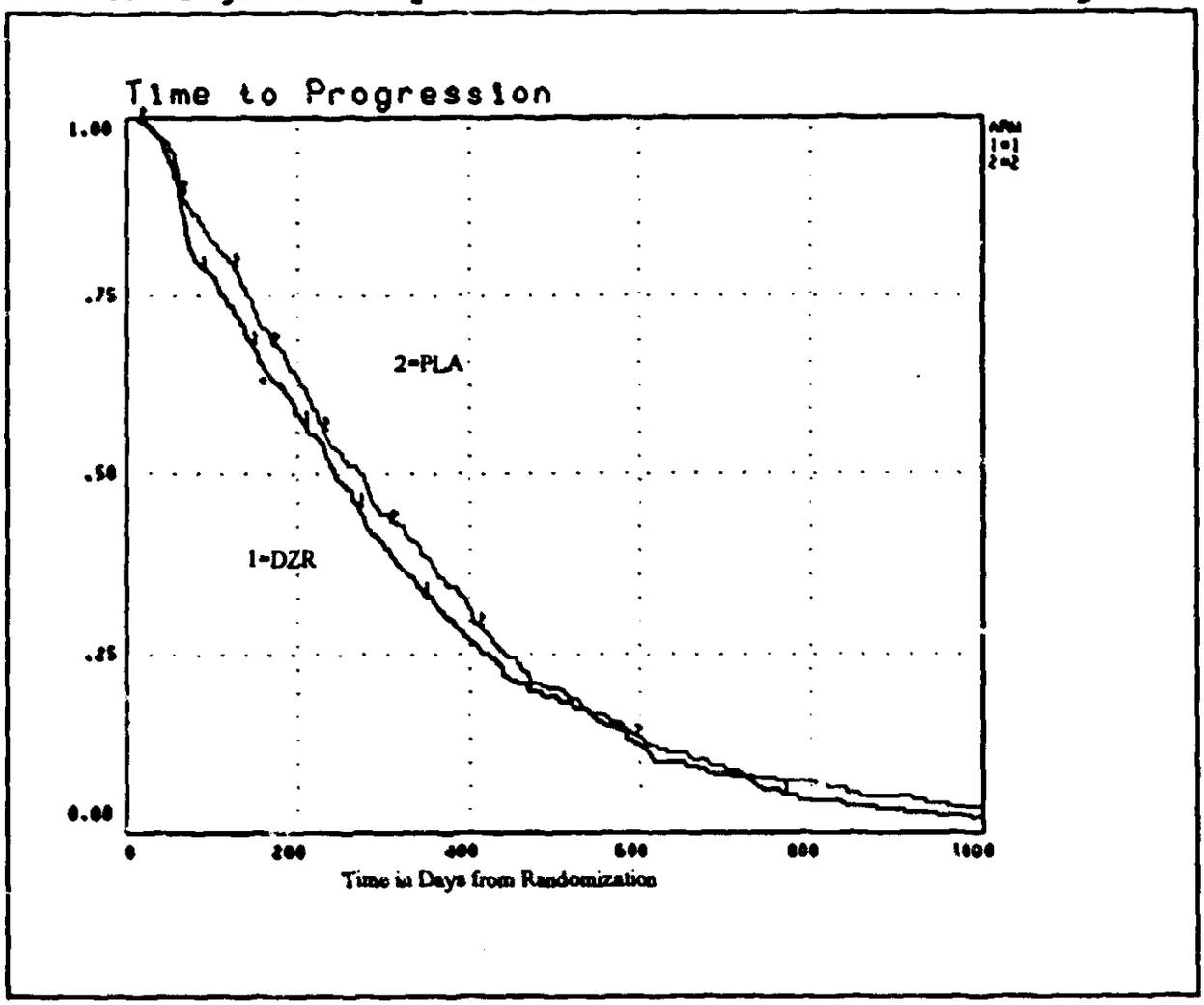
[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Study Arm	Total # Patients	# Progressed	# Censored	Median Time to Progression	Statistical Test*
1-DZR	458	379	79	244	LR: 0.11 GW: 0.06
2-PLACEBO	500	381	119	272	

*P value for Log Rank (LR) and Gehans-Wilcoxon tests as generated by NCSS Statistical Package.

Time to Progression by arm in all Randomized Patients



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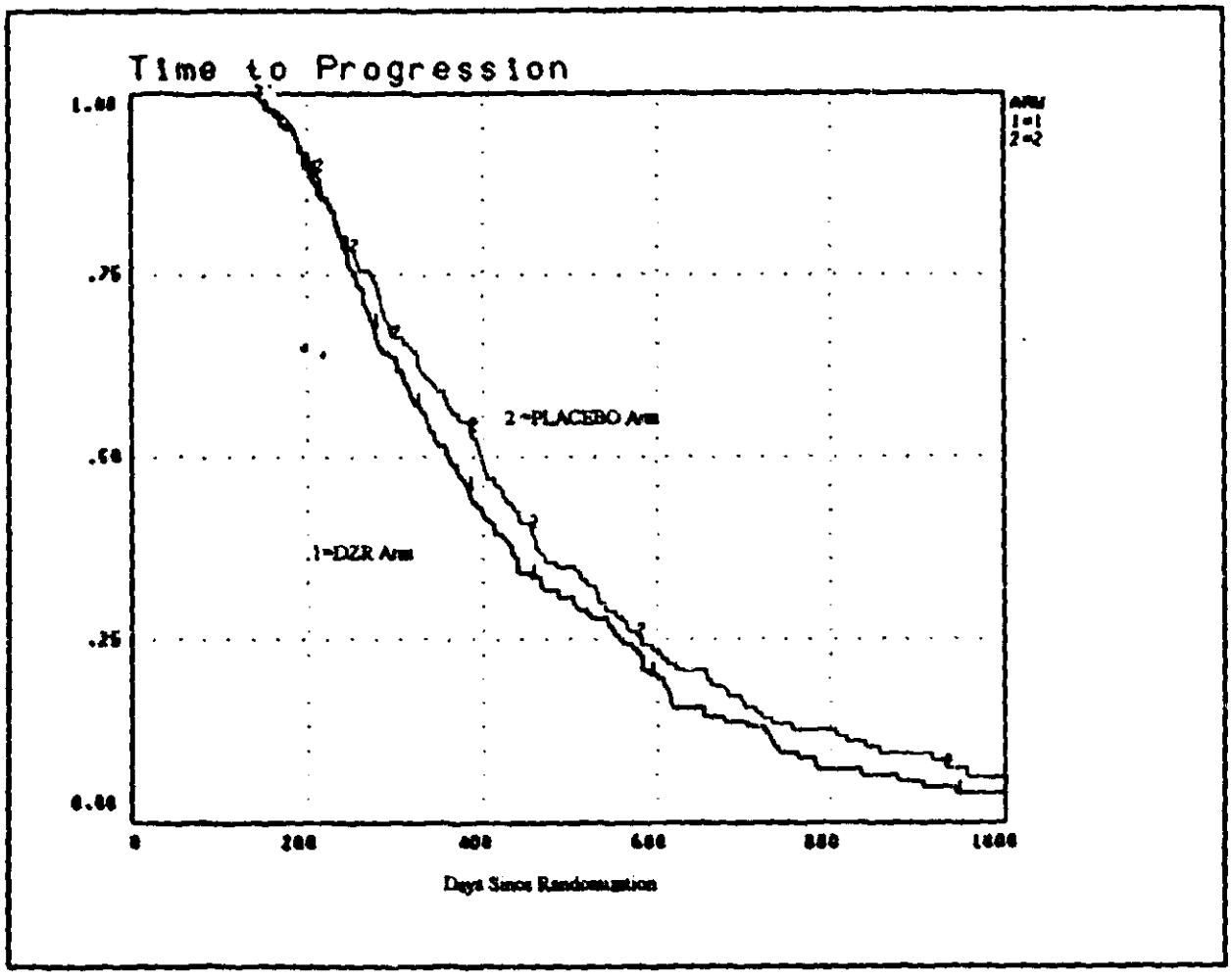

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Study Era	Total # Patients	# Progressing	#Censored	Median TTP*	Statistical Test**
1-Early	79	69	10	411	LR = 0.07
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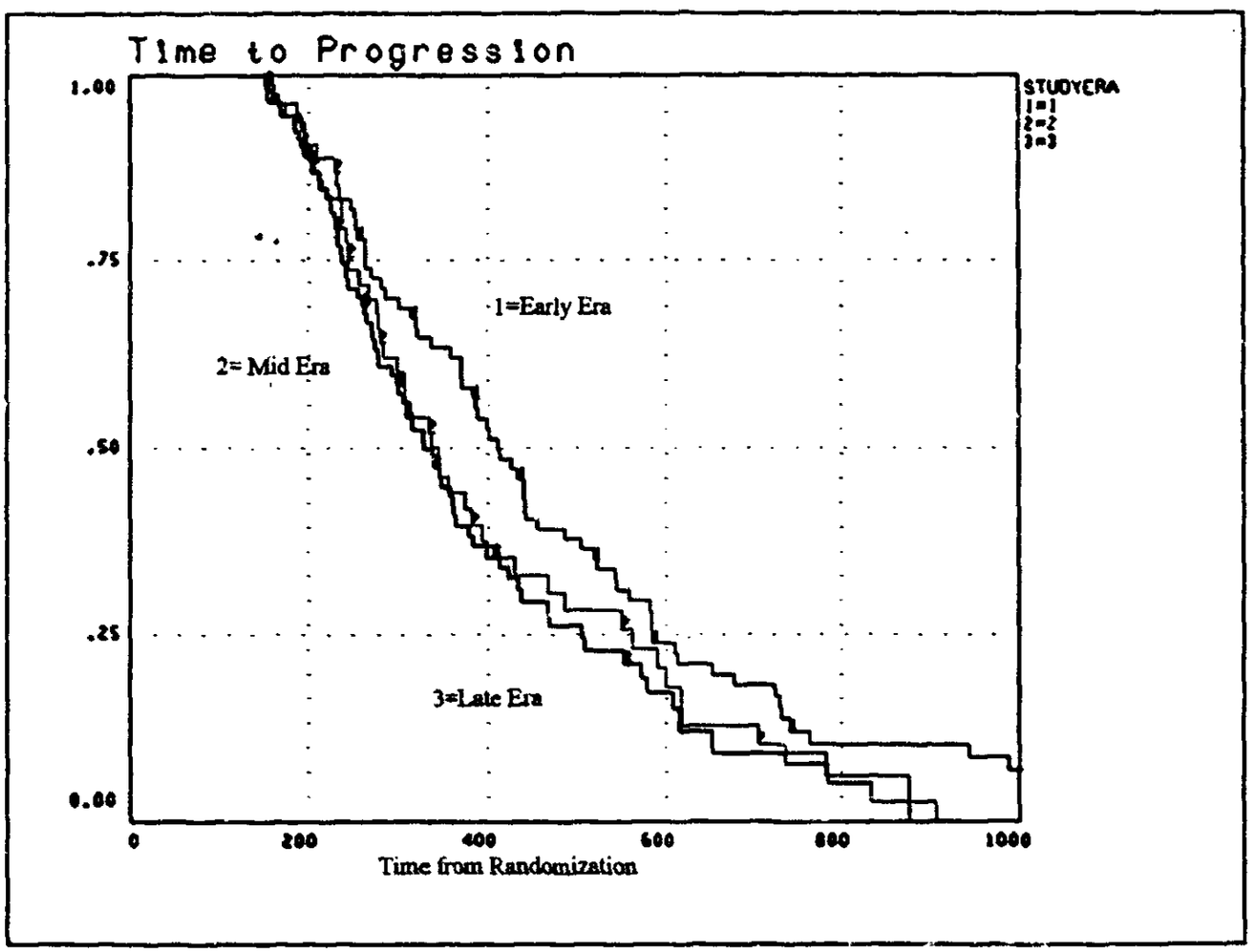

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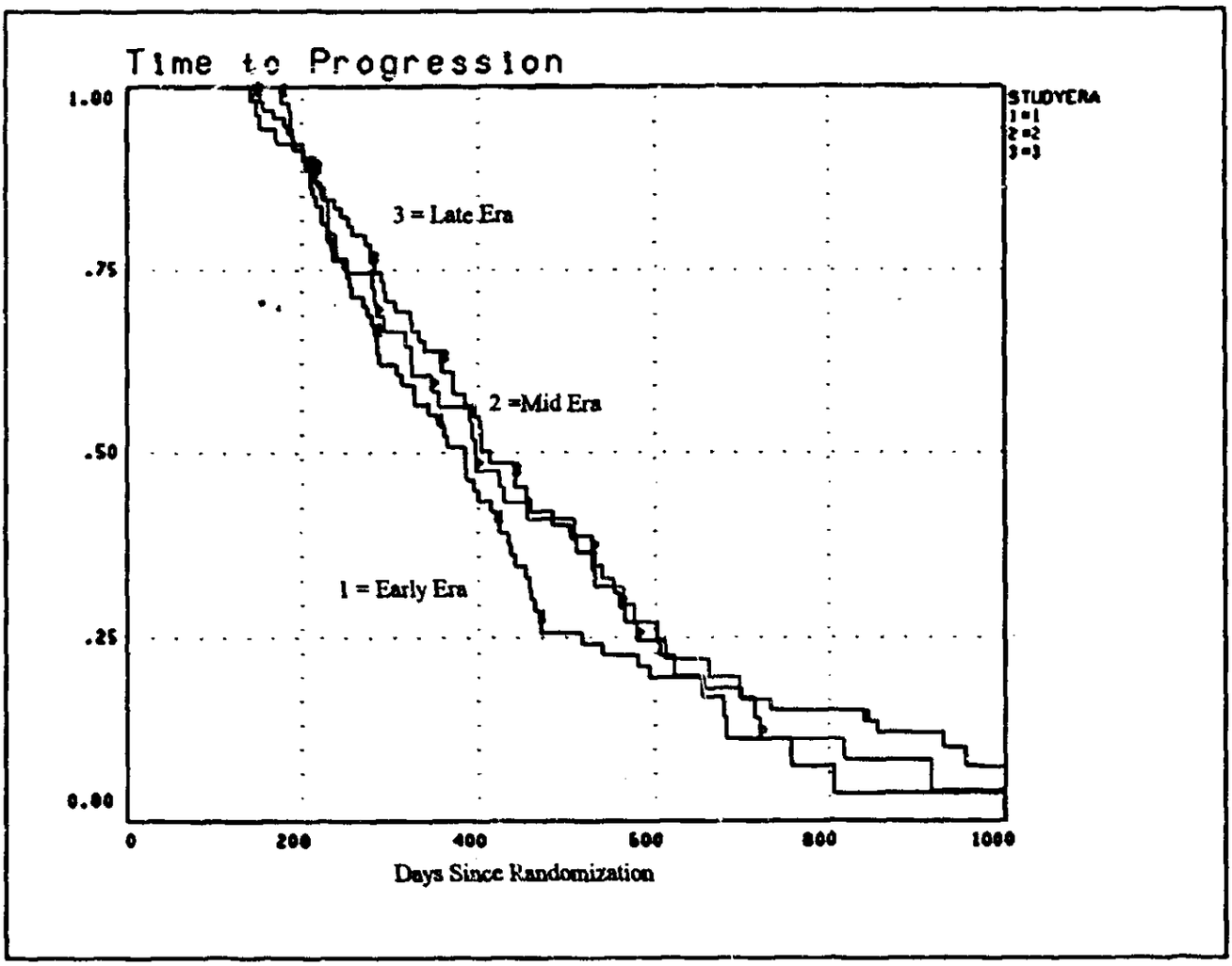

Appendix D: Reviewer Analysis of Survival:

1. Numbers of patients in different Survival Analyses:

'Course-7' Analyses

Randomized ARM	Time Period of Randomization				
		1.Early	2.Mid	3.Late	Total
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	2.PLA	99	56	102	257
	Both Arms	180	112	204	496

'All-Patient' Analyses

Randomized ARM	Time Period of Randomization				
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	1.DZR	195	121	173	489
	2.PLA	210	129	180	519
	Both Arms	405	250	353	1008

2. Diagram for Visualizing Comparisons:

Comparison Diagram (example DZR vs PLA)

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

(All = all pts., CS=Course 7 pts).

Patient Population:	All Patients
Groups compared:	PLA vs DZR
Comparison within:	All Periods

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			All

Reviewer analysis of survival of DZR group versus PLA group in All patients.

VARIABLE	DZR	PLA	STATISTICAL TEST	
			Statistic	Result
No. Patients	489	519		
No. Events	340	339		
Median Event Time (from Rand. Time)	557 d	568	LR	P = 0.26
Hazard Ratio (PLA:DZR)	0.92		GW	P = 0.27
95% c.i. of HR	0.79	1.07		
Adjusted Hazard Ratio*	0.96			
95% c.i. of Adjusted HR	0.82	1.11	WCS	P = 0.56

* Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	Course 7 Patients
Groups compared:	DZR arm vs PLA arm
Comparison within:	All periods

	Period	1	2	3	
ARM	DZR	X	X	X	
	PLA	Y	Y	Y	
		Population			CS

Reviewer analysis of survival by randomized Arm in 'Course 7' patients

VARIABLE	DZR	PLA	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	239	257		
No. Events	151	148		
Median Event Time (from Rand. Time)	706	748	LR	P = 0.58
Hazard Ratio	0.94			
95% c.i. of HR	0.75	1.18	GW	P = 0.98
Adjusted Hazard Ratio[a]	1			
95% c.i. of Adjusted HR	0.8	1.25	WCS	P = .99

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	DZR and PLA arms
Comparison within:	Late Period of Randomization(3)

	Period	1	2	3
ARM	DZR			X
	PLA			Y
		Population		All

Reviewer analysis of survival of DZR group versus PLA group in Late Period

VARIABLE	DZR arm (DZR/DZR)	PLA arm (PLA/DZR)	STATISTICAL TEST	
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No. Patients	173	180		
No. Events	100	82		
Median Event Time (from Rand. Time)	585	748	LR	P = .07
Hazard Ratio (PLA:DZR)	0.76		GW	P = .28
95% c.i. of HR	0.57	1.02		
Adjusted Hazard Ratio [a]	0.82			
95% c.i. of Adjusted HR	0.61	1.1	WCS	P = .18

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	All Patients
Groups compared:	Periods 1,2,3
Comparison within:	Combined Arms

	Period	1	2	3	
ARM	DZR	X	Y	Z	
	PLA	X	Y	Z	
		Population			All

Reviewer analysis of survival versus time period in All patients.

VARIABLE	Early Period	Middle Period	Late Period	STATISTICAL TEST	
				Statistic[b]	Result
No. Patients	405	250	353		
No. Events	318	179	182		
Median Event Time (from Rand. Time)	536	548	631	LR	0.05
				GW	0.04

GW = Generalized Wilcoxon. LR = Logrank

Patient Population:	Course 7 Patients
Groups compared:	Periods 1,2,3
Comparison within:	Combined Arms

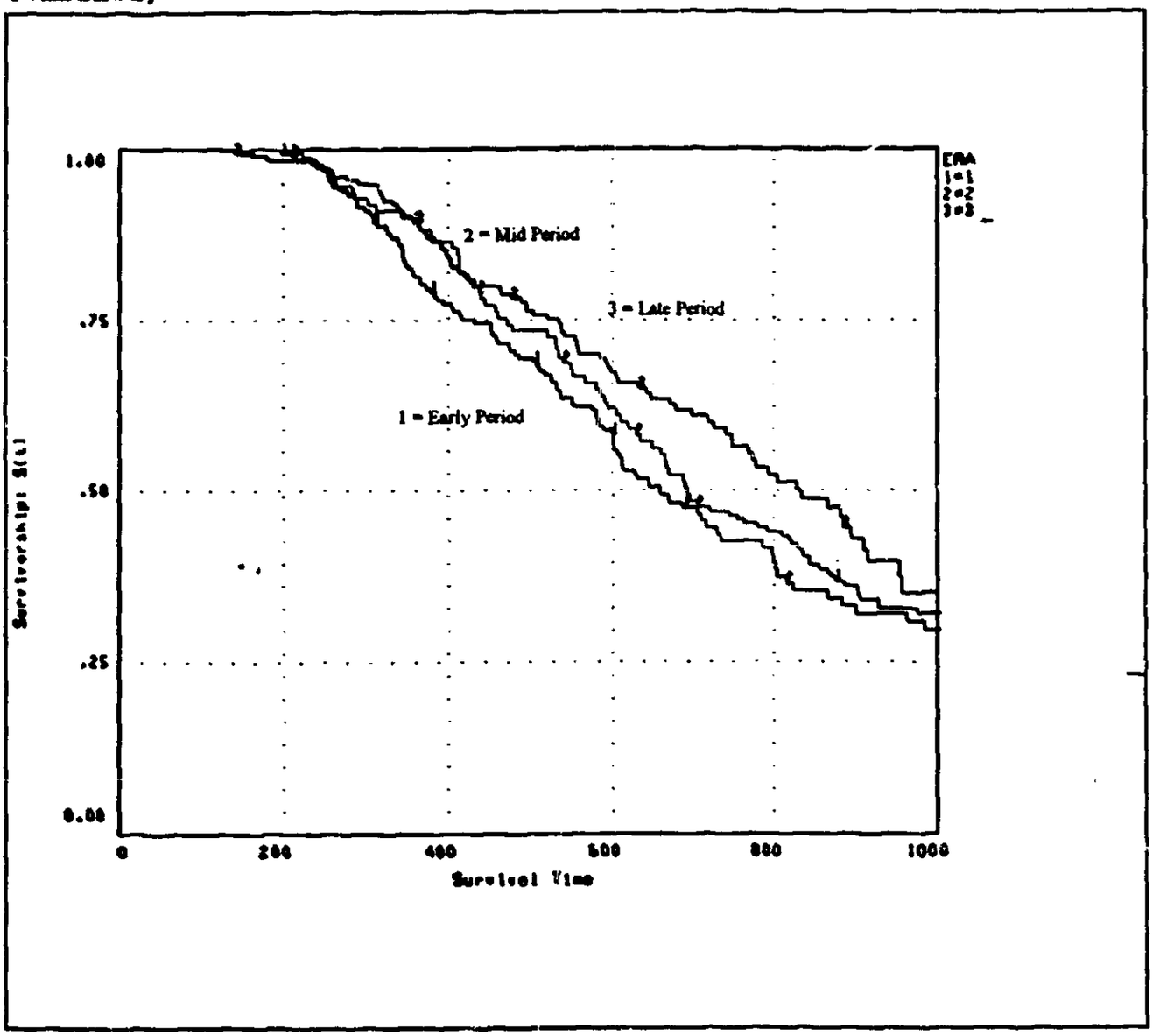
	Period	1	2	3	
ARM	DZR	X	Y	Z	
	PLA	X	Y	Z	
		Population			CS

Reviewer analysis of survival versus period of randomization in Course 7 patients

VARIABLE	Early Period	Middle Period	Late Period	STATISTICAL TEST	
				Statistic[b]	Result
No. Patients	180	112	204		
No. Events	136	76	87		
Median Event Time (from Rand. Time)	659	691	829	LR	P = 0.23
				GW	P = 0.11

GW = Generalized Wilcoxon. LR = Logrank

Survival by Period of Randomization in Course-7 patients (Arms Combined)



Patient Population:	**All Patients**
Groups compared:	**Randomization Period 1 Pts. vs. Randomization period 3**
Comparison within:	**Combined Ar...**

	Period	1	2	3	
ARM	DZR	X		Y	
	PLA	X		Y	
		Population			All

Reviewer analysis of survival in Period 1 versus Period 3 patients.

VARIABLE	Period 1 (early)	Period 3 (late)	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	405	353		
No. Events	318	182		
Median Event Time (from Rand. Time)	536	631	LR	P = 0.03
Hazard Ratio (late:early)	0.9		GW	P = 0.02
95% c.i. of HR	0.82	0.98		
Adjusted Hazard Ratio[a]	0.91			
95% c.i. of Adjusted HR	0.83	1	WCS	P = 0.05

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Patient Population:	Course 7 patients
Groups compared:	Randomization Period 1 vs. Randomization Period 3
Comparison within:	DZR Arm

	Period	1	2	3	
ARM	DZR	X		Y	
	PLA				
		Population			CS

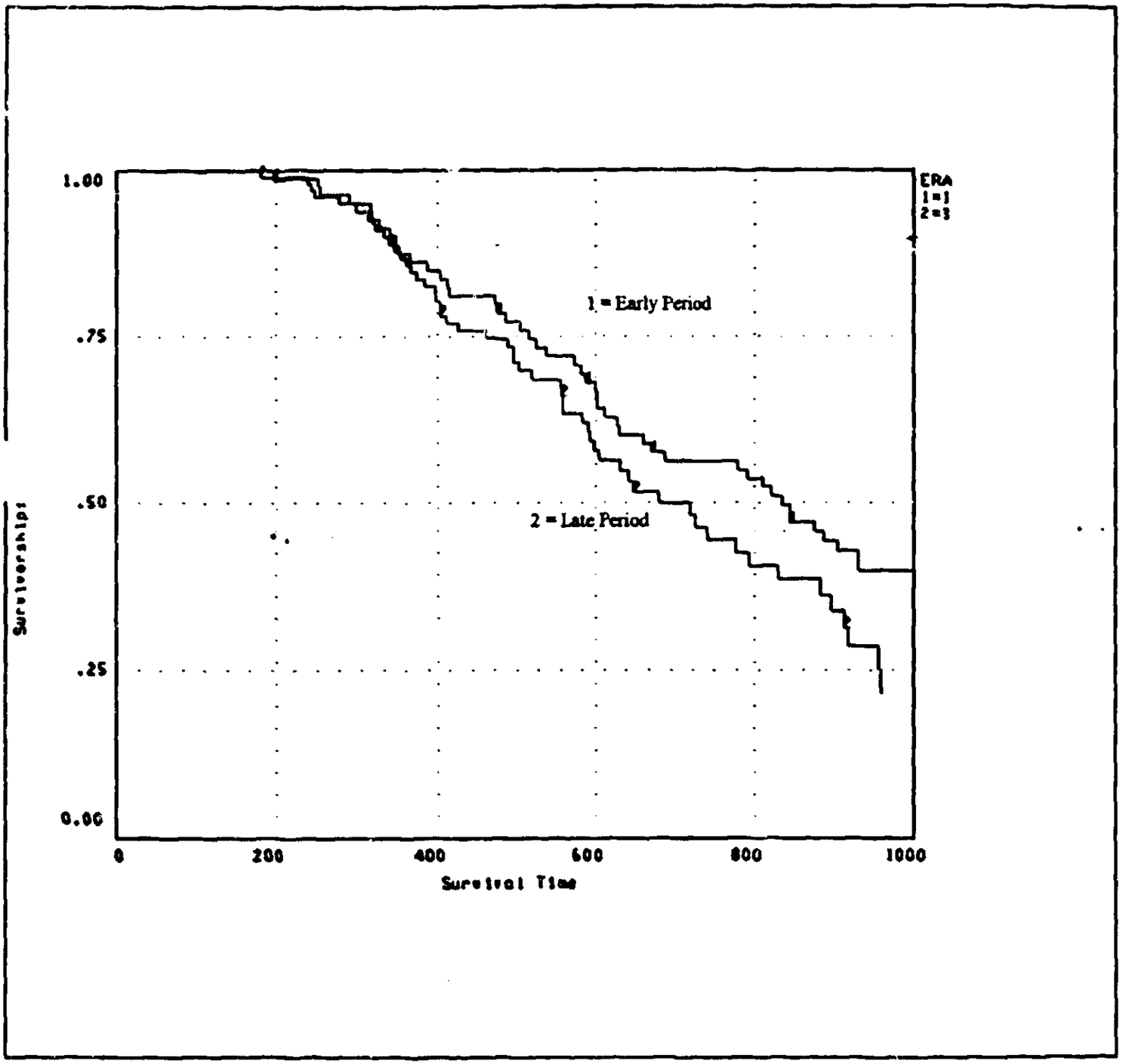
Reviewer analysis of survival in Course-7 patients randomized to DZR in Period 1 versus Period 3.

VARIABLE	Period 1 (early) DZR/DZR	Period 3 (late) DZR/DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	81	102		
No. Events	59	54		
Median Event Time (from Rand. Time)	832	680	LR	0.12
Hazard Ratio (late:early)	1.18		GW	0.22
95% c.i. of HR	0.97	1.44		
Adjusted Hazard Ratio[a]	1.19			
95% c.i. of Adjusted HR	0.98	1.46	WCS	0.085

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Survival of DZR Arm, Course-7 patients, early period vs late period of Randomization.



Patient Population:	Course 7 patients
Groups compared:	Randomization Period 1 Pts. versus Randomization period 3
Comparison within:	PLA Arm

	Period	1	2	3	
ARM	DZR				
	PLA	X		Y	
		Population			CS

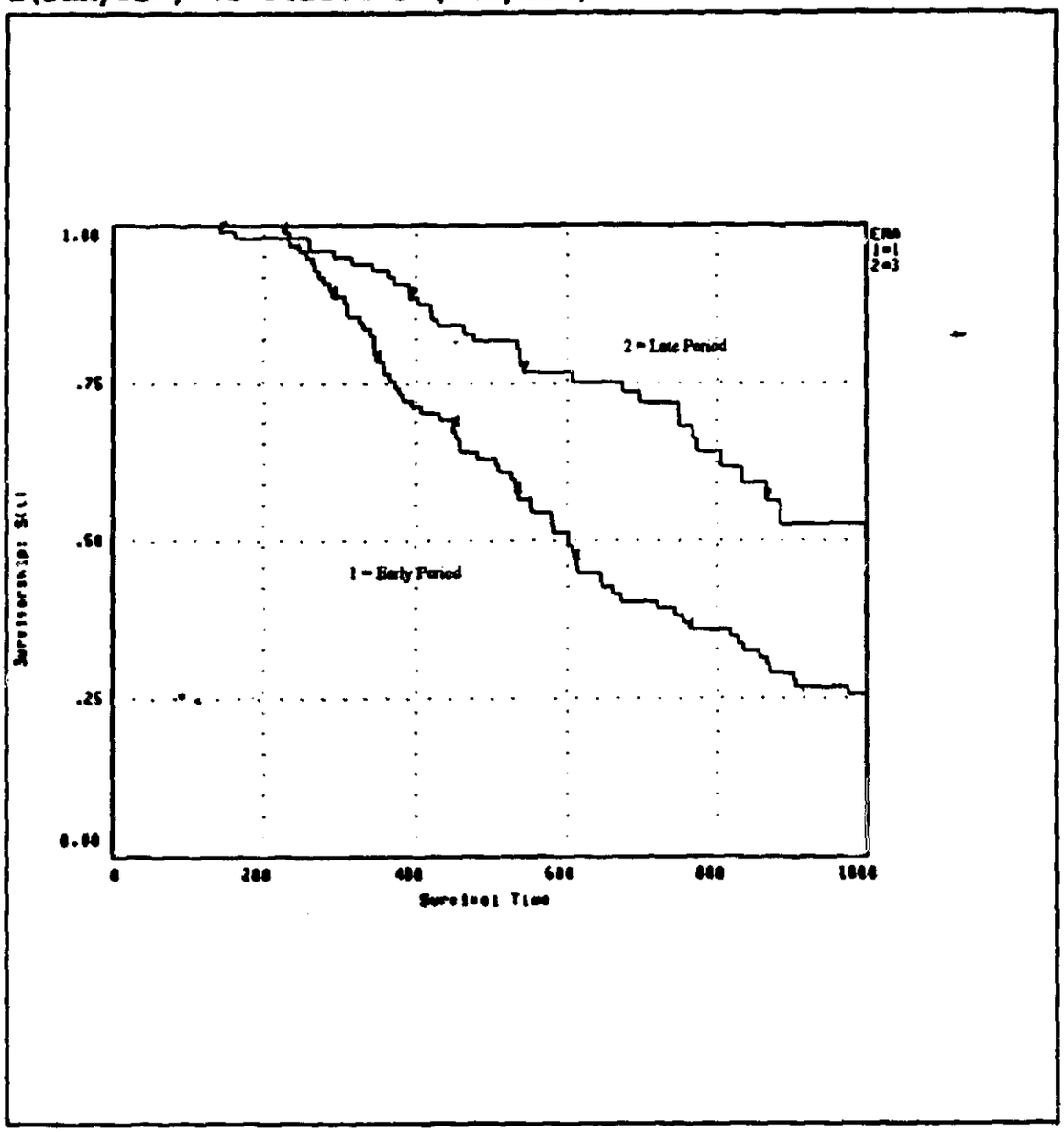
Reviewer analysis of survival in Course-7 patients randomized to Placebo in Period 1 versus Period 3.

VARIABLE	Period 1 (early) PLA/PLA	Period 3 (late) PLA/DZR	STATISTICAL TEST	
			Statistic[b]	Result
No. Patients	99	102		
No. Events	77	33		
Median Event Time (from Rand. Time)	601	897	LR	0.001
Hazard Ratio (late:early)	0.67		GW	0.002
95% c.i. of HR	0.83	0.55		
Adjusted Hazard Ratio[a]	0.71			
95% c.i. of Adjusted HR	0.87	0.57	WCS	P = 0.001

[a] Adjusted for age, performance status, and number of tumor sites.

[b] GW = Generalized Wilcoxon. LR = Logrank WCS=Wald chi-squared.

Survival in Placebo arm, course-7 patients; Period 1(PLA/PLA) vs Period 3 (PLA/DZR).



MEDICAL OFFICER REVIEW #2
(Supplementary review to MOR#1 based on previously unreviewed information in original NDA submission and on information in more recent submissions)

1. General Information:

1.1 NDA# 20-212

1.1.2 Review: M.O. Review #2

1.1.3 Submission date February 7, 1992

1.1.4 Date of Review June 24, 1992

1.2 Drug Name

1.2.1 Generic name: dexrazoxane

1.2.2 Proposed trade name: Zinecard

1.2.3 Other names: ICRF-187/ADR-529

1.3 Sponsor ADRIA LABORATORIES

1.4 Pharmacologic Category: Cardioprotectant

1.5 Proposed indication:
"for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration"

1.6 Dosage form and directions for use:
Lyophilized powder for IV injection, reconstituted with M/6 Sodium Lactate Injection, USP to be given IV at a 10:1 ratio prior to doxorubicin.

1.7 NDA Drug Classification: 1A

1.8 Related IND

Attachments for MOR #2 are in my office.
Ellen

MOR

2.0 TABLE OF CONTENTS

3 Material Reviewed

In addition to material in the original NDA submission, submissions by ADRIA responding to requests for information with the following letter date are covered in this review:

March 2, April 3
April 9, 16, 24
May 1, 8, 11, 12, 14, 22, 22
June 1, 2, 4, 5, 8, 12, 16, 16

4. Pharmacocology

Material reviewed:

Sponsor's summary V1.66 pp 117-143
Agency biopharmaceutics draft review (M. Hossain, Ph. D.
Agency Integrated summary of preclinical pharmacology and toxicology.

Location of study reports: V 1.58 to V 1.65

a. Pharmacokinetics

Animal studies

Disposition has been studied in rats, rabbits, and dogs. Drug is rapidly cleared from the plasma. Tissue levels of C14 labeled DZR in these animals were less that 2X plasma levels in most tissues of these animals except for liver and kidney (which were about 40X and 10X plasma levels respectively). Brain levels were less than 1 (p 02-0120). In animals DZR kinetics demonstrated linearity, with low partitioning into tissues and rapid renal and metabolic elimination.

Animal studies with C14 labelled DZR suggest that the non-renal component of clearance is from metabolism, probably hydrolysis. The size of the estimate of Vss of about 20 L/m2 in humans suggests that distribution is largely confined to total body water. The fraction bound to plasma proteins in animals and humans was less than 5%.

In animals, fecal excretion of DZR over 72 hours was between 5-10%.

Human pharmacokinetic studies

In study 1, (Narang et al, ADRIA LABS, V1.58 0113) cohorts of 3 patients were given DZR in escalating doses from 60 mg/M^2 to 900 mg/m2 30 minutes prior to Doxorubicin. AUC increased linearly with dose. In study 2, DZR was evaluated at a fixed dose of 500 mg/M^2 in 12 patients. Results are summarized in table 4 on p 02-0126.

Terminal half-life was 2.1 to 2.5 hours, clearance was 6.3 to 9.6 L/Hr/m2, and Vss was 22 to 25.6 L/m2. Renal clearance was estimated to be 34% of total clearance.

The two oriental patients had systemic clearances > 2 standard deviations above the mean of the other patients. Since renal clearance was similar to that of the other patients, the difference appears to be have been from in creased metabolic clearance.

In one patients with biliary drainage, biliary excretion mirrored plasma levels suggesting no significant excretory role.

PK interaction with doxorubicin:

In ADRIA study 1 by Narang, doxorubicin pharmacokinetics were evaluated at a dose of 50 mg/M^2 of doxorubicin in 7 patients with and without prior DZR at a 10:1 ratio. Pharmacokinetic parameters of doxorubicin (t 1/2 74 and 63 hours and for doxorubicinol (60 and 59 hours) were unchanged by prior DZR.

DZR kinetics was studied in children ages 9 to 21 years of age (Holcenberg et al Cancer Treatment Rep 70:703, 1986). Half life was about 2/3 of adults (113 to 117 minutes) and clearance was about 50% greater (190 ml/min/m2). This is consistent with the higher MTD found in pediatric studies.

b. Pharmacodynamics

Mice, rats, rabbits, guinea pigs, and dogs had evidence of dose-dependent cardioprotection from doxorubicin toxicity. There was a decrease in incidence and severity of cardiac lesions at ADR-529/doxorubicin ratios of 5:1 to 20:1.

In mouse tumor models, there was no decrease in mean survival time when ADR-529 was given in conjunction with doxorubicin, epirubicin, or idarubicin; in some models additive tumor reduction was noted. As mentioned in MOR#1, in the BL BX7 human tumor xenograft, there was a reduction in time to tumor regrowth in the group treated doxorubicin with concurrent ADR-529 compared to the group receiving DOX alone.

Studies in mice show cardioprotection when DZR is given from 30 minutes before up to 15 minutes after doxorubicin. In mice the ED50, based on mean heart scores, was 22 mg/kg when given with weekly doxorubicin at 8 mg/kg/wk.

The sponsor compares DZR concentrations and clearances in mice and man in table 7.

Table 7

DZR Concentration and Cardioprotection Effect

Tissue	DZR Concentration (μg/mL)			CL_s (Man/Mice)
	In-vitro	*In-vivo*		
	Rat	Mice[a]	Man	
Plasma	-	33[b]	36-21[c]	
Heart	-	17[b]	-	
Myocytes	>25[d]	-	-	
CL_s (mL/min/Kg)	22[e]	13[a]	4[e]	>3
DZR (mg/Kg)	-	65	12	5

[a]Total ^{14}CDZR.
[b]Predicted concentration for a ED_{75} in mice.
[c]Based on unchanged DZR.
[d]Concentration at which exposure >3 hours showed protection.
[e]For 1.73 m^2 and 70 Kg man (2).

It is noted that the concentration for ED75 in mice is similar to the concentration in man within 30 minutes after the doxorubicin infusion when the 10:1 ratio of DZR had been given. This exceeds the level of 20-30 mcg/ml found to be necessary in vitro in rats and in vivo in mice 30 to 60 minutes after doxorubicin for cardioprotection to occur. The ED75 dose in mice is 65 mg/kg of DZR or about 8 times the doxorubicin dose.

In-vitro studies in rat heart myocytes suggests that 1-3 hours exposure to ≥ 25 mcg of DZR is needed to protect from doxorubicin cardiotoxicity.

c. Toxicology

In acute toxicity studies in dogs high dose ADR-529 (4000 mg/M^2) caused hepatotoxicity manifested by elevation of AST/ALT. In lower doses concurrent with doxorubicin, however, ADR-529 was protective to liver and kidney. Target organs for toxicity in most species were marrow and testes; toxicity was more severe in these organs with doxorubicin and epirubicin.

ADR-529 was not mutagenic in the ames assay, it was clastogenic in the mouse micronucleus test. It was teratogenic in rats below maternally toxic levels (12 mg/M^2) and fetotoxic in rabbits at maternotoxic levels (200 mg/M^2).

The pharmacologists have recommended changes in the labeling for tumor protection, pregnancy category and description, and carcinogenicity/mutagenicity.

5 Structurally related compounds

Razoxane

Reviewed: Summary (02 1980, summary tables from (08 4348-4379.

Razoxane is the racemic mixture including the D (dexrazoxane) and L isomers. Preclinical studies suggested that it could inhibit formation of metastases, reduce anthracycline cardiotoxicity, and potentiate effects of chemotherapy and radiation.

Solid tumor trials involved 2000 patients. Side effects included predominantly myelosuppression, but also GI side effects(nausea, vomiting, mucositis, and diarrhea), alopecia, and occasional dermatitis. In studies with radiotherapy, an increase in local toxicity was noted, including esophagitis and pneumonitis.

Trials with radiotherapy suggested an increase in local toxicity, including esophagitis and pneumonitis.

In Britain it was used for psoriasis. Longterm use was associated with atypia in myeloid precursors. AML was reported 6 cases treated for GI malignancies, all in patients taking the drug for well over 1/2 years. 5 cases were detailed in the psoriatic literature, again after chronic use. The estimated incidence is 2-3 patients per 1000 treated. All cases have occurred in patients taking the drug for at least 8 months.

Reviewer's Comments:
This information on carcinogenesis is present in the proposed labeling. One might consider including the incidence as noted above.

6 [redacted]-Pivotal studies of ADR-529

Antitumor studies

There were 5 phase I trials using dx3, dx5, 48 hr ci or weekly schedules. The dose-limiting toxicity in adults was myelosuppression, that in children (dX3 then weekly) was

hepatotoxicity.

MTD was 7 grams/wk on weekly X4 schedule, 1 gram/m2 /d on daily X 3 given q 4 weeks, 1 gram /m2 on 48 hr ci schedule, 1 gm/m2/d on daily X 5 q 3 weeks, and 3500 mg/M^2 in the pediatric daily X3 q 3 week schedule. Patients with previous nitrosourea therapy were showed more severe myelosuppression. In children, hepatotoxicity and abnormalities of lab tests of coagulation were noted. Despite the higher doses, myelosuppression was erratic and scarcely detectable, except that hemoglobin decreased 2-5 g/dl at higher doses through an unknown mechanism.

Reviewer's Comments:

There seems to be some schedule dependence. This should be kept in mind when considering that doxorubicin is used in clinical practice on different schedules. The above data suggests that if given daily X3, the amount of drug used in the initial NDA studies (1gm/m2 or 20X 50 mg/m2) would approach the MTD. Also just one such dose spread over a 48 hr ci would be at MTD without any other drugs. This schedule dependent toxicity should definitely go in labeling with an appropriate cautionary note.

The sponsor cites a reference suggesting that the decreased sensitivity of children to myelotoxicity might be at least partially due to increased clearance and increased volume of distribution in children.

Reviewer's Comments:

This should be kept in mind for possible emphasis in labeling. Separate studies are needed in children: It might be that children would need a different dose for cardiac protection.

One study noted an increase in iron and zinc urinary excretion, an increase in iron serum levels and a decrease in serum zinc levels.

Phase II trials included head and neck cancer, Kaposi's sarcoma, renal cancer, colorectal cancer, and lung cancer. There were 3 responses total (2 head and neck, one Kaposi's sarcoma). There were no responses in 56 pediatric patients in a broad phase II study.

Reviewer's Comments:

Note that we have no phase II data in breast cancer.

Cardioprotection studies

The 5 studies evaluating ADR-529 as a cardioprotectant are summarized in table 1 (4330-4334). Most are small or incomplete. The one of note is a placebo controlled randomized trial at (T83-1050)

The study report form the 1990 annual report located in volume 5.1 of IND was reviewed. The protocol was a randomized double-blind study of patients receiving doxorubicin either as a

single agent or in a combination regimen. Randomization was to ICRF (10 mg/kg) or placebo concurrent with doxorubicin. 94 patients were randomized, however due to dropout for progression, only 28 patients Dox doses greater than 300 mg/M^2 and median cumulative dose was only 200 mg/M^2. Many different regimens and diseases (including leukemia) were included. Cardiac function was monitored by a variety of methods. Although there were trends in the data suggesting a protective effect, the study was considered underpowered and the population and treatment regimens too heterogeneous to provide any definitive answers about efficacy, antitumor effect, and toxicity.

A pediatric protocol(utilizing ifosfamide, cytoxan, vincristine, and ADR-529 in the treatment of neuroectodermal tumors uses a total Doxorubicin dose of 410 mg/M^2. Patients are randomized to DOX with or without ADR-529. Cardiac function is being monitored at intervals on both arms. There are only 28 patients onstudy. According to table 1, grade 4 leukopenia is universal in both arms, however grade 4 thrombocytopenia was noted in 11/15 on ADR-529 and 3/13 on control.

CALGB 8983 is an uncontrolled study to which ADR-529 is added after the MTD of DOX plus GMCSF has been determined. 10 patients are on study, and no patients have yet received ADR-529.

Overall summary (Sponsor's summary, p 08-4324)

Safety data is discussed for 2000 cancer patients with razoxane, the racemate. Side effect included myelosuppression (mainly leukopenia), nausea, vomiting, mucositis, and diarrhea with occasional alopecia and dermatitis. A rare but serious side-effect of chronic use is AML.

Safety data is discussed for 167 patients (including 46 children) in phase I studies of ADR-529, 212 patients (including 56 children) in phase II studies, 92 patients in cardioprotection studies, 125 in foreign studies with epirubicin. In all, data on 600 patients receiving ADR-529 is presented. Side-effects in high doses include myelosuppression, primarily leukopenia but also to a lesser extent thrombocytopenia and anemia, transient LFT elevation (SGOT, SGPT, and bilirubin), transient elevation of amylase, and in one study an increase in urinary copper and iron excretion. Also seen in some studies were GI side-effects (anorexia, nausea, vomiting, stomatitis) and alopecia.

Reviewer's Comments:

To summarize regulatory points:

-data on leukemogenic effect of razoxane,the racemate, is in the proposed labeling.
-we have no phase II data of ADR-529 in breast cancer.
-data in this section support the myelosuppressive potential of ADR-529.
-There is a suggestion of schedule dependent toxicity that might be considered for labeling.

-Data on PK differences in children might be considered for labeling. There will be a desire to use this in children given recent information on chronic cardiotoxicity. Emphasis on lack of clinical data and differences in pharmacokinetics might be emphasized in labeling.

Published Clinical Literature

Additional bibliographies and abstracts are listed on p 4430-4444. There are many references to including analyses at 56, 67, and 82 patients. Only the 67 patient citing gives cardiac data.

7 Sponsors integrated summary of safety

Material reviewed: Vol 78 Text pp 4489-4520
Tables pp 4527-4830

In Volume 78 the sponsor presents summary tables based on data in the 4 pivotal trials. In this review I will describe the tables and give my impressions. In addition I will comment on the impressions of the sponsor. The format for data reporting is the same as that used in trial 88001; see my review of this trial for more details. Details are repeated in the sponsor's text (pp 4489-4520).

The studies and schedules are summarized in table 1.1-1.3. Attached is table 2.1 which summarizes extent of drug exposure. In all the ADRIA-sponsored trials in breast cancer, 10:1 and 20:1, the median number of courses is 5 or 6, whereas in the 20:1 study the median number is 9 on ADR-529 and 8 on placebo. The combination of the 10:1 data in table 2.2 is especially revealing. Despite dropouts due to cardiac monitoring, the number of courses on the ADR-529 arm never exceeds the number on the placebo arm. In the lung cancer study, the median number of courses was 6 on placebo and 4 on ADR-529. However the sponsor notes that in the 20:1 data base in breast cancer, patients receiving 10 or more courses makes up 46% of the ADR-529 arm and only 22% of the placebo arm. This was not noted in the larger 10:1 data base where equal numbers received 10 or more courses (overall 14%).

Reviewer's Comments:
This certainly raises questions about efficacy of the 20:1 ratio vs the 10:1 ratio. Part of the difference could be the influence of the data base in the 20:1 data.

Table 3.1 (pp 4534-4545) summarizes the patient characteristics. In the 20:1 breast cancer trials median age was about 55 in both the ADRIA-sponsored trial (88001) and in the trial(88011). In the 10:I breast cancer trials (006 and 001) median ages were 56-59. Overall 1/4 to 1/5 of the patients in the 10:1 data base were black. PS 2 patients made up about 20% of both the ADRIA and 20:1 data base in breast cancer and only 2% of the 10:1 data base. The rest of the patients in both 10:1 and 20:1 data bases were split fairly equally between PS 0 and PS 1.

Incidence of toxicity in all courses in the 20:1 data base in breast cancer is described in table 4.A.1. (p 4549 ff). The only findings showing significant differences were sepsis (see attached table 4.A.1) 19 vs 5 cases, p = 0.005) and neurotoxicity (10 cases vs 1 case, p=0.004), both more common in the ADR-529 arm.

Incidence of toxicity in the first course in the 20:1 data base

is described on p 4547 ff. There were no significant differences.

Incidence of toxicity in all courses in the 10:1 data base in breast cancer is described in table 4.A.2.(p 4549 ff). Significantly more common on the ADR-529 arm were fever (50 vs 38 cases),pain on injection (yes ADR-529 12 vs yes placebo 2, p=0.003), infection (28 vs 15, p=0.014). Less common on the ADR-529 arm were nausea (yes 153 on ADR-529 vs 199 on placebo , p=0.041) and vomiting. There were 16 reports of neurotoxicity on ADR-529 versus 10 on placebo (0=0.11).

An excerpt from table 4.A.3 shows that infection, sepsis, fever, and neurotoxicity remained significantly more common for breast cancer during course 1 when data from all ratios was combined. When the lung cancer data from 002 were combined, incidence of alopecia in course 1 was significantly more common on the placebo arm (81 vs 56, p=0.006).

For the all courses analysis for the combined 10:1 and 20:1 ratios in the lung cancer study, esophagitis was more common on the placebo arm (11 vs 2, p=0.02).

For the all courses analyses in table 4.b.1 (p4566 ff) for 20:1 in breast cancer sepsis (32 vs 14, p=0.015) and fever were significantly more common in the ADR-529 arms.

For the all courses analyses in table 4.b.2 (p4566 ff) for 10:1 in breast cancer, more common on the ADR-529 arm was pain on injection (27 vs 10, p=0.001); sepsis and infection were not significantly different. More common on the placebo arm were nausea (249 vs 199, p=0.014), vomiting (153 vs 215, p<0.001), esophagitis (24 vs 11, p=0.06), stomatitis (126 vs 87, p=0.034) and CHF (13 vs 2, p=0.008).

Tables on pp 4582-4617 compare toxicities by grade. Review of these did not reveal additional insight.

Reviewer's Comments:
This overview suggests less of a clinical problem with sepsis at the 10:1 ratio. Protection from mucositis and from nausea and vomiting is suggested at the 10:1 ratio. Protection from alopecia in course 1 with CAV is suggested in the lung study. Neurotoxicity was more prominent in the 20:1 ADR-529 arms, with no significant differences seen in the 10:1 arms. Pain on injection was more common on ADR-529 in with both ratios.

Adverse reactions reported by COSTART terminology are summarized item by item by trial, disease, and dose ratio in tabular form on pp 4618 to 4830. Page by page examination of the numbers reported in each arm in the various presentations did not reveal new insight

Sponsor's summary of clinical toxicity overview

"The overall impression gained by examining the clinical toxicity profile of breast cancer patients receiving the 10:1 ratio treatment [redacted] on the DZR arm had a higher incidence of infections and fever on injection than did patients on the PLA arm. The incidence of urticaria was also somewhat higher on the DZR arm, but the number of patients with this event was too low [redacted] that the clinical relevance [redacted] breast cancer studies, patients on the PLA arm experienced a significantly higher incidence and significantly higher average severity of symptoms related to the gastrointestinal tract (esophagitis, stomatitis, dysphagia, nausea, vomiting). This difference was not observed in the lung cancer study. There was also a highly significant difference in the incidence of congestive heart failure on the PLA arm experiencing more events."

Reviewer's Comments:
I am in agreement with this summary. Attached is the sponsor's summary table of frequencies of clinical toxicities for all courses of the 10:1 breast cancer patients in 88001 and 88006. This is a correction table. The originally submitted table had 42% ADR-529 vs 34% placebo in the infection and/or sepsis category; the replacement table submitted soon after NDA submission has 31% ADR-529 versus 28% placebo in this table. I imagine the difference involves duplicate counts of the patients with both infection and sepsis, but this should be clarified.

Attached table 8.A.1 shows the nadir granulocyte count in course 1 in the 20:1 breast cancer patients. The mean in the ADR-529 arm was 200 in the 001 trial compared 500 in the placebo arm. However in the study, the nadir was dramatically higher in both arms (1000). The reason for this difference is not clear.

Attached table 8.A.2 shows the combined data from the 10:1 breast cancer patients in the 001 and 006 trials. The mean granulocyte count nadir was 600 in the ADR-529 arm versus 900 in the placebo arm. Attached table 8.A.5 displays grade of myelosuppressive toxicity in course 1. 131/220 (60%) had grade 4 toxicity on ADR-529 versus 117/257 (46%) on placebo. As shown in table 8.5.B, over all courses, most patients had at least one course of grade 4 granulocytopenia (ADR-529 172/232 or 74%; placebo 172/271 63%). For platelets there was a clear difference in patients with no toxicity as shown in attached tables 9.A.5 and 9.B.5. Overall, 89/236 (37%) had less than grade I platelet suppression on ADR-529 versus 144/272 (53%) on placebo.

Chemistries

Chemistries are presented by mean, median, and by grade for individual study, for disease, and dose ratio in tables on pp

4909-4944. No differences in the 2 arms were noted.

On-study deaths

These are summarized in tables on pp 4945-4956. Attached is table 8.B.4 which lists the individual onstudy deatns. Attached table 13.B.2 summarizes the findings in the 10:1 breast cancer patients. Onstudy deaths were similar on the 2 arms (3%).

In the 10:1 lung and breast cancer patients, there were 6 deaths attributed to sepsis or infection in the DZR arm (6/249 or 2.4%) versus none on the control arm.

Sponsor's summary of onstudy deaths:

On p 4510 the sponsor summarizes the data related to the 10:1 patients:

> "Of the 14 deaths (eight in the breast studies, six in the lung study) at the 10:1 ratio on the DZR arm six patients died of sepsis or infection." On the PLA arm of the 14 patients who died, "...No patient was considered to have died of sepsis or infection."

> "It appears that the excess number of deaths on study on the DZR arm in the 20:1 ratio was observed mainly on the lung study. The incidence of deaths at the 10:1 ratio was similar on the two treatment arms. However, at both the 20:1 and 10:1 ratio, sepsis or infection was more frequently listed as the course of death in DZR than PLA treated patients (13 DZR, 3 PLA)."
>
> "At the 10:1 ratio the incidence of deaths on study was similar for the two treatment arms, but deaths due to sepsis were somewhat more frequent on the DZR than the PLA arm, 2% and 0%."

Offstudy

Reasons for going off-study are summarized in attached table 14.2 for the 10:1 breast cancer studies.

Sponsor's conclusions:

Excerpts from conclusions on p 4520. :

> "There was a statistically significant difference in the incidence of infection after the first course of therapy, with DZR patients experiencing the higher incidence. This difference was not statistically significant when all courses were considered."

"Initially, when the 20:1 ratio of DZR to DOX was used, there was a higher incidence of death on study on the DZR than on the PLA arm. This was particularly evident in the lung study and led to reduction in the dose ratio of DZR to DOX from 20:1 to 10:1. At the 10:1 ratio, the incidence of deaths on study was similar for the two treatment arms but deaths due to sepsis were somewhat more frequent on the DZR than the PLA arm, 2% and 0%, respectively."

"It may, therefore, be concluded that the addition of DZR to FAC or CAV reduces the incidence of CHF and possibly of some gastrointestinal toxicities. It does contribute to a somewhat more severe but reversible myelosuppression and patients receiving the combination are at slightly higher risk than control patients of experiencing death due to sepsis."

8 Sponsor's Integrated Summary of Efficacy

Material reviewed:

pp 08-04445 to 04488

ADRIA presents summary tables of efficacy data in the pivotal trials. These might be useful and hence the most useful are appended, however discussions in MOR#1 address these issues in a more thoughtful fashion. The pooling of results is questionable. In table 7.A.1 I note that the incorrect response results from the 20:1 data are used (As in the report the "evaluable" analysis is actually closer to what be routinely considered the intent-to-treat analysis for response since it only included patients with measurable disease.) In the survival table (10.A.1) I have added the more complete survival analyses from the report. The one in the table is the onstudy survival which has very few events. In the time to disease progression table (8.A.1) the more favorable onstudy analysis has been used. I again have substituted the more complete analysis. In all the pooled analyses presented for the 20:1 data in breast cancer one should remember that suboptimum analyses from the trial are included.

Overall there were 1022 patients in the pivotal studies, 534 in the 10:1 trials in breast cancer, trials 001 and 006.

From table 4a one notes that there in the 10:1 breast cancer studies, there were 15 cases of CHF; 13 of these in the control arm. The overall incidence in the control arm was 13/285 or 4.5%.

The overview and pooling results do not materially alter the summary and conclusions in MOR#1.

9 Responses to MOR requests for information

These are listed by letter date of submission.

April 9

This submission contains updated tabulations of response, toxicity, and baseline prognostic factors.

April 16

It is noted that all p values are 2-sided. The distribution of 10:1 dosing for the "20:1 patients" is given. Randomization is discussed. Formulation change is discussed. Under response 28 onstudy deaths are listed and case report forms are submitted.

April 24

Response to 12/10 FAX

This contains an analysis of disagreement in response between ADRIA and investigator. There is no evidence of systematic bias on ADRIA's part.

Response 1.1

This contains cover sheet for CRF's submitted with NDA, explaining reasons submitted.

Response 9

This lists individual LVEF offstudy events according to classification (>0.10 to below institutional norm, etc.).

Response 12.1

This lists the neurotoxicity adverse reactions by COSTART terminology of the 20:1 ratio versus control and individual patients (39 versus 18 events, 27 versus 14 patients). These do not appear serious (anxiety, depression, hypertonia, insomnia, etc.) and don't appear qualitatively different from those on the control arm.

Response 12.2

This lists the patients in course 1 with neurotoxicity in the 10:1 88001 study, where there was a statistical difference (8% vs 2%).

Response 12.3

The 4 patients in 88001 with ophthalmic difficulties are listed

and CRF's are supplied.

Response 31

Onstudy deaths are listed with causes of death.

May 1

Response to 2/28/92 FAX

This contains the discussion of the contribution of doxorubicin to the CAF regimen in breast cancer. A comprehensive table on pp 20-32 of this report lists major studies of doxorubicin in breast cancer with response rates, durations, survival, patient numbers and trial design. On p 18 (copy attached) is a comparison of CAF to CMF. This lists 7 studies of CAF vs CMF. In 5 there was a significant difference in response, on the order of 15-20%. Survival was significantly prolonged in one study with trends in 4 others. In each case, the point value of median survival was higher on the CAF arm.

From ADRIA's review, the median response rates were 35% for cytoxan, 35% for 5FU, 45% for doxorubicin, 50% for CF, 53% for CMF, and 55% for CAF. Variations in response to CAF ranged from 11% to 94%. The conclusion was that it was unlikely that small or moderate variations or reductions in drug doses would affect eventual outcome in patients with breast cancer. The exact contribution of doxorubicin could not be precisely defined.

Response to 4/9/92 FAX

In response 1, appendices for studies 88006 and 88001 are resubmitted unblinded. In response 5 a discussion addresses the potential for censoring for progression to affect the cardiac event analysis. In response 9.4, the patients at 500 mg/M^2 of doxorubicin with a drop of at least 0.10 are listed. For 88001 10:1 patients there are 15 on ADR-529 versus 5 on doxorubicin, for 88006 there are 7 versus 1. Response 21 notes that patients progressing prior to 3 months were exclude from the "evaluable" patients analysis of response. Response 27 gives a histogram presentation of doxorubicin dosing on each arm of the studies. Response 28 includes additional case report forms for onstudy deaths, and locates the other CRF's in the submission.

May 8

This submission includes case records and internal evaluation forms for NYU Study 88011. In addition issues related to patients with baseline LVEF less than 10% above lower limit of normal are addressed.

May 12, 1992

This submission contains corrected overview tabulations of survival and cardiac endpoints that have been minor modifications.

May 14

Response 7

This contains listing of additional therapy in patients going offstudy for reasons other than progression.

Response 9.1

This contains a description of all patients going offstudy for CHF. Most appear legitimate; but few were hospitalized.

Response 9.6a

Few patients on either arm actually had a second verifying LVEF for going offstudy.

Response 9.9

This clarifies that patients going offstudy with both progression and cardiac toxicity were included in both analyses.

Response 32

An explanation of the data monitoring committee analyses is given. It is noted that the actual data set upon which stopping was based was purged from the computer. The reconstructed analysis is given with p=0.005 for LVEF at 500 mg/M^2.

"Response to 5/5/92 FAX"

Multivariate analysis is done for the small cell trial. No major difference is noted with adjustment.

May 22, 1992

This submission attempts to address the differences in table 34b (offstudy) and table 7B(cardiac LVEF). This was better clarified in a later submission. Response 15.1 presents response duration; it also provides time to progression in non-responders. There are no significant differences between the arms. It does not support the speculation in the NDA that the responses on the placebo might have been less meaningful.

The meta-analysis for response, time to progression, and survival

are included in this submission.

June 1

This submission answers questions about the 88011 NYU trial. Details of measurable disease status are given. The 12 patients excluded from the evaluable patients analysis included 5 patients with measurable disease, one on the control arm and 4 on the ADR-529 arm (one possible response). So, the real intent to treat analysis would add these patients to the denominator of the evaluable patients analysis presented by The result would be slightly worse for ADR-529 than the "evaluable" patients analysis; as already noted, this the "evaluable" patients analysis was worse for ADR-529 than the analysis mis-labeled as "intent-to-treat" (which included many patients without measurable disease).

June 2

Response rate updates are given and tabulations submitted on patients recently accrued to 88001 and 88006. If one uses the corrected denominators for equal accrual cutoff, the response in 001 is 26/52 (50%) for DZR and 34/60(57%) for PLA.

June 4

This contairs more replies on 88011 study. The median dosing of doxorubicin is provided and shows no hint of a lesser doxorubicin dose on the DZR arm. The number of patients with the primary endpoint of a 10% LVEF drop at or beyond 450 mg/M^2 of doxorubicin is presented and is favorable for DZR (14/32 on DZR versus 39/43 on control).

Data listings which were supposed to be in NDA are provided (response, cardiac, etc.). Listings of tumor measurements are provided by f r the first time (item 3). The involvement in allowing to audit the NYU trial is documented in item 4.

June 5 4-month Safety update, update of survival and progression

This was reviewed in some detail prior to the ODAC meeting. Except for the update of survival and time to progression there was no information of note compared to that in the safety overview in the NDA. The data are summarized in the following tables. They show that when all 10:1 patients in the 88001 trial are evaluated with the new data cutoff date of 3-31-92, time to progression is significantly inferior on the ADR-529 arm, whether you censor at crossover (HR = 0.79 p=0.05 by LR) or not (HR = 0.79 p=0.03 by LR). The hazard ratio for progression is similar in the 88006 update but with fewer events, the difference is not statistically significant. The Hazard ratio for survival P:D is 1.0 for the 88001 trial and 0.77 for the 88006 trial.

Progression update

Trial 88001, patients censored at crossover to DZR

 # patients 509

 # events 258

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
0.79	(0.62, 1.00)	0.05	0.013

==========

Trial 88001, patients uncensored at crossover to DZR

 # patients 509

 # events 313

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
0.79	(0.63, 0.98)	0.03	0.016

Progression update

Trial 88006, patients censored at crossover to DZR

 # patients 296

 # events 124

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
0.83	(0.58, 1.20)	0.31	0.41

==========

Trial 88006, patients uncensored at crossover to DZR

 # patients 296

 # events 167

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
0.79	(0.61, 1.12)	0.21	0.29

Survival update

Trial 88001, patients censored at crossover to DZR

 # patients 509

 # deaths 185

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
1.01	(0.75, 1.35)	0.96	0.61

==========

Trial 88001, patients uncensored at crossover to DZR

 # patients 509

 # deaths 211

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
1.03	(0.78, 1.37)	0.82	0.79

Survival update

Trial 88006, patients censored at crossover to DZR

 # patients 296

 # deaths 102

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
0.77	(0.52, 1.14)	0.20	0.36

==========

Trial 88006, patients uncensored at crossover to DZR

 # patients 296

 # deaths 121

Hazard ratio(P:D)	95%ci	Log rank	Wilcoxon
0.81	(0.26, 1.17)	0.26	0.23

June 8

This contains tabulations for the update of survival in the 4-months safety update.

June 12

This contains the explanation of how information on survival was left out of the summary tables of literature on Razoxane.

June 16

"Response 9.8"

This shows that patients who had repeat LVEF after a LVEF event did not generally have continued decline of LVEF. Those on the DZR arm improved significantly more than did those on the control arm.

Response 20 and 23

This discusses differences in response analyses of sponsor, investigator, and consultant. It shows no evidence that decisions were biased for or against ADR-529.

Response 25.2

This shows that # of courses with AGC < 500 was significantly greater on DZR for all studies, and is clear in all 10:1 data bases. This occurred both early (courses 1-3) and late (courses 6-9).

June 16

Response 1 and 2

This lists the updated response patients giving accrual and data cutoff dates. It reveals that determination of measurability classification is not presented strictly as stratified, but involves a later assessment of what was on the baseline assessment form. In updated the updated cohorts, 10/111 in study 001 and 11/68 in 88006 have a measurability classification which is different from that stratified. This is more than in previous cohorts (10 out of more than 300 patients in in the original 88001 data base and 4 in 88006 as submitted to NDA originally). This supports the unreliability of this interim response assessment.

Response 4

This resolves the issue of apparent poor data quality in LVEF measurements. Most patients with missing data had LVEF at other times (450 instead of 400). Actually 96% of patients had data or a reasonable reason for missing data.

Response 5

This explains the discrepancy in the offstudy table 34b. As I suspected it used a different data cutoff date for # of courses received. Hcwever the actual offstudy analyses used the correct cutoff of 4-30-91.

10 Analyses of Case Report Forms

Given the view of the ODAC, an extensive validation of data from CRF's was not undertaken. A few issues were addressed:

Ophthalmology ADR's:

The May 1 submissior. contains the 5 cases of ophthalmology ADR's on the ADR-529 arm of study 88001. These were examined:

10:1 ratio

1102 Had hoarseness on course 1 and L ptosis on course 2. Patient had 1 supraclavicular nodal involvement. Attribution is unclear, I suspect L. Horner's syndrome secondary to tumor.

30116 Had fluctuating Diplopia on courses 1,2, 6, 7. Possible left 6th nerve paresis. Had tumor in bone of L orbit.

30204 Had dizziness and diplopia on course 18; none in courses 19 and 20.

40203 Listed as "visual field defect" which consisted of transient tunnel vision, nausea and vomiting after a surgical procedure after cycle 9.

20:1

28202 Had diplopia on courses 32 and 33, none on courses 34 or 36.

Reviewer's Comments:
In aggregate these cases do not suggest an underlying ophthalmic toxicity of ADR-529.

Claim that patients discontinued therapy because of fear of cardiac toxicity.

The May 1 submission contains the CRFs for the 9 patients on ADR-529 that refused therapy after 10 or more courses. The sponsor had made the assertion that "fear of cardiotoxicity" played a role in the patients going offstudy. My examination of the offstudy sheet from the CRF's did not support this assertion as it was not listed as a reason anywhere on any of them. Most listed patients as being tiring of the side-effects of chemotherapy.

Onstudy deaths

The April 16th submission lists the onstudy deaths and the case report forms are found in the May 1 submission. In examining the 20:1 cases not attributed to sepsis, etiology is often not clear. Several deaths occurred with the first or with early courses. Often cause of death is attributed by the investigator to progressive disease, but seldom is it definite. Sepsis seems just as reasonable a possibility. Nadir counts were not available for most of these patients due to the design of the 20:1 trials.

Many of the 10:1 deaths not attributed to sepsis on the DZR arm were also examined. Again many of the deaths occurred with the first course of therapy. Similarly some of these deaths could have been do to sepsis. Severe granulocytopenia was apparent just prior to death in many cases. For instance, case 6211 had AGC of 250 on 11/6 and died on 11/11 after the first course of therapy. There seems to be little firm basis for attributing this to progressive disease. Again case 77101 had a nadir AGC of 10 nadir on 9/29 and died on 10/1 after the first course. Again the investigator attributed death to progression, but in such cases it is difficult to exclude sepsis. On the control arm, patient 4201 died after the first course on 11/21/89 5 days after a nadir AGC of 140. Death attributed to progression could have been from the pleural effusion as listed; sepsis and ARDS is also a possibility. In summary, one can not be confident that onstudy causes of death as listed are accurate when attributed to progressive disease. If progression had been well documented, the patients would not have been onstudy.

Tumor and Cardiac endpoints

Several CRF's from the 88001 10:1 trial were examined to verify data in tabulations and in the PC patient profiles on response, time to progression, survival, and cardiac LVEF. Data on these endpoints had been accurately transcribed. A larger sample of CRF data was not validated given ODAC recommendations against approval at this time.

11 Advisory Committee Recommendations

At the meeting of ODAC on June 19, 1992, the advisory committee unanimously voted that the NDA was not approvable at this time. In another unanimous vote it was agreed that there was not adequate assurance that ADR-529 did not decrease the antitumor effect of doxorubicin. There was a diversity of opinion on what additional evidence would be enough to counter the findings suggesting tumor protection using the 10:1 ratio in the 88001 trial.

12 Regulatory Recommendations

1. The findings in this review do not change the overall findings noted in MOR #1. The findings of ODAC further support the recommendation against approval at this time.

2. A meeting should be scheduled with the company to discuss plans for the current trials and for future trials.

3. The clinical portion should use the same language as in the question to ODAC:

   "There is not adequate assurance that Zinecard does not decrease the antitumor effect of doxorubicin."

Grant A. Williams, MD

John R Johnson, MD
6-26-92

NDA 20212
HFD-150 div file
HFD-150/GWilliams
HFD-150/ECutler

Cutler

Attachments, MOR #2

NDA 20-212

Attachment

TABLE 2.1

Extent of Drug Exposure

U.S. Controlled Trials in Breast Cancer

20:1 Patients

	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Number Randomized	67	54	0	0	76	74	143	128
Number On-Study	1	0	0	0	1	0	2	0
Number Off-Study	66	54	0	0	75	74	141	128
Number of Patients Per Course								
0	67	54	0	0	76	74	143	128
1	66	53	0	0	76	74	142	127
2	60	50	0	0	73	72	133	122
3	55	49	0	0	70	68	125	117
4	41	42	0	0	68	66	109	108
5	35	39	0	0	64	63	99	102
6	35	34	0	0	59	63	94	97
7	32	24	0	0	58	57	90	81
8	31	24	0	0	52	49	83	73
9	27	14	0	0	50	42	77	56
10	24	10	0	0	46	32	70	42
>10	24	5	0	0	42	23	66	28
Median Number of Courses	6	6	0	0	11	9	9	8

TABLE 2.1

Extent of Drug Exposure

U.S. Controlled Trials in Lung Cancer

20:1 Patients

	088002	
	DZR	PLA
Number Randomized	26	25
Number On-Study	0	0
Number Off-Study	26	25
Number of Patients Per Course		
0	26	25
1	26	25
2	23	20
3	20	20
4	15	18
5	13	16
6	12	14
7	11	10
8	10	9
9	10	5
10	10	3
>10	3	1
Median Number of Courses	5	6

TABLE 2.2

Extent of Drug Exposure

U.S. Controlled Trials in Breast Cancer

10:1 Patients

	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Number Randomized	168	181	81	104	0	0	249	285
Number On-Study	31	17	12	21	0	0	43	38
Number Off-Study	137	164	69	83	0	0	206	247
Number of Patients Per Course								
0	168	181	81	104	0	0	249	285
1	165	181	79	100	0	0	244	281
2	149	169	71	94	0	0	220	263
3	142	162	65	89	0	0	207	251
4	115	141	51	74	0	0	166	215
5	102	120	47	62	0	0	149	182
6	84	107	38	54	0	0	122	161
7	60	74	31	42	0	0	91	116
8	44	67	27	37	0	0	71	104
9	38	51	22	32	0	0	60	83
10	32	39	15	27	0	0	47	66
>10	23	21	12	20	0	0	35	41
Median Number of Courses	6	6	5	6	0	0	5	6

TABLE 2.2

Extent of Drug Exposure

U.S. Controlled Trials in Lung Cancer

10:1 Patients

	088002	
	DZR	PLA
Number Randomized	73	82
Number On-Study	4	7
Number Off-Study	69	75
Number of Patients Per Course		
0	73	82
1	71	82
2	59	75
3	50	71
4	39	55
5	36	50
6	33	45
7	26	30
8	20	20
9	17	13
10	13	8
>10	9	5
Median Number of Courses	4	6

TABLE 2.3

Extent of Drug Exposure

U.S. Controlled Trials in Lung Cancer

All Patients

	088002	
	DZR	PLA
Number Randomized	99	107
Number On-Study	4	7
Number Off-Study	95	100
Number of Patients Per Course		
0	99	107
1	97	107
2	82	95
3	70	91
4	54	73
5	49	66
6	45	59
7	37	40
8	30	29
9	27	18
10	23	11
>10	12	6
Median Number of Courses	4	6

TABLE 4.A.1
(Cont)

CLINICAL TOXICITIES - COURSE 1

U.S. Controlled Trials in Breast Cancer

Incidence of Toxicities

20:1 Patients

Toxicity	Grade	088001 DZR	088001 PLA	088006 DZR	088006 PLA	088011 DZR	088011 PLA	Total DZR	Total PLA	Statistical* Test
Vomiting:	Yes	31	20	0	0	0	0	31	20	X^2=1.17
	No	34	33	0	0	0	0	34	33	p=0.28
	No Data	0	0	0	0	75	73	75	73	
Diarrhea:	Yes	7	8	0	0	8	5	15	13	X^2=0.19
	No	58	45	0	0	6	13	64	58	p=0.66
	No Data	0	0	0	0	61	55	61	55	
Fatigue/ Malaise:	Yes	33	31	0	0	10	12	43	43	X^2=0.57
	No	32	22	0	0	6	7	38	29	p=0.45
	No Data	0	0	0	0	59	54	59	54	
Fever:	Yes	19	9	0	0	12	8	31	17	X^2=1.84
	No	46	44	0	0	43	33	89	77	p=0.18
	No Data	0	0	0	0	20	32	20	32	
Sepsis:	Yes	16	4	0	0	3	1	19	5	X^2=7.97
	No	49	49	0	0	9	16	58	65	p=0.005
	No Data	0	0	0	0	63	56	63	56	
Infection:	Yes	12	4	0	0	13	15	25	19	X^2=2.16
	No	53	49	0	0	8	11	61	60	p=0.14
	No Data	0	0	0	0	54	47	54	47	
Neurotoxicity:	Yes	1	1	0	0	9	0	10	1	X^2=8.36
	No	64	52	0	0	7	15	71	67	p=0.004
	No Data	0	0	0	0	59	58	59	58	

* Mantel-Haenszel test (not performed if many sparse cells)
** Interpret with caution due to sparse cells

TABLE 4.A.3
(Cont)

CLINICAL TOXICITIES - COURSE 1

U.S. Controlled Trials in Breast Cancer

Incidence of Toxicities

All Patients

Toxicity	Grade	088001 DZR	088001 PLA	088006 DZR	088006 PLA	088011 DZR	088011 PLA	Total DZR	Total PLA	Statistical* Test
Vomiting:	Yes	105	117	40	60	0	0	145	177	X^2=2.46
	No	125	114	39	40	0	0	164	154	p=0.12
	No Data	0	1	0	0	75	73	75	74	
Diarrhea:	Yes	26	27	8	9	8	5	42	41	X^2=0.33
	No	204	205	70	91	6	13	280	309	p=0.56
	No Data	0	0	1	0	61	55	62	55	
Fatigue/ Malaise:	Yes	91	93	33	44	10	12	134	149	X^2=0.07
	No	139	139	46	56	6	7	191	202	p=0.80
	No Data	0	0	0	0	59	54	59	54	
Fever:	Yes	59	35	10	12	12	8	81	55	X^2=6.27
	No	171	197	68	88	43	33	282	318	p=0.012
	No Data	0	0	1	0	20	32	21	32	
Sepsis:	Yes	35	16	5	6	3	1	43	23	X^2=8.45
	No	194	216	73	94	9	16	276	326	p=0.004
	No Data	1	0	1	0	63	56	65	56	
Infection:	Yes	33	16	7	3	13	15	53	34	X^2=8.41
	No	197	215	71	96	8	11	276	322	p=0.004
	No Data	0	1	1	1	54	47	55	49	
Neurotoxicity:	Yes	14	4	3	7	9	0	26	11	X^2=7.58
	No	216	228	75	93	7	15	298	336	p=0.006
	No Data	0	0	1	0	59	58	60	58	

* Mantel-Haenszel test (not performed if many sparse cells)
** Interpret with caution due to sparse cells

TABLE 4.B.2
(Cont)

CLINICAL TOXICITIES - ALL COURSES

U.S. Controlled Trials in Breast Cancer

Incidence of Toxicities

10:1 Patients

Toxicity	Grade	088001 DZR	088001 PLA	088006 DZR	088006 PLA	088011 DZR	088011 PLA	Total DZR	Total PLA	Statistical* Test
Vomiting:	Yes	103	137	50	78	0	0	153	215	χ^2=12.73
	No	62	42	29	22	0	0	91	64	p<0.001
	No Data	0	1	0	0	0	0	0	1	
Diarrhea:	Yes	33	40	20	28	0	0	53	68	χ^2=0.38
	No	132	140	58	72	0	0	190	212	p=0.54
	No Data	0	0	1	0	0	0	1	0	
Fatigue/ Malaise:	Yes	93	108	59	71	0	0	152	179	χ^2=0.08
	No	72	72	20	29	0	0	92	101	p=0.78
Fever:	Yes	63	59	23	34	0	0	86	93	χ^2=0.25
	No	102	121	55	66	0	0	157	187	p=0.62
	No Data	0	0	1	0	0	0	1	0	
Sepsis:**	Yes	30	28	13	15	0	0	43	43	χ^2=0.53
	No	134	152	65	85	0	0	199	237	p=0.47
	No Data	1	0	1	0	0	0	2	0	
Infection:	Yes	40	35	19	17	0	0	59	52	χ^2=2.48
	No	125	145	59	83	0	0	184	228	p=0.12
	No Data	0	0	1	0	0	0	1	0	
Neurotoxicity:	Yes	24	18	15	17	0	0	39	35	χ^2=1.52
	No	141	162	63	83	0	0	204	245	p=0.22
	No Data	0	0	1	0	0	0	1	0	

* Mantel-Haenszel test (not performed if many sparse cells)
** Interpret with caution due to sparse cells

TABLE 8.A.1

MYELOSUPPRESSION - ABSOLUTE GRANULOCYTE COUNT ($x10^3/mm^3$)

U.S. Controlled Trials in Breast Cancer
Course 1

20:1 Patients

	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Expected Nadir Counts: Day 1 - 15 (±3)								
Mean	0.2	0.5	0.0	0.0	1.0	1.0	1.0	1.0
Median	0.1	0.8	0.0	0.0	0.5	0.6	0.5	0.6
St.Dev.	0.2	0.4	0.0	0.0	1.3	1.1	1.2	1.1
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	0.4	0.8	0.0	0.0	6.7	5.0	6.7	5.0
n	4	3	0	0	69	66	73	69
Expected Recovery Counts: Day 22 (±3)								
Mean	4.8	4.6	0.0	0.0	4.9	4.2	4.9	4.4
Median	4.4	4.1	0.0	0.0	4.6	3.8	4.5	3.8
St.Dev.	2.9	2.7	0.0	0.0	2.2	2.5	2.5	2.6
Min	1.0	1.0	0.0	0.0	0.4	0.9	0.4	0.9
Max	18.1	15.6	0.0	0.0	11.1	13.0	18.1	15.6
n	57	49	0	0	62	51	119	100

TABLE 8.A.5

MYELOSUPPRESSION – ABSOLUTE GRANULOCYTE COUNT

U.S. Controlled Trials in Breast Cancer
Course 1

10:1 Patients

Grade	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Expected Nadir Counts: Day 1 – 15 (±3)								
0	5	18	5	8	0	0	10	26
1	11	11	4	11	0	0	15	22
2	14	23	6	6	0	0	20	29
3	29	40	15	23	0	0	44	63
4	88	69	43	48	0	0	131	117
No Data	16	17	2	4	0	0	18	21
Expected Recovery Counts: Day 22 (±3)								
0	130	135	60	73	0	0	190	208
1	9	10	2	10	0	0	11	20
2	6	5	3	8	0	0	9	13
3	4	7	2	1	0	0	6	8
4	0	0	0	1	0	0	0	1
No Data	14	21	8	7	0	0	22	28

TABLE 9.A.5

MYELOSUPPRESSION - PLATELETS

U.S. Controlled Trials in Breast Cancer
Course 1

10:1 Patients

Grade	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Expected Nadir Counts: Day 1 - 15 (±3)								
0	90	132	51	76	0	0	141	208
1	48	24	19	16	0	0	67	40
2	6	2	3	1	0	0	9	3
3	6	4	1	2	0	0	7	6
4	1	0	0	1	0	0	1	1
No Data	12	16	1	4	0	0	13	20
Expected Recovery Counts: Day 22 (±3)								
0	149	153	70	94	0	0	219	247
1	1	4	0	2	0	0	1	6
2	0	0	0	0	0	0	0	0
3	0	1	0	1	0	0	0	2
4	1	0	0	0	0	0	1	0
No Data	12	20	5	3	0	0	17	23

TABLE 9.8.5

MYELOSUPPRESSION - PLATELETS

U.S. Controlled Trials in Breast Cancer
All Courses

10:1 Patients

Grade	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Expected Nadir Counts: Day 1 - 15 (±3)								
0	61	94	28	50	0	0	89	144
1	71	53	37	29	0	0	108	82
2	10	12	6	8	0	0	16	20
3	12	10	4	7	0	0	16	17
4	6	5	1	4	0	0	7	9
No Data	3	5	0	2	0	0	3	7
Expected Recovery Counts: Day 22 (±3)								
0	148	150	68	86	0	0	216	236
1	7	16	4	11	0	0	11	27
2	2	5	1	0	0	0	3	5
3	0	0	0	1	0	0	0	1
4	1	1	0	0	0	0	1	1
No Data	5	7	3	2	0	0	8	9

TABLE 8.A.2

MYELOSUPPRESSION – ABSOLUTE GRANULOCYTE COUNT ($x10^3/mm^3$)

U.S. Controlled Trials in Breast Cancer
Course 1

10:1 Patients

	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Expected Nadir Counts: Day 1 – 15 (±3)								
Mean	0.6	0.9	0.6	0.8	0.0	0.0	0.6	0.9
Median	0.4	0.6	0.4	0.5	0.0	0.0	0.4	0.5
St.Dev.	0.8	1.1	0.6	0.7	0.0	0.0	0.7	1.0
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	5.9	8.1	2.5	3.2	0.0	0.0	5.9	8.1
n	147	161	73	96	0	0	220	257
Expected Recovery Counts: Day 22 (±3)								
Mean	4.4	4.5	4.4	4.1	0.0	0.0	4.4	4.3
Median	4.1	3.4	3.8	3.6	0.0	0.0	4.1	3.5
St.Dev.	2.6	3.3	2.6	3.0	0.0	0.0	2.6	3.2
Min	0.6	0.6	0.8	0.3	0.0	0.0	0.6	0.3
Max	15.8	23.3	12.9	24.0	0.0	0.0	15.8	24.0
n	149	157	67	93	0	0	216	250

TABLE 6.B.5

MYELOSUPPRESSION - ABSOLUTE GRANULOCYTE COUNT

U.S. Controlled Trials in Breast Cancer
All Courses

10:1 Patients

Grade	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Expected Nadir Counts: Day 1 - 15 (±3)								
0	0	6	0	1	0	0	0	7
1	4	1	1	8	0	0	5	9
2	18	17	5	6	0	0	23	23
3	20	44	12	16	0	0	32	60
4	115	105	57	67	0	0	172	172
No Data	6	6	1	2	0	0	7	8
Expected Recovery Counts: Day 22 (±3)								
0	91	79	43	47	0	0	134	126
1	22	33	10	16	0	0	32	49
2	22	27	8	14	0	0	30	41
3	22	28	10	15	0	0	32	43
4	1	5	2	5	0	0	3	10
No Data	5	7	3	3	0	0	8	10

TABLE 13.8.2

Deaths On Study

U.S. Controlled Trials in Breast Cancer
All Courses

10:1 Patients

	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Number Randomized	168	181	81	104	0	0	249	285
Number (%) Of Deaths	4(2)	6(3)	4(5)	3(3)	0(0)	0(0)	8(3)	9(3)

TABLE 13.B.4
LISTING OF PATIENTS WHO DIED ON STUDY

Study No.	Patient No.	Age	Sex	Treatment Arm	Ratio DZR:DOX	No. of Courses at Time of Death	Cause of Death	Investigators' Attribution of Relationship to Study Drugs
088001	6201							
088001	6205							
088001	9101							
088001	28101							
088001	46201							
088001	49202							
088001	50202							
088001	62101							

N/A - Not Assessed
*Considered to be possibly related by sponsor.

TABLE 13.B.4
Cont'd.
LISTING OF PATIENTS WHO DIED ON STUDY

Study No.	Patient No.	Age	Sex	Treatment Arm	Ratio DZR:DOX	No. of Courses at Time of Death	Cause of Death	Investigators' Attribution of Relationship to Study Drugs
088001	6211							
088001	19202							
088001	20109							
088001	77101							
088001	4201							
088001	22206							
088001	33202							
088001	34104							

N/A - Not Assessed
*Considered to be possibly related by sponsor.

TABLE 13.8.4
Cont'd.
LISTING OF PATIENTS WHO DIED ON STUDY

Study No.	Patient No.	Age	Sex	Treatment Arm	Ratio DZR:DOX	No. of Courses at Time of Death	Cause of Death	Investigator's Attribution of Relationship to Study Drugs
088001	45302							
088001	54102							
088002	5202							
088002	9101							
088002	17201							
088002	28101							
088002	48201							
088002	26201							
088002	5209							

N/A - Not Assessed
*Considered to be possibly related by sponsor.

TABLE 13.B.4
Cont'd.
LISTING OF PATIENTS WHO DIED ON STUDY

Study No.	Patient No.	Age	Sex	Treatment Arm	Ratio DZR:DOX	No. of Courses at Time of Death	Cause of Death	Investigators' Attribution of Relationship to Study Drugs
088002	8202							
088002	36205							
088002	61204							
088002	102202							
088002	147203							
088002	50201							
088002	54205							
088002	78201							

N/A - Not Assessed
*Considered to be possibly related by sponsor

TABLE 13.B.4
Cont'd.
LISTING OF PATIENTS WHO DIED ON STUDY

Study No.	Patient No.	Age	Sex	Treatment Arm	Ratio DZR:DOX	No. of Courses at Time of Death	Cause of Death	Investigators' Attribution of Relationship to Study Drugs
088002	114201							
088002	114205							
088006	11405							
088006	27201							
088006	48202							
088006	54205							
088006	11307							
088006	11408							

N/A - Not Assessed
*Considered to be possibly related by sponsor.

TABLE 13.B.4
Cont'd.
LISTING OF PATIENTS WHO DIED ON STUDY

Study No.	Patient No.	Age	Sex	Treatment Arm	Ratio DZR:DOX	No. of Courses at Time of Death	Cause of Death	Investigators' Attribution of Relationship to Study Drugs
088006	42101							
088011	7							
088011	10							
088011	28							
088011	16							
088011	54							
088011	75							
088011	81							
088011	119							

N/A - Not Assessed
*Considered to be possibly related by sponsor.

TABLE 14.2

PATIENT DISPOSITION

U.S. Controlled Trials in Breast Cancer

10:1 Patients

	088001		088006		088011		Total	
	DZR	PLA	DZR	PLA	DZR	PLA	DZR	PLA
Number Randomized	168	181	81	104	0	0	249	285
Number On-Study	31	17	12	20	0	0	43	37
Number Off-Study	137	164	69	84	0	0	206	248
Primary Reason Off-Study (%)								
Progressive Disease	70(51)	59(36)	32(46)	36(43)	0(0)	0(0)	102(50)	95(38)
Cardio-toxicity	13(9)	46(28)	8(12)	22(26)	0(0)	0(0)	21(10)	68(27)
Adverse Experience	5(4)	4(2)	3(4)	3(4)	0(0)	0(0)	8(4)	7(3)
Refusal	23(17)	21(13)	10(14)	10(12)	0(0)	0(0)	33(16)	31(13)
Protocol Violation	10(7)	13(8)	5(7)	7(8)	0(0)	0(0)	15(7)	20(8)
Death	4(3)	6(4)	4(6)	3(4)	0(0)	0(0)	8(4)	9(4)
Lost to Follow-up	1(1)	2(1)	1(1)	0(0)	0(0)	0(0)	2(1)	2(1)
Randomized, not Treated	2(1)	0(0)	0(0)	0(0)	0(0)	0(0)	2(1)	0(0)
Other	9(7)	13(8)	6(9)	3(4)	0(0)	0(0)	15(7)	16(6)

5.0 SUMMARY TABLES

TABLE 1A
IDENTIFICATION OF CLINICAL STUDIES
Breast Cancer Studies

Trial	Dosing Ratio (DZR:DOX)	Number of Randomized Patients[a]		
		DZR	PLA	Total
088001	20:1	67	54	121
088011	20:1	76	74	150
	Subtotal:	143	128	271
088001	10:1	168	181	349
088006	10:1	81	104	185
	Subtotal:	249	285	534
			GRAND TOTAL:	805

[a]Randomized by January 14, 1991.

TABLE 1B
IDENTIFICATION OF CLINICAL STUDIES
Lung Cancer Study

Trial	Dosing Ratio (DZR:DOX)	Number of Randomized Patients[a]		
		DZR	PLA	Total
088002	20:1	26	25	51
088002	10:1	73	82	155
			GRAND TOTAL:	206

[a]Randomized by January 14, 1991.

TABLE 3A
TIME TO CARDIAC EVENT
Breast Cancer Studies

Statistic	Clinical Study					
	088001		088006		088011	
	D	P	D	P	D	P
20:1 Patients						
No. Patients	67	54			65	61
No. Events (%)	9 (13%)	25 (46%)			11 (17%)	33 (54%)
Median (Days)	--[a]	456			--[a]	455
Hazard Ratio (P:D)	6.177				5.070	
95% C.I. of (P:D)	(2.692, 14.174)				(2.495, 10.303)	
p-value (logrank)	<0.001				<0.01	
p-value (Wilcoxon)	0.034				<0.01	
10:1 Patients						
No. Patients	168	181	81	104		
No. Events (%)	19 (11%)	52 (29%)	7 (9%)	22 (21%)		
Median (Days)	1157	503	--[a]	600		
Hazard Ratio (P:D)	2.875		2.297			
95% C.I. of (P:D)	(1.654, 4.997)		(0.978, 5.396)			
p-value (logrank)	<0.001		0.048			
p-value (Wilcoxon)	0.16		0.46			

[a]The median was inestimable as the estimated distribution of time to cardiac event did not cross .50.

TABLE 3B
TIME TO CARDIAC EVENT
Lung Cancer Study

Statistic	Clinical Study 088002	
	D	P
20:1 Patients		
No. Patients	26	25
No. Events (%)	4 (15%)	9 (36%)
Median (Days)	--[a]	450
Hazard Ratio (P:D)	2.263	
95% C.I. of (P:D)	(0.670, 7.641)	
p-value (logrank)	0.18	
p-value (Wilcoxon)	0.62	
10:1 Patients		
No. Patients	73	82
No. Events (%)	9 (12%)	24 (29%)
Median (Days)	--[a]	500
Hazard Ratio (P:D)	2.281	
95% C.I. of (P:D)	(1.055, 4.936)	
p-value (logrank)	0.029	
p-value (Wilcoxon)	0.053	

[a]The median was inestimable as the estimated distribution of time to cardiac event did not cross .50.

TABLE 4A
TIME TO CONGESTIVE HEART FAILURE
Breast Cancer Studies

	Clinical Study			
	088001		088006	
	D	P	D	P
20:1 Patients				
No. Patients	67	54		
No. Events (%)	0 (0%)	2 (4%)		
Median (Days)	--[a]	--[a]		
Hazard Ratio (P:D)	79.653			
95% C.I. of (P:D)	(2.903, >100)			
p-value (logrank)	0.010			
p-value (Wilcoxon)	0.011			
10:1 Patients				
No. Patients	168	181	81	104
No. Events (%)	2 (1%)	10 (6%)	0 (0%)	3 (3%)
Median (Days)	1150	--[a]	--[a]	--[a]
Hazard Ratio (P:D)	10.776		5.161	
95% C.I. of (P:D)	(1.356, 85.648)		(0.491, 54.350)	
p-value (logrank)	0.005		0.17	
p-value (Wilcoxon)	0.045		0.17	

[a]The median was inestimable as the estimated distribution of time to congestive heart failure did not cross .50.

TABLE 48
TIME TO CONGESTIVE HEART FAILURE
Lung Cancer Study

	Clinical Study 088002	
	D	P
20:1 Patients		
No. Patients	26	25
No. Events (%)	0 (0%)	1 (4%)
Median (Days)	--[a]	--[a]
Hazard Ratio (P:D)	21.020	
95% C.I. of (P:D)	(0.322, >100)	
p-value (logrank)	0.16	
p-value (Wilcoxon)	0.16	
10:1 Patients		
No. Patients	73	82
No. Events (%)	2 (3%)	5 (6%)
Median (Days)	--[a]	--[a]
Hazard Ratio (P:D)	1.977	
95% C.I. of (P:D)	(0.382, 10.222)	
p-value (logrank)	0.41	
p-value (Wilcoxon)	0.49	

[a]The median was inestimable as the estimated distribution of time to congestive heart failure did not cross .50.

TABLE 5A
MEAN CHANGE FROM BASELINE IN LVEF VALUES (%)
Breast Cancer Studies

Study	Cumulative Doxorubicin Dose (mg/M^2)							
	300		400[a]		500		550	
	D	P	D	P	D	P	D	P
20:1 Patients								
088001	0.0 (34)[b]	-6.4** (34)	-0.2 (28)	-9.9** (21)	-2.3 (22)	-12.9** (8)	-2.0 (23)	-20.8** (5)
088011	-1.3 (60)	-4.8* (56)	-2.5 (32)	-13.9** (42)			-1.6 (31)	16.6** (13)
10:1 Patients								
088001	-2.5 (77)	-3.9 (99)	-0.3 (33)	-7.0** (65)	-1.3 (26)	-8.9** (34)	-1.7 (21)	-11.8** (19)
088006	-4.0 (33)	-5.0 (46)	-4.1 (21)	-7.9 (35)	-3.8 (12)	-10.0* (23)	-0.7 (10)	-12.4** (17)

[a]450 mg/M^2 in 088011
[b]( ) = number of patients.
*p<0.05, **p<0.01 (Wilcoxon rank sum test), DZR vs. PLA.

TABLE 5B
MEAN CHANGE FROM BASELINE IN LVEF VALUES (%)
Lung Cancer Study

Study	Cumulative Doxorubicin Dose (mg/M²)							
	300		400		500		550	
	D	P	D	P	D	P	D	P
2D:1 Patients								
063002	-2.9 (10)[a]	-9.4* (14)	3.4 (7)	-14.3** (9)	-1.6 (7)	-13.7* (3)	-5.7 (3)	-9.0 (1)
1D:1 Patients								
088002	-4.2 (31)	-6.3 (40)	-0.1 (17)	-7.1* (15)	-1.4 (12)	-7.0 (6)	-3.5 (8)	-5.5 (4)

[a] ( ) = number of patients.
*p<0.05, **p<0.01 (Wilcoxon rank sum test), DZR vs. PLA.

TABLE 7.A.1
RESPONSE RATES
Breast Cancer Studies
Intent-to-Treat Patients

	Clinical Study					
	088001		088006		088011	
	D	P	D	P	D	P
20:1 Patients						
No. Patients[a]	66	54			~~76~~	~~74~~
No. Responses (%)[b]	38 (58%)	29 (54%)			~~26 (47%)~~ 24/49 (50%)	~~33 (45%)~~ 24/41 (59%)
Difference in Rates[c]	4%				~~-2%~~ -9%	
95% C.I. of % Difference[c]	(-14%, 22%)				~~(-14%, 18%)~~ (-30, 12)	
p-value[d]	0.67				0.73	
10:1 Patients						
No. Patients[a]	141	152	54	69		
No. Responses (%)[b]	67 (48%)	96 (63%)	31 (57%)	36 (52%)		
Difference in Rates[c]	-15%		5%			
95% C.I. of % Difference[c]	(-27%, -4%)		(-13%, 23%)			
p-value[d]	0.007		0.56			

[a]Number of randomized patients with bidimensional, measurable disease. — NOT True for 88011
[b]# of complete responses plus # of partial responses (plus # of "improved" in 088011)
[c]p̂ (DZR) minus p̂ (PLA).
[d]Pearson chi square.

TABLE 7.A.3
POOLING OF RESPONSE RATES
Breast Cancer Studies

	Dosing Ratio (DZR:DOX)					
	20:1[e]		10:1[f]		All Patients	
	D	P	D	P	D	P
Intent-to-Treat Patients						
No. Patients[a]	142	128	195	221	337	349
No. Responses (%)[b]	74 (52%)	62 (48%)	98 (50%)	132 (60%)	172 (51%)	194 (56%)
Difference in Rates[c]	4%		-10%		-5%	
95% C.I. of % Difference[c]	(-8%, 16%)		(-20%, 0%)		(-12%, 2%)	
p-value[d]	0.55		0.052		0.23	
Evaluable Patients						
No. Patients[a]	100	98	160	196	260	294
No. Responses (%)[b]	59 (59%)	51 (58%)	91 (57%)	130 (66%)	150 (58%)	181 (62%)
Difference in Rates[c]	1%		-9%		-4%	
95% C.I. of % Difference[c]	(-13%, 15%)		(-20%, 1%)		(-12%, 4%)	
p-value[d]	0.88		0.07		0.35	

[a]Number of randomized patients with bidimensional, measurable disease.
[b]# of complete responses plus # of partial responses (plus # of "improved" in 088011)
[c]$\hat{p}$ (DZR) minus $\hat{p}$ (PLA).
[d]Pearson chi square.
[e]Includes 088001 and 088011.
[f]Includes 088001 and 088006.

TABLE 7.B.1
RESPONSE RATES
Lung Cancer Study
Intent-to-Treat Patients

	Clinical Study	
	088002	
	D	P
20:1 Patients		
No. Patients[a]	26	25
No. Responses (%)[b]	15 (58%)	17 (68%)
Difference in Rates[c]	-10%	
95% C.I. of % Difference[c]	(-36%, 16%)	
p-value[d]	0.45	
10:1 Patients		
No. Patients[a]	67	76
No. Responses (%)[b]	30 (45%)	45 (59%)
Difference in Rates[c]	-14%	
95% C.I. of % Difference[c]	(-30%, 2%)	
p-value[d]	0.09	

[a]Number of randomized patients with measurable disease.
[b]# of complete responses plus # of partial responses.
[c]$\hat{p}$ (DZR) minus $\hat{p}$ (PLA).
[d]Pearson chi square.

TABLE 7.B.3
POOLING OF RESPONSE RATES
Lung Cancer Study

	Dosing Ratio (DZR:DOX)					
	20:1[c]		10:1[c]		All Patients	
	D	P	D	P	D	P
Intent-to-Treat Patients						
No. Patients[a]	26	25	67	76	93	101
No. Responses (%)[b]	15 (58%)	17 (68%)	30 (45%)	45 (59%)	45 (48%)	62 (61%)
Difference in Rates[c]	-10%		-14%		-13%	
95% C.I. of % Difference	(-36%, 16%)		(-30%, 2%)		(-29%, 3%)	
p-value[d]	0.45		0.09		0.07	
Evaluable Patients						
No. Patients[a]	19	20	43	62	62	82
No. Responses (%)[b]	15 (79%)	17 (85%)	29 (67%)	42 (68%)	44 (71%)	59 (72%)
Difference in Rates[c]	-6%		-1%		-1%	
95% C.I. of % Difference[c]	(-30%, 18%)		(-19%, 17%)		(-16%, 14%)	
p-value[d]	0.70		0.97		0.90	

[a]Number of randomized patients with measurable disease.
[b]# of complete responses plus # of partial responses.
[c]p (DZR) minus p (PLA).
[d]Pearson chi square.
[e]088002.

TABLE 8.A.1
TIME TO DISEASE PROGRESSION
Breast Cancer Studies

	Clinical Study					
	088001		088006		088011[a]	
	D	P	D	P	D	P
20:1 Patients						
No. Patients	67	54			76	74
No. Events (%)	52 (78%)	49 (91%)			48 (63%)	32 (43%)
Median (days)	231	225			~~208~~	~~272~~
Hazard Ratio (P:D)	1.179				~~1.060~~ 0.81	
95% C.I. of (P:D)	(0.795, 1.749)				~~(0.659, 1.704)~~	(0.55, 1.2
p-value (logrank)	0.41				0.81	
p-value (Wilcoxon)	0.73				0.97	
10:1 Patients						
No. Patients	168	181	81	104		
No. Events (%)	89 (53%)	96 (53%)	43 (53%)	51 (49%)		
Median (days)	232	243	199	232		
Hazard Ratio (P:D)	0.908		0.949			
95% C.I. of (P:D)	(0.680, 1.212)		(0.630, 1.431)			
p-value (logrank)	0.51		0.80			
p-value (Wilcoxon)	0.09		0.43			

[a]Data summary reflects on-study assessments.

TABLE 8.B.1
TIME TO DISEASE PROGRESSION
Lung Cancer Study

	Clinical Study 088002	
	D	P
20:1 Patients		
No. Patients	26	25
No. Events (%)	22 (85%)	22 (88%)
Median (days)	195	241
Hazard Ratio (P:D)	0.908	
95% C.I. of (P:D)	(0.496, 1.659)	
p-value (logrank)	0.75	
p-value (Wilcoxon)	0.49	
10:1 Patients		
No. Patients	73	82
No. Events (%)	51 (70%)	59 (72%)
Median (days)	183	183
Hazard Ratio (P:D)	1.021	
95% C.I. of (P:D)	(0.696, 1.496)	
p-value (logrank)	0.92	
p-value (Wilcoxon)	0.52	

TABLE 8.B.2
POOLING OF TIME TO DISEASE PROGRESSION DATA
Lung Cancer Study[a]

	Dosing Ratio (DZR:DOX)					
	20:1		10:1		All Patients	
	D	P	D	P	D	P
No. Patients	26	25	73	82	99	107
No. Events (%)	22 (85%)	22 (88%)	51 (70%)	59 (72%)	73 (74%)	81 (76%)
Median (days)	195	241	183	183	188	192
Hazard Ratio (P:D)	0.908		1.021		1.00	
95% C.I. of (P:D)	(0.496, 1.659)		(0.696, 1.496)		(0.724, 1.301)	
p-value (logrank)	0.75		0.92		1.00	
p-value (Wilcoxon)	0.49		0.52		0.42	

[a]088002.

TABLE 10.A.1
SURVIVAL
Breast Cancer Studies

	Clinical Study					
	088001		088006		088011[a]	
	D	P	D	P	D	P
20:1 Patients						
No. Patients	67	54			76	74
No. Events (%)	50 (75%)	45 (83%)			3 (4%)	5 (7%)
Median (days)	517	502			-[b]	-[b]
Hazard Ratio (P:D)	1.197				~~0.592~~ 1.05	
95% C.I. of (P:D)	(0.798, 1.796)				(0.552, 10.267)	
p-value (logrank)	0.38				0.23	
p-value (Wilcoxon)	0.71				0.40	
10:1 Patients						
No. Patients	168	181	81	104		
No. Events (%)	58 (35%)	69 (38%)	30 (37%)	25 (24%)		
Median (days)	526	537	420	526		
Hazard Ratio (P:D)	1.022		0.604			
95% C.I. of (P:D)	(0.720, 1.449)		(0.355, 1.028)			
p-value (logrank)	0.90		0.06			
p-value (Wilcoxon)	0.88		0.12			

[a]Data summaries reflect on-study assessments.
[b]The median was inestimable as the estimated survival distribution did not cross .50.

TABLE 10.A.2
POOLING OF SURVIVAL DATA
Breast Cancer Studies

	Dosing Ratio (DZR:DOX)					
	20:1[a]		10:1[b]		All Patients	
	D	P	D	P	D	P
No. Patients	67	54	249	285	316	339
No. Events (%)	50 (75%)	45 (83%)	88 (35%)	94 (33%)	138 (44%)	139 (41%)
Median (days)	517	502	474	437	490	52[illegible]
Hazard Ratio (P:D)	1.197		0.878		0.976	
95% C.I. of (P:D)	(0.798, 1.796)		(0.655, 1.176)		(0.770, 1.237)	
p-value (logrank)	0.38		0.38		0.84	
p-value (Wilcoxon)	0.71		0.29		0.31	

[a]Includes 088001 only.
[b]Includes 088001 and 088006.

TABLE 10.B.1
SURVIVAL
Lung Cancer Study

	Clinical Study 088002	
	D	P
20:1 Patients		
No. Patients	26	25
No. Events (%)	25 (96%)	23 (92%)
Median (days)	277	307
Hazard Ratio (P:D)	0.911	
95% C.I. of (P:D)	(0.516, 1.611)	
p-value (logrank)	0.75	
p-value (Wilcoxon)	0.48	
10:1 Patients		
No. Patients	73	82
No. Events (%)	48 (66%)	49 (60%)
Median (days)	280	312
Hazard Ratio (P:D)	0.972	
95% C.I. of (P:D)	(0.649, 1.457)	
p-value (logrank)	0.89	
p-value (Wilcoxon)	0.15	

TABLE 10.B.2
POOLING OF SURVIVAL DATA
Lung Cancer Study[a]

	Dosing Ratio (DZR:DOX)					
	20:1		10:1		All Patients	
	D	P	D	P	D	P
No. Patients	26	25	73	82	99	107
No. Events (%)	25 (96%)	23 (92%)	48 (66%)	49 (60%)	73 (74%)	72 (67%)
Median (days)	277	307	280	312	278	307
Hazard Ratio (P:D)	0.911		0.972		0.954	
95% C.I. of (P:D)	(0.516, 1.611)		(0.649, 1.457)		(0.687, 1.325)	
p-value (logrank)	0.75		0.89		0.78	
p-value (Wilcoxon)	0.48		0.15		0.11	

[a]088002.

APPENDIX A.

RESPONSE TO THE QUESTION BY

In a recent communication, [redacted] has forwarded a document which summarizes his review of studies which have compared CAF to CMF in the treatment of breast cancer. The applicable table and references cited are as follows:

A. Table

Study Design	Study	Ref.	No. of Patients	Response Rate (%) CMF	Response Rate (%) CAF	Response (p)	Duration (weeks) CMF	Duration (weeks) FAC	Duration (p)	Survival (weeks) CMF	Survival (weeks) FAC	Survival (p)

*CMF q 4 wk = Cyclophosphamide 100 mg/m² p.o. d1-14; Methotrexate 40 mg/m² i.v. d1,8; 5 Fluorouracil i.v. 500/600 mg/m² d1,8.

+CAF q 4 wk = Cyclophosphamide 100 mg/m² p.o. d1-14; Doxorubicin 20 mg/m² (ref. 40) / 25 mg/m² (ref. 38,41) / 30 mg/m² (ref 37,39) i.v. d1,8; 5 Fluorouracil 500/600 mg/m² I d1,8.

@ Two arms of a three-arm study comparing CAFVP with a continuos or intermittent schedule of CMFVP.

**CMFVP q 4 wk = Cyclophosphamide 400 mg/m² i.v. d1; Methotrexate 30 mg/m² i.v. d1,8; 5 Fluorouracil 400 mg/m² i.v. d1,8; Vincristine 1 mg i.v. d1,8; Prednisone 20 mg p.o. d1-7.

++CAF q 3 wk = Cyclophosphamide 500 mg/m² i.v. d1; Doxorubicin 50 mg/m² i.v. d1; 5 Fluorouracil 500 mg/m² i.v. d1.

APPENDIX B.

REFERENCES CITED IN TABLE A

37. **Bull JM, Tormey DC, Carbone PP, et al:** A randomized comparative trial of Adriamycin® verses methotrexate in combination drug therapy. *Cancer.* 41:1649-1657. 1978.

38. **Aisner J, Weinberg V, Perloff M, et al:** Chemotherapy versus chemoimmunotherapy (CAF vs CAFVP vs CMF each MER) for metastatic carcinoma of the breast. A CALGB study. *J. Clin. Oncol.* 5:1523-1533. 1987.

39. **Cunning FJ, Gelmans R, Horton J:** Comparison of CAF versus CMFP in metastatic breast cancer; analysis of prognostic factors. *J. Clin. Oncol.* 3:932-940. 1985.

40. **Muss HB, White DR, Richard F, et al:** Adriamycin vs methotrexate in five-drug combination chemotherapy for advanced breast cancer. *Cancer.* 42:2141-2148. 1978.

41. **Tormey DC, Wenlberg VE, Leone LA, et al:** A comparison of intermittent vs continuous and of Adriamycin vs methotrexate 5-drug chemotherapy for advanced breast cancer. *Am. J. Clin. Oncol.* 7:231-239. 1984.

42. **Smalley RV, Lefante J, Bartolucci A, et al:** A comparison of cyclophosphamide, Adriamycin and 5-Fluorouracil, vincristine and prednisone (CMFVP in patients with advanced breast cancer. *Breast Cancer Res. Treat.* 3:209-220. 1983.

MEDICAL OFFICER REVIEW #1
(preliminary, see introductory comments)

1. General Information:

1.1 NDA# 20-212

1.1.2 Review: M.O. Review #1 (preliminary)
1.1.3 Submission date February 7, 1992
1.1.4 Date of Review May 25, 1992

1.2 Drug Name

1.2.1 Generic name: dexrazoxane
1.2.2 Proposed trade name: Zinecard
1.2.3 Other names: ICRF-187/ADR-529

1.3 Sponsor ADRIA LABORATORIES

1.4 Pharmacologic Category: Cardioprotectant

1.5 Proposed indication:
"for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration"

1.6 Dosage form and directions for use:
Lyophilized powder for IV injection, reconstituted with M/6 Sodium Lactate Injection, USP to be given IV at a 10:1 ratio prior to doxorubicin.

1.7 NDA Drug Classification: 1A

1.8 Related IND

Attachments for MOR #1 are in my office

2.0 TABLE OF CONTENTS

3 Material Reviewed

The individual Study reports and appendices of the pivotal studies 88001, 88002, 880-6, and 88011 were the primary material reviewed for this preliminary review. This review is presented prior to complete review of all data in order to be available in time for Advisory Committee scrutiny. Not yet reviewed in depth by the medical officer are the pharmacology (under review by Agency pharmacology and biopharmacology reviewers), background clinical literature, and safety and efficacy overviews.

4 Chemistry/Manufacturing Controls

See Chemistry review by Dr. Tolgyesi. A change in formulation involved the need to use a different diluent (1/6 M Sodium Lactate instead of saline) occurred midway through clinical trials. This change is not thought to be of clinical importance.

5 Animal Pharmacology/Toxicology

See reviews by W. Schmidt PhD and W.Coulter PhD. Additional details will be presented in MOR #2 after further review.

Pharmacodynamics

There continues to be academic debate regarding the cardioprotective mechanism of ADR-529. One explanation suggests that it inhibits free radical formation by chelating Fe III ions, inhibiting oxidation of membrane lipids. It was also found to have antineoplastic activity and was studied under an IND for this indication.

Dr. Coulter reviewed the various pharmacodynamic studies in his 64 page review dated 4-30-92. Many animal tumor models were reviewed which failed to show an inhibition in of doxorubicin anti-tumor effect. However the intrinsic anti-tumor activity of ADR-529 itself in some of these models could have hidden a protective effect.

Attached is data from one study which supports the possibility that ADR-529 at some ratios could inhibit the antitumor effect of doxorubicin. This study is described on p 59-61 of the review, and refers to a study in nude mice of transplanted subcapsular renal tumors from a human breast cancer cell line. Several ratios (5:1, 10:1, 15:1, and 20:1) of ADR-529 were tested with doxorubicin. All combinations showed equal initial tumor shrinkage in response to doxorubicin, but the 10:1 ratio showed a significantly faster tumor regrowth. See attached copy of page 60.

Pharmacokinetics

Kinetics appears to be linear. The terminal half life is 2 to 4 hours. Studies suggest that ADR-529 given prior to doxorubicin as in the clinical studies does not affect pharmacokinetic parameters of doxorubicin. Clearance appears to involve both renal elimination and a metabolic degradation.

6 Clinical Background

It is important to consider the contribution of doxorubicin to the efficacy of regimens used in the pivotal studies (CAV for small cell, CAF for breast cancer). The company has been asked to address this issue.

ICRF was first studied as an anticancer agent under IND . The MTD was 1000-1250 mg/day qdX3 every 4 weeks. Toxicities included myelosuppression in adults and hepatotoxicity in children.

Parts of the NDA dealing with these issues will be reviewed in more detail in MOR# 2.

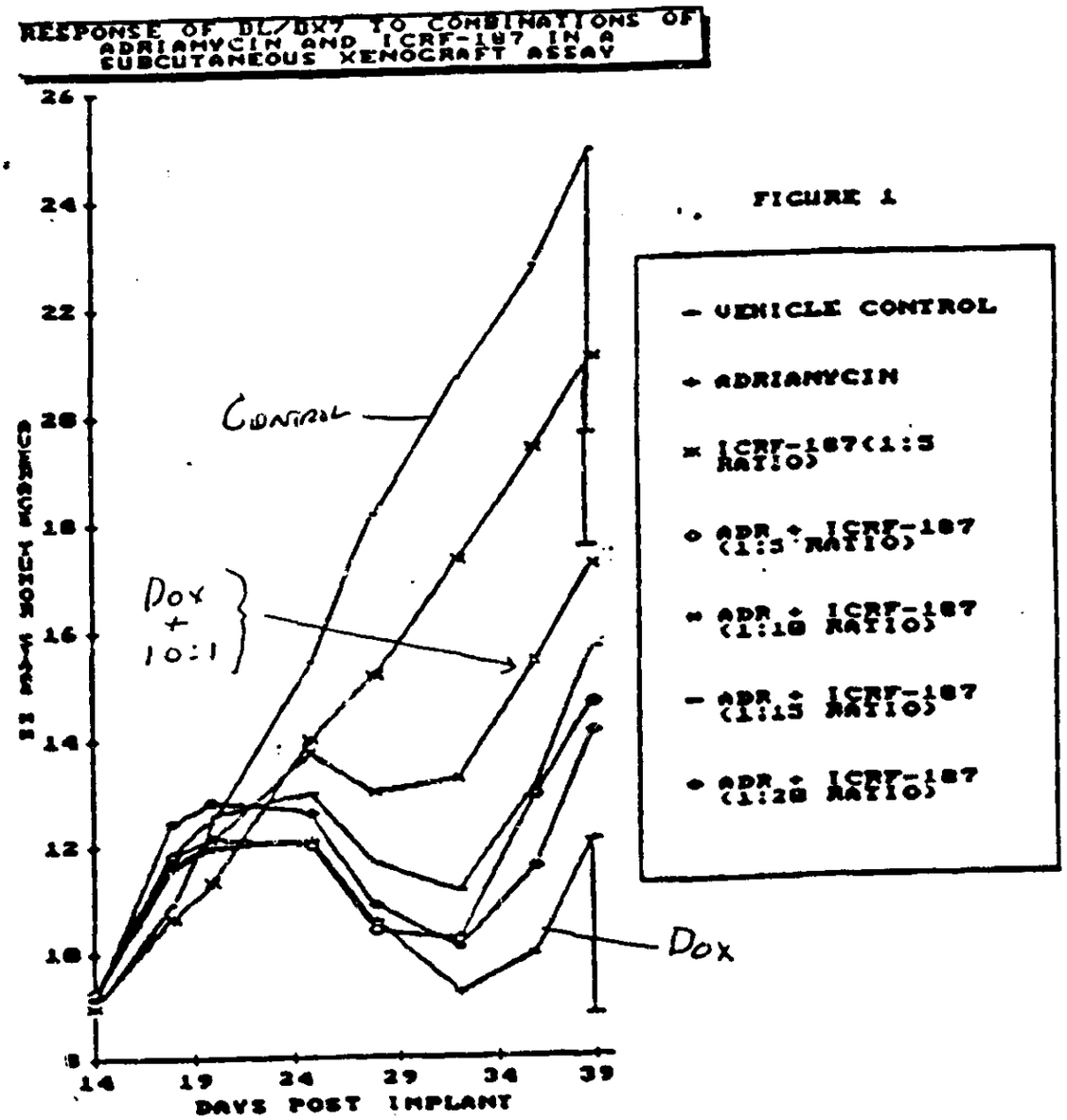
7 Description of Clinical Data Sources

The primary sources of data for this review were the individual Study reports. Original protocols were also examined in both the ADRIA and IND's. Literature descriptions of the studies were also examined.

Tumor Size Measurements (L + M)/2

Gp	Day 14	Day 28	32	Day 36	Day 39
1	8.95±1.99	18.15±4.09	20.65±4.84	22.70±4.62	24.85±4.76
2	9.15±1.14	10.55±2.32	9.20±2.61	9.95±3.20	12.05±3.44
3	8.95±1.42	15.15±1.18	17.30±1.85	19.35±2.35	21.05±2.71
4	9.25±1.33	10.40±3.00	10.21±3.69	12.93±3.76	14.64±3.16
5	9.20±1.68	13.00±3.65	13.35±4.20	15.40±5.22	17.20±5.00
6	9.25±1.47	11.65±3.14	11.10±3.73	13.10±3.69	15.65±4.80
7	9.15±1.18	10.85±2.32	10.06±2.63	11.58±3.33	14.08±3.88

There were 3 deaths in Group 4 (Day 32) and 4 deaths in Group 7 (2 Day 32, 2 Day 36). Body weight loss was 18% in G2, 26% in G4, 10% in G5. 22% in G6, and 29% in G7. Figure 1 (p. 05-01767) shows a graph of the tumor response to treatment and depicts the increase in BL/BX7 tumor growth once treatment is withdrawn.



8 Clinical studies: Introductory comments

This Medical Officer review is being submitted prior to complete evaluation of the NDA to facilitate communication to the Advisory Committee before the meeting on June 18. The primary focus of Medical Officer review has to this point been analysis of the protocols and evaluation of the data as presented by ADRIA in individual study reports. Numerous additional analyses and questions have been communicated to ADRIA LABS regarding the trials. Summary tabulations, enabling individual data verification, in the format requested by the reviewer nearly a year before NDA submission, were not submitted until well after the NDA submission in February. Prior to considering the review complete additional time will be needed to verify validity of data (summary tabulation to CRF), review ADRIA responses to questions communicated, and review other portions of the application (clinical use outside of pivotal studies, overview of safety and efficacy, etc.). It is hoped that the bulk of this process will be completed prior to the June 18 meeting. Reviewer comments in this preliminary review are made based on the assumption that the data and analyses presented by ADRIA LABS in the application are largely valid.

Due to changes in the midst of 2 of the trials, the historical aspects of the application are complex. I suggest that the reader begin with the reviewer summary and conclusions of the 88001 trial to help with orientation. There are a large number of attachments from the individual study reports to allow individual review of pertinent summary data. These are numbered according to the corresponding page of reference in the review. The volume of these attachments is increased by the fact that 88001 and 88002 trials are each actually 2 trials, a 20:1 ratio (ADR-529 to doxorubicin) trial and a 10:1 ratio trial analysis. However, their inclusion will allow one to examine with some detail complimentary analyses between 20:1 and 10:1 trials.

Briefly the trials are:

88011: This was the initial trial by Spyerer et al at NYU, an unblinded randomized controlled trial in breast cancer, of FAC with or without a 20:1 ratio of ADR-529. Reports have been published in NEJM and more recently in JCO. The study was audited and reviewed for ADRIA by

88001: This was the initial ADRIA sponsored trial in breast cancer, a randomized, double-blinded trial of FAC with placebo or with ADR-529 at a 20:1 ratio. When an overview of trials suggested an increased death rate from myelosuppression on ADR-529 arms, the trial was restarted using a 10:1 ratio, and patients originally receiving the 20:1 ratio crossed over to 10:1. During

the 10:1 portion of this trial, on the recommendation of a data monitoring committee, this part of the trial was stopped after an interim analysis because the committee considered that cardiac protection had been proven and that it would be unethical to proceed. All patients in all trials were thereafter offered ADR-529 after a cumulative dose of 350 mg/M^2 of doxorubicin.

88002 This trial was of similar design to 88001, but in patients with small cell lung cancer being treated with CAV. It similarly was restarted with the 10:1 ratio and was subsequently stopped at the time 88001 was stopped on advice of the monitoring committee.

88006 This was a second trial in breast cancer started by ADRIA at the time of redesign of 88001, also using a 10:1 ratio. It also was stopped at the time of the monitoring committee recommendation.

So, in reality, there are 2 essentially complete trials, an older complete 20:1 study from NYU (88011) and the ADRIA sponsored 10:1 trial (88001) stopped at a planned interim analysis. There are fragments of 10:1 trials in breast cancer (88006) and lung cancer (88002) and fragments of 20:1 (with some patients also receiving 10:1) data in each of these trials. In addition, ADRIA will update us on response data (unreviewed) from ongoing extensions of the 88001 and 88006 trials. These trials now have new cardiac-endpoint objectives, and all patients cross over to ADR-529 after 350 mg/M^2 of doxorubicin.

There are many ways one could approach the complex array of data in this application. I consider the 88001 trial to be complete, and to be the only complete trial using the proposed 10:1 ratio. The results of this trial have raised serious doubts about the selectivity of ADR-529 at this ratio in protecting the heart but not tumor. With such doubt firmly established, I suggest that formal proof of net benefit be required of this agent in the setting to be used through trials designed to strictly demonstrate equivalence of tumor-associated efficacy outcomes. I do not think additional increments of information on response rates or assumptions regarding extrapolation of data from the 20:1 drug ratio or extrapolation of data from animals should be sufficient.

8.1 Trial #1, Clinical Study 88001

TITLE: ADR-529 as a Cardioprotective in a Phase III Randomized Trial of FAC versus FAC + ADR-529 in the Treatment of Disseminated Carcinoma of the Breast

DATE OF ORIGINAL PROTOCOL: July 28, 1987

DATE OF AMENDMENT NO. 1: November 25, 1987
DATE OF AMENDMENT NO. 2: February 12, 1989
DATE OF AMENDMENT NO. 3: April 12, 1989
DATE OF AMENDMENT NO. 4: January 14, 1991

Historical considerations

Amendment #1 11-25-87 It is more appropriate to consider these changes as part of the original protocol since they occurred so near beginning of study.

Amendment #2 2-12-89
Amendment #3 4-12-89

These are the major amendments defining the protocol as it was applied to the "10 to 1" patients in this study, providing the framework for the data reported in the NDA for these patients.

Amendment #4 1-14-91

This amendment primarily outlined post-NDA handling of accrual, etc. for patients continuing on in new studies.

Other dates of import:

2-88 Study was begun, 20:1 dose

11-2-88 Dose was modified to 10:1 due to excess of deaths from myelosuppression, amendment #2 outlines these changes.

2-6-89 Data monitoring committee and interim analysis plan added.

4-24-89 88006, Second Breast Cancer Study started.

10-13-89 Data monitoring committee (DMC) began viewing cardiac data in addition to the other toxicity data at quarterly meetings.

Trial 88001

11-5-90 DMC recommends ADR-529 to both arms after 300 mg/M^2 of Doxorubicin.

12-6-90 Meeting with FDA. Decision to end accrual for initial endpoint.

1-14-91 Accrual cutoff date for NDA.

3-27-91 PreNDA meeting, attended by FDA, ADRIA, and ODAC chairman Craig Henderson, MD. Agreement that NDA would be submitted.

3-31-91 Data cutoff date for NDA.

4-31-91 Data cutoff for response endpoint.

8.1.1 Objective:

The 3 objectives have been constant from the original protocol:

-To demonstrate cardioprotective effect of ADR-529.
-To determine if ADR-529 alters response rate to FAC.
-To assess the safety of doxorubicin plus ADR-529.

8.1.2 Design:

The protocol was for a multicenter randomized double blind placebo controlled trial of FAC (5FU, Adriamycin, and cyclophosphamide) with ADR-529 versus FAC plus placebo. Stratification occurred, after early 1989, on the basis of measurable vs non-measurable and presence of cardiac risk factors:

Cardiac risk factors were to be considered one or more of the following:

-mediastinal radiation
-Age > 65
-History of heart disease (MI, significant arrhythmia, angina).
-Hypertension requiring medication.
-Diabetes mellitus requiring medication.
-Baseline MUGA scan 1-10% above the lower limit of normal for the institution.

8.1.3 Protocol

8.1.3.1 Details in Protocol Amendments:

Study was begun. 2-88

Amendment #2 2-12-89
Amendment #3 4-12-89

The following are significant changes outlined in these two amendments defining the 10:1 portion of this trial:

-The ratio of ADR-529 to Doxorubicin was changed from 20:1 to 10:1.
-Interim analysis plan was added.
-Safety committee plan was submitted.
-Non-measurable disease patients were included.
-Stratification was begun:

a. Cardiac risk factors versus no cardiac risk factors.
b. Measurable disease versus non measurable disease.

-Formulation was changed from free amine to sodium salt. To keep the Ph similar, lactate was substituted for saline as diluent. See Chemistry section and chemistry review.

Amendment #4 1-14-91

Data from patients accrued after this amendment were not considered part of the trials submitted for this NDA.
-All patients receive ADR-529 after 300 mg/M2 of doxorubicin.

-Patients were to be accrued to answer the question of whether a difference in cardiotoxicity can be detected in patients given ADR-529 from the start versus those given it only after 300 mg/M^2. Response data from some of these patients may be available in 4-month safety update.

8.1.3.2 Eligibility:

-women with unresectable or metastatic breast cancer were eligible.
-Initially measurable disease was a requirement, however after the major amendment in early 1989, both measurable and evaluable disease was allowed (with stratification on this criterion).
-No prior chemotherapy was allowed for metastatic disease.
-No prior anthracycline or anthracene was allowed even as an adjuvant.
-ECOG performance status was required to be 0,1,or 2. PS 2 was excluded at the time of the change to the 10:1 ratio. With the PreNDA amendment (#4), PS 2 was again allowed. So, the bulk of the patients getting 10:1 in the NDA were PS 0 or PS 1.
-Other details of eligibility are listed on p 08-00671.

Reviewer's Comments:
In considering differences in response or efficacy results in various portions of this trial, and in considering generalizability of results or appropriateness of combination via meta-analysis, it is important to remember the various changes occurring with the early 1989 amendment which lowered the ratio of ADR-529 to 10:1, excluded PS 2 patients, included non-measurable disease patients, and changed formulation. With the 4th amendment, the Pre-NDA amendment, (involving no data in this review) again PS 2 patients are included.

8.1.3.3 Number of patients:

Initially the protocol was to have 72 "response evaluable patients per arm" or 38 patients per arm who had received 500 mg/M2 of doxorubicin, which ever was largest. The DMC recommended on 11-5-90 that accrual need not proceed further due to interim analysis establishing the cardioprotective effect.

The basis of the 38 patient size was to give 80% power to detect a 10% change in LVEF at 500 mg/M2, the primary objective of the trial. The primary dose of interest was to be this dose although 150 mg/M2, 300 mg/M2 and every 100 mg/M2 thereafter were to be evaluated also.

The secondary objective was to compare response rates on the 2 arms. In the patients with measurable disease, 72 patients were required to have a power of 80% to detect a 20% decrease in response rates using a one-tailed test for equality of proportions.

Other comparisons of interest included dropouts for heart failure, dropouts for LVEF decline to at least 20% from baseline, a decline to 10% below normal limit, or decline to at least 5% below normal limit.

Reviewer's Comments:
Note that the original design did not describe an analysis of time to "cardiac event."

The sample size for the ongoing trial described in amendment #4, is based on continuing accrual until 24 cardiac events occur. Breast cancer response data from some of these patients may be available in the 4-month safety update.

8.1.3.4 Randomization

Central randomization occurred by phone M-F in Eastern Time Zone. Although the study report states that individual lists were prepared for each center, the sponsor states that they were kept centrally.

8.1.3.5 Procedure

Both arms received CAF intravenously every 3 weeks:
Cytoxan 500 mg/M^2 day 1
doxorubicin 50 mg/M^2 day 1
5FU 500 mg/M^2 day 1

According to randomization, patients were to receive either

placebo or ADR-529 in a volume of 50 ml/M2 of M/6 sodium lactate by slow IV push prior to, but within 30 minutes of the doxorubicin administration.

As noted in the amendment section, on 1-14-91 after a cumulative 300 mg/M2 of doxorubicin had been given, all patients were to be given ADR-529 with the next doxorubicin dose. This would only have affected such patients from 1-14-91 until 4-31-91 for the original NDA submission, but would affect more data in the safety update; this would include data extending until early 1992.

Dose modification

Doxorubicin and ADR-529 doses were not to be altered. Cytoxan and 5FU doses were to be decreased by 100 mg/M^2 each for granulocytopenic fever or day 22 granulocyte count below 1500 or platelet count below 90k. Subsequent dose reductions for similar reasons were to be by 50 mg/M^2.

Patients were to continue therapy until cardiac toxicity or disease progression.

Reviewer's Comments:
I am not sure that I agree with dose-adjustment only for neutropenic sepsis or day 22 counts. I think I would have adjusted for severe myelosuppression without neutropenic sepsis. It might be worth discussing this with ODAC, and also doing an analysis of patients with low nadirs with the first few courses. How many of them got into trouble with the next course?

Criteria for early termination for cardiac toxicity are described in section 8.4:

-congestive heart failure, manifest by at least 3 of the following::

- -cardiomegaly by X-ray
- -Basilar rales.
- -Cardiac S3 Gallop
- -PND and/or orthopnea and/or exertional dyspnea.

-LVEF decline by 0.10 to below normal for institution.

-Decline in LVEF by at lease 0.20 from baseline regardless of relation to institutional norm.

-Decline by 0.05 in LVEF below lower limits of institutional norm.

Reviewer's Comments:
According to these criteria, a relatively minor change from 0.51 to 0.45 could cause pt to go off study. ADRIA has been queried on the accuracy of LVEF tests. It is noted that the initial primary endpoint was to be decline by 0.10 in LVEF. In reality, the stopping occurred on the basis of time to cardiac event, an aggregate of the various cardiac events.

Concomitant therapy

Although no systemic hormonal therapy and no off-study chemotherapy was allowed, local radiation to painful bone metastases and CNS radiation were allowed.

Reviewer's Comments:
It was not well-specified what patients going off-study for toxicity were to do regarding further therapy ie no therapy until progression, etc.

Evaluation schedule:

Attached is the schedule of evaluation. Note that assessment for response occurs every 3 courses while patients were receiving study drugs and every 3 months during followup until relapse.

Reviewer's Comments:
This evaluation schedule might not be very sensitive for detection of earliest progression, and might tend to be slightly asymmetric if there were an imbalance of patients going off-study for cardiac progression. In other words after going offstudy for any reason including cardiac toxicity evaluation is every 12 weeks instead of every 9 weeks). This would bias the evaluation in favor of the arm with more going offstudy. An evaluation of when people progressed, at regularly scheduled visits or earlier, on the 2 arms would be of interest. Here, maintenance of the blind might be of import. Early evaluation due to hematologic toxicity and subsequent hospitalization might bias the evaluation against the toxic arm.

Cardiac toxicity evaluation consisting of MUGA scan and evaluation of cardiac signs and symptoms were to be evaluated after 150 mg/M^2, 300 mg/M^2, 400 mg/M^2, and every 50 mg/M^2 thereafter.

With the amendment changing dose to 10:1 a couple of changes were made. If the patient was going off study only for a MUGA scan change, a rapeat NUGA scan was required. The weekly CBC was

reintroduced for the first 4 cycles at this time also.
Reviewer's Comments:
The quality of the MUGA scan data may be better after this amendment. Likewise, data for comparative effect on myelosuppression will be more complete after this amendment.

8.1.3.6 Efficacy criteria

Response

Response criteria were fairly standard:

CR
PR 50% decrease in sum of products.
PD any of the following:
-25% increase in SOP for non-responders.
-50% increase in SOP after response
-new lesions.
-"unequivocal progression" of non-measurable disease.

Only lytic bone lesions were considered measurable. Definitions of response in bone are given on p 08-00685.

Reviewer's Comments:
One wonders about the verifiability of "unequivocal progression." Here importance of blind would be important. Note that there are different criteria for progression after response and after non-response. Stratification might be appropriate, but given that response is a non-randomized phenomenon, I am not sure about this. It might be appropriate to look at response rates, time to progression in non-responders and in responders. Note that a duration of response of one month was not part of the partial response definition like in most oncology trials; moreover, no-one month reanalysis was required. Those with more easily noted lesions might tend to have progression noted sooner.

Cardiac efficacy

In section 10.2 under Efficacy Criteria the following is the introductory statement:

"The two treatment arms will be compared with respect to percent decline from baseline in LVEF and incidence of cardiac toxicities at similar cumulative doses of Adriamycin."

It then goes on to list the 5 conditions (one clinical: congestive heart failure and 4 permutations of MUGA scan changes. See the discussion of offstudy criteria for cardiac toxicity or p 08-00683 for details.)

Regarding the cardiac efficacy criteria, the statistical discussions seem clear that a primary endpoint is named as a change by 10% in EF to below the institutional norm. Several other cardiac endpoints are also to be evaluated. Again I note that initially the primary endpoint was going to be LVEF decrease of at least 0.10 at 500 mg/M^2.

Reviewer's Comments:

The protocol does not seem clear how to analyze the patients going off study for cardiac toxicity prior to 500 mg/M^2. The protocol seems well-designed to detect a difference in clinical and MUGA scan changes. The clinical significance of the magnitude of such changes is less clear, and might involve considerations such as how beneficial continued therapy with adriamycin was at the point of LVEF decline, how specific the protective effect was, and whether the LVEF changes were reversible or would have led to significant clinical changes. Unblinding might affect the clinical evaluation of CHF but is unlikely to affect the LVEF evaluation.

8.1.3.7 Statistical considerations

The initial statistical section (volume 2.1 of IND) states that LVEF will be assessed at baseline, 300 mg, etc. "The primary dose of interest will be 500 mg/M^2." The test was to be an analysis of variance, though mention was also made of a 2-sided t-test. The secondary objective of comparing response rates was to be a one-tailed test for equality of proportions with alpha of 0.05. In addition, the incidence of dropouts for heart failure, decline of LVEF by 20%, decline of at least 10% to below norm, and decline in LVEF to 5% below norm was to be compared.

Reviewer's Comments:

This seems to have been somewhat illogical. The primary comparison was to exclude those having toxicity the earliest. Although not specified in the protocol, time to cardiac event is more logical. However, since the events, later to be put forward in aggregate by ADRIA as an endpoint, were only identified as offstudy criteria instead of efficacy endpoints, their suitability for defining efficacy may not have been as closely considered as would that of a primary endpoint.

Methods for comparing safety, survival, etc. were not specified. A group sequential plan was introduced with the dose change in the 2-89 amendment. Again it was stated that the main variable of interest was change from baseline in LVEF at dose of 500 mg/M^2. The minimum difference of interest was 0.10. The plan was to be a one-sided asymmetric group sequential plan described by DeMets and Ware (referenced, p 08-00634). No more than 5 analyses were

anticipated. Evaluations were to occur every 6 evaluable patients "per treatment" (reaching 500 mg/M2). Nominal significance levels were 0.00005, 0.003, 0.013, 0.027, and 0.042. The 2-sample Wilcoxon tank sum test was to be used to compare LVEF differences at interim evaluations. A blocked Wilcoxon rank sum test was to be used when there were notable imbalances in known cardiac risk factors. If the upper boundary was crossed, accrual was to continue.

Reviewer's Comments:

Note that within the statistical section of the protocol, the interim plan for analysis and the final plan use different tests. From amendment #2: (2-12-89):

"Addition: The Safety Committee shall consist of three to five independent experts in the conduct of clinical trials. At least one biostatistician and two medical oncologists shall be included on the Committee. None of the members shall be involved as investigators in this Adria Laboratories sponsored clinical study. The Safety Committee shall meet a minimum of four times per year to review the safety of ADR-529 trials. If, at any time, the Committee feels that the safety of patients is being compromised by participating in the trials, the trials will be closed to further accession. A representative of the Regulatory Department of Adria Laboratories shall serve as an ongoing ex-officio member of the committee to record the deliberations of the committee including meeting minutes.

The following table from the report summarizes the reported meetings of the DMC,

TABLE 5.5

Timing of Data Monitoring Committee Meetings

Meeting No.	Date of Meeting	Data Presented
1	January 6, 1989	S
2	April 14, 1989	S
3	July 14, 1989	S
4	October 13, 1989	S, CV
5	January 12, 1990	S, CV
6	April 27, 1990	S, CV
7	August 3, 1990	S, CV, T
8	November 5, 1990	S, CV, T
9	February 1, 1991	S, CV, T
10	May 10, 1991	S, CV, T

S=Safety (includes deaths),
CV=Cardioprotection data,
T=Antitumor data.

Reviewer's Comments:
According to my count, the CV data was analyzed for the 5th time on 11-5-90, when the decision to stop was made. It is not clear to me why ADRIA considers that there were fewer analyses for efficacy.

8.1.4 Results

(20:1) 8.1.4 Results of 20:1 Drug Ratio

(20:1) 8.1.4.1 Investigators

The 36 investigators who enrolled 121 patients are listed on p 08-00814. 67 were randomized to ADR-529 and 54 to the control. Table 1 A (p 08-00472) lists the number of patients at each center.

Reviewer's Comments:
The most patients on an arm at one center is 4. There are 8 centers with no patients on the placebo arm and 3 centers with no patients on the ADR-529 arm.

(20:1) 8.1.4.2 Baseline characteristics

Baseline characteristics are listed in attached table 2A, p 00477. Median age was 55-56 years, and 12-17 % of the patients were black. As noted in table 3A, (attached) cardiac risk factors were similar in the 2 arms, with history of heart disease slightly favoring placebo and history of hypertension favoring ADR-529. Baseline disease characteristics are compared in attached table 4A. The median disease free interval was longer on the placebo arm (median 670 d vs 440d, p 0.052). Prior hormonal therapy was more common on the placebo arm (p = 0.049). Otherwise the 2 arms were comparable (# of disease sites, performance status, prior surgery, RT, adjuvant chemotherapy (31-37%), dominant disease site, ER and PR receptors, and baseline blood counts.

Reviewer's Comments:
Given the imbalance in a few important prognostic factors for response, a multivariate analysis was requested for response and survival data. The analyses were not substantially changed.

(20:1) 8.1.4.3 Data Sets Analyzed

As shown in attached table 6.3.3, nearly all 67 ADR-529 and 54 placebo patients were included in cardiotoxicity time to event data, response, progression, disease-free survival, survival, quality of life, and course 1 toxicity analyses. The number of

patients evaluated for LVEF changes was equal up to the 300 mg/M^2 dose of doxorubicin (34 patients each at this dose). Thereafter the numbers rapidly dropped off on the placebo arm:

Dox (mg/M^2)	ADR-529	placebo
400	28	21
500	22	8
550	23	5
600	19	1

The biggest drop in these data is between 400 and 500 mg/M^2, predominantly in the placebo arm.

(20:1) 8.1.4.4 Cardiac effects

This data is summarized in attached Table 5A and Figure 1A. There were 9 events in the ADR-529 arm and 25 in the placebo arm, with p 0.001 by log rank when analyzed by dose of doxorubicin. Hazard ratio was 6.18 (95% ci 2.7 to 14). There were 2 episodes of congestive heart failure in the placebo arm versus none in the ADR-529 arm. Attached table 7A details the LVEF changes versus dose of doxorubicin. As shown, the difference between the mean of LVEF of patients remaining on study steadily increased from 2.9 at baseline to 16.7 at 550. Similarly, the individual patient changes from baseline were compared and the medians of these changes increased from 0.6 at 150 mg/M^2 to 18.8 at 550 mg/M^2.

Reviewer's Comments:
These data are quite impressive. We must remember that the trial was not stopped for efficacy, so we might not hold the p values to interim standards. Again I mention that the time to cardiac event of any cause seems like a very reasonable way to analyze the data, it was never specified in the protocol. The primary analysis was to be comparison of LVEF at 500mg/M^2 of doxorubicin. An important assumption in the time to event analysis is that censoring for other reasons such as response was not related to the cardiac endpoint. For instance, if the poor performance status patients on one arm all progressed, and poor PS was related to cardiac sensitivity, the analysis of cardiac toxicity might be confounded. I have asked ADRIA to consider this and whether an analysis could clarify the issue.

(20:1) 8.1.4.5 Antitumor Efficacy

Response rates

As mentioned earlier, 21% of the ADR-529 arm and 13% of the placebo arm were not considered evaluable for response. Reasons are located in appendix VIII (p 08-01977) and are summarized in the attached table from p 08-00443. Almost all were for inevaluable because they had not received the required 3 courses. The intent to treat and evaluable patients response analyses are shown in table attached table 8A. In the intent to treat analysis:

	ADR-529	placebo
Response rate	58% (38/66)	54% (29/54)
95% ci of difference	(-14%, 22%)	
p value	p = 0.67	

The evaluable patients analysis looks more favorable for the ADR-529 arm (67% vs 57%) but still is not statistically different (p = 0.31).

Reviewer's Comments:
In trying to assess the potential for the drug to cause inhibition of antitumor effect, it is likely that the analysis closest to theoretical optimum would lie somewhere between the intent the analysis and the evaluable patient analysis. It is quite reasonable to consider the 11 patients off study without tumor assessment (7 A vs 4 P) as failures. However, it is likely that a fraction of the other 11 patients (7 A vs 4 P) would have responded. This includes the 4 deaths (4 A vs 0 P).

Progression

Neither time to disease progression (displayed in attached Figure 3A) nor disease free survival were significantly different:

	Time to Progression	Dz Free Survival
Median ADR-529	231 d	227 d
Median placebo	235	225 d
Hazard Ratio [p:A] and 95% ci	1.18 [0.80, 1.75]	1.07 [0.75, 1.58]
p value (LR)	0.41	0.66
P value (GW)	0.73	0.96

Reviewer's Comments:
For purposes of analyzing anti-tumor effect inhibition, the time to progression, which censors for death, is probably appropriate. In this analysis, the data point in favor of ADR-529 with wide confidence intervals.

(20:1) 8.1.4.6 Survival

Survival was similar on the 2 arms as shown in attached table 11A.

Reviewer's Comments:
This survival data is quite mature (about 80% dead in both arms). Despite the early deaths, the point value of the hazard ratio (1.20) favors ADR-529 with lower bound of 95% ci at 0.80.

(20:1) 8.1.4.7 Quality of Life

There were very few events on either arm for "time to performance status ≥ 3 on study' or time to decrease in body weight ≥ 15%. For time to PS ≥ 3 any time in study, there were 57 recorded events, Hazard ratio P:D 1.38 (0.82-2.24), with p = 0.34.

(20:1) 8.1.4.8 Toxicity

Clinical toxicities

Clinical toxicities DURING COURSE 1 are summarized in Table 12 A (08-00500). Sepsis (25% vs 8%) was significantly more common in the ADR-529 arm. Although reports of esophagitis and dysphagia were slightly more common on the placebo arm, the mean severities of those reported were significantly less on the ADR-529 arm. Clinical toxicities were not statistically different in incidence and severity for alopecia, skin reactions, hemorrhage,CHF, anorexia, N&V, diarrhea, Fatigue, fever, infection, and neurotoxicity. Although fever (p=0.10) and infection (p=0.09) were more common on the ADR-529 arm the differences were not statistically significant.

The most severe toxicities present during course 1 were compared and are presented in table 13A on are found on pp 00502-00506 along with statistical comparisons (wilcoxon rank sum). Differences included with fever (p=0.10) and sepsis (p=0.013) which were more common on the ADR-529 arm. Grade 2 sepsis occurred in 22% of the ADR-529 arm compared to 8% of the placebo arm.

Similarly these analyses were done over ALL COURSES. Table 14A presents the comparative incidence; sepsis (38% vs 17%, p< 0.01), fever (49% vs 34%), and infection (35% vs 17%, p < 0.05) were more common on the ADR-529 arm. Mean severity scores were similar for clinical toxicities on the 2 arms.

The tables comparing most severe toxicity grades over all courses are on pp 00516-00521. Again differences approaching statistical significance included sepsis, infection, and fever (see attached excerpt from table 15A). Grade 3 sepsis was 34% vs 17%, and Grade 4 was 5% vs 0%.

Adverse reactions reported DURING COURSE 1 are compared by COSTART terminology on pp 00528-00532. These were similar except for a higher incidence of asthenia on the placebo arm (31% vs 16%). The comparisons for incidence of adverse experiences for ALL COURSES are found on pp 00541-00548. These are generally similar on the 2 arms. Under Ner/CNS/B on p 00545(attached), in aggregate there are many more reports on the ADR-529 arm. ADRIA has been asked to clarify how many of these events involve separate patients and how many are separate occurrences. Skin rash was slightly more

common on the ADR-529 arm (10% vs 4%). Again asthenia was more common on the control arm (39% vs 25%).

Laboratory tests

Renal and Hepatic

Renal and hepatic enzyme values after course 3 (the time of first scheduled repeat measurements) are compared in table 18A, pp 00560-564, for the 97 patients (82%) for whom data was available at this point. Comparison of grades by wilcoxon rank sum tests and comparisons of highest values by wilcoxon rank sum showed no statistical differences. Comparison of most severe grade over all studies is presented in table 20A on pp 566-570. There were 3 patients in the placebo arm with elevated bilirubin compared to none in the ADR-529 arm (p=0.06). This is of questionable significance. Similarly there were 5 versus 1 patients with LDH elevations of at least grade II in the placebo arm.

Myelosuppression

Data on nadirs for the 20:1 group is scant since the study was not designed to collect such data until the amendment including the change to the 10:1 ratio. However, data on recovery by day 22 was recorded in 111 patients (92%). The mean counts on day 22 of course one are presented in tables 25A, 26A,27A, and 28A (pp 580-584). These were not statistically different. The data for all courses is summarized in these same tables. Overall there is a trend for higher day 22 medians of WBC, platelet count, and AGC on the ADR-529 arm.

Reviewer's Comments:
This phenomenon could occur as a result of differences in the timing or height of rebound or from differential dropout of patients sensitive to myelosuppression.

(20:1) 8.1.4.9 Dosing intensity

Dose intensity expressed in mg/M2/course was not statistically different by WRS for the 2 arms (table 29A, attached). This table also demonstrates also that cumulative doses of the 3 agents were not statistically different in the 2 arms. However, it is noted that the ranges extend to higher values on the ADR-529 arm, since

more patients continued on this arm for an extended period of time. Median dosing intensity was about 93-94% for doxorubicin and 89-91% for the other agents.

Attached table 31 A demonstrates no differences in # of patients with dose reductions or dose delays when compared by WRS. About half of each arm had no reductions or delays. Repeated delays were more common on the ADR-529 arm. 10 patients (15%) on ADR-529 versus 2 controls (4%) had 5 or more delays.

(20:1) 8.1.4.10 Patient disposition

Patient disposition is outlined in attached table 33A. Note the excess in patients going off the ADR529 arm for progressive disease (38% vs 28%), patient refusal (20% vs 11%), and death (11% vs 2%) in the ADR-529 arm. On the other hand, patients went off-study more often for cardiac toxicity in the placebo arm (44% vs 14%). These are displayed course by course in attached table 34A. 8 cardiac toxicities, 6 on the ADR-529 arm occur at course 3. This is prior to a time one would expect such changes, and details will be requested on these patients. Cardiac off-study events on the placebo arm occur primarily at course 6 and beyond. The differences in patient refusal come from the 9 patients refusing on the ADR-529 arm at course 10 or later. The reasons for going off here may be of interest, since this number (9) exceeds the number going off for progression (2) on the ADR-529 arm; furthermore, this prolonged therapy forms the justification for using a chemoprotectant in the first place. These patients may give a clue to the quality of life imparted by prolonged chemotherapy. The KM curve for time to off-study is shown in attached figure 9A. The Hazard ratio is 1.7 with $p = 0.009$ by LR. However, one notes that the curves cross at the area of the median. Obviously our assumptions of proportional hazards are not valid here. Early the hazard is higher on the ADR-529 arm probably from myelosuppressive complications, deaths, progression, and cardiac toxicity. Later, the hazard is higher on the placebo due to an excess of off-study for cardiac toxicity.

(10:1) 8.1.4. 10:1 Drug Ratio Results

(10:1) 8.1.4.1 Investigators

The 36 investigators who enrolled 349 patients, 168 randomized to ADR-529 and 181 to placebo, are listed on p 08-00814. Table 1A on p 00474 lists the number of patients at each center according to arm. The following is derived from the numbers in this table:

#Pts/site	#sites
1-5	24
≥ 10	12
≥ 15	7
20-30	4

Reviewer's Comments:
From this table it is obvious that even auditing the four largest centers will not assess more than 1/4 to 1/3 of the patients. A verification of the randomization process by audit is essential. The pattern of imbalance of patient numbers (imbalance of 13 in 20:1 data corrected in the opposite direction in the 10.1 data) suggested that the same blocked stratification lists were used after the dose ratio change. ADRIA confirmed that this did occur.

(10:1) 8.1.4.2 Baseline characteristics

Baseline characteristics are listed in table 2B, p 00479. Median age was 55-58 years, and 12-17% of the patients were black (17% on placebo arm). As noted in table 3A, (attached) cardiac risk factors-diabetes was the only risk factor which was significantly different in the 2 arms, with 6% on the placebo arm versus 12% on the ADR-529 arm ($p = 0.05$). Age > 65, prior mediastinal radiation therapy, and LVEF ≤ 10% above lower limit of normal favored the placebo arm, but the differences were not statistically significant. Baseline disease characteristics are compared in attached tables 4B. The median disease free interval was similar on the 2 arms. In this trial, PS 2 patients were excluded. 44% of ADR-529 vs 51% of placebo were asymptomatic ($p = 0.14$). Previous adjuvant chemotherapy had occurred in 43% of the ADR-529 arm vs 35% of the placebo arm. 45% of the ADR-529 arm had ER negative tumors versus 55% of the placebo arm ($p = 0.15$). About 16% of both arms did not have measurable disease. Baseline granulocyte

count was lower on the ADR-529 arm (median 4600 vs 4900, p = 0.043). Baseline platelets and hemoglobin were comparable.

Reviewer's Comments:
Once again, given imbalance in a few prognostic factors, a multivariate analysis was requested for response and survival data and made no substantial differences in the analyses.

(10:1) 8.1.4.3 Data Sets Analyzed

As shown in the attached table 6.3.2, all patients were included in cardiotoxicity time to event, progression, disease-free survival, survival, and quality of life analyses. For the intent to treat analysis of response, 141 of ADR-529 arm and 152 of the placebo arm had measurable disease. The evaluable patients' response analysis excluded 25 (18%) ADR-529 patients and 15 (10%) of the placebo patients with measurable disease.

Reviewer's Comments:
The reason for nearly 20% exclusion of measurable disease on the ADR-529 arm certainly bears examination to see if the dropouts represent ADR-529 toxicity.

The per cent of patients evaluated for LVEF changes was similar up to the 300 mg/M^2 dose of doxorubicin. Between 300 mg/M^2 and 400 mg/M^2 over half of the ADR-529 arm were lost from evaluation (44 of 77) compared to only about one third of the placebo arm (34 patients of 99). The numbers evaluated in each arm were similar until the 600 mg/M^2 dose.

	ADR-529	placebo
Baseline	168	181
150	131	147
300	77	99
400	33	65
500	26	34
550	21	19
600	17	8

Recall the 20:1 data:

	ADR-529	placebo
300	34	34
400	28	21
500	22	8
550	23	5
600	19	1

Reviewer's Comments:
There is a clear difference in the pattern between the 10:1 and 20:1 patients. Note the loss of more ADR-529 patients from analysis prior to loss of placebo patients in the 10:1 analysis, which is the opposite of what occurred in the 20:1 group. It throws some doubt on the validity of the analyses of LVEF in small residual groups onstudy. Note also how few patients are out to 600 mg/M^2 where a difference in the arms in duration of therapy with FAC becomes clear.

It is remarkable that at the 400 mg/M^2 doxorubicin level, almost twice as many patients on the placebo arm were analyzed for LVEF than on the ADR-529 arm (65 or 36% versus 33 or 20%). Even more puzzling is the fact that according to the offstudy table 34B, 53 patients were on the ADR-529 arm for the 9th course (450mg/M^2 of doxorubicin) and the above suggests only 33 were included in LVEF measurements at 400 mg/M^2. ADRIA has been asked to explain this apparent discrepancy.

(10:1) 8.1.4.4 Cardioprotection

This data is summarized in attached Table 5B and Figure 1B. There were 19 events in the ADR-529 arm and 52 in the placebo arm, with p<0.001 by log rank when analyzed by dose of doxorubicin. Hazard ratio was 2.88 (95% ci 1.65, 5.00) There were 10 episodes of congestive heart failure in the placebo arm versus 2 in the ADR-529 arm. Time to CHF is depicted versus doxorubicin dose and Kaplan Meier Plot in attached Figure 2 B. Hazard ratio was 10.8 (1.4, 85) and p = 0.005 by LR. Note that most of these events occurred beyond 500 mg/M^2. 1% (2/168) in the ADR-529 arm versus 6% (10/181) in the placebo arm (10/181) developed CHF on-study.

Reviewer's Comments:

Note that the events curve begins to separate at the 400 mg/M² point and continues to separate whereas the CHF curves do not separate until after 500 mg/M². When combined with the previous observation of differences in censoring at the 400 mg/M² one must interpret the results with caution. Again one must assume no major relationship between cardiac prognosis and other reasons for dropout. The case report forms for the reported CHF events will be examined to assess the validity and clinical nature of these events.

Attached table 7B details the LVEF changes versus dose of doxorubicin. As shown, the difference between the mean of LVEF of patients remaining on study steadily increased from 8.3% at baseline to 10.5% at 550. Similarly, the individual patient changes from baseline were compared and the medians of these changes increased from 6.7% at 400 mg/M² (P=0.005) to 10.1% at 550 mg/M² (P=0.007). For the 60 patients making it out to 500 mg/M², the difference was 7.6% (p=0.003).

Reviewer's Comments:
Again, these data are impressive but are not as extreme as were the 20:1 data. There is the additional concern about unbalanced dropouts at a crucial stage (300-400 mg/M² of doxorubicin). This portion of the trial was stopped for efficacy, so we should hold the p values to interim standards and consider the protocol specified endpoint. See similar comments in the 20:1 section. The delta LVEF at 500 mg/M² considered to be of clinical import at the time of protocol design was 10%, whereas the mean difference value noted in the analysis is 7.6%.

(10:1) 8.1.4.5 Antitumor Efficacy

Response rates

There were 141 patients on ADR-529 arm and 152 on the placebo arm with measurable disease. Of these, 14 on the ADR-529 arm and 9 on the placebo arm were without off-study tumor assessment. In other words, ADRIA considered 10% and 6% to be inevaluable, respectively. Reasons are located in appendix VIII (p 08-01980) and are summarized in the attached table 6.5.1.2.

Again the main reason for not being evaluable because was not receiving the required 3 courses. The intent to treat and

evaluable patients response analyses are shown in table attached table 8B. In the intent to treat analysis the response rate was 63% in the placebo arm and 48% in the ADR-529 arm (p=0.007). It is also noted that the number progressing at this analysis was also in favor of placebo (21% vs 11%). The evaluable patients analysis is similar as shown in the same table.

Reviewer's Comments:
The finding of a highly statistically significant difference in response rates, lower in the ADR-529 arm in the single study with the most patients having measurable disease using the NDA proposed ratio of 10:1 is disturbing. This is rather strong evidence that ADR-529 is inhibiting anti-tumor effect.

Progression

Data from time to progression are displayed in table 9B (attached). Neither time to disease progression (displayed in attached Figure 3B) nor disease free survival were significantly different. The corresponding Kaplan Meier plot is in attached figure 3B. Although the medians and the curves after the medians appear identical, the curves appear dissimilar prior to the median, with recurrences occurring sooner on the ADR-529 arm. To put the curves in perspective with the "time" to cardiac event curves, the median here represents about 10 courses of q21 day therapy or about 500 mg/M^2 of doxorubicin if given on schedule. At this dose of 500 mg/M^2 of doxorubicin, only about 20% of patients were still on study according to the previously discussed table onstudy table from p 00439. The early difference is reflected in the p value of the Wilcoxon statistic, p = 0.09, which is weighted to be more sensitive to early portions of time to event data; the logrank statistic does not even approach statistical significance (p = 0.51). The lower bound of the 95% ci for the hazard ratio is 0.68.

Disease free survival is depicted in attached table 10B and figure 3B.

Reviewer's Comments:
Though the arms are not statistically different, the data suggests a tendency toward earlier progression on the ADR-529 arm. This gives further credence to the observation of a statistical difference in response rates.

(10:1) 8.1.4.6 Survival

As noted in the table 11B (attached) the attached KM curve, survival, with 35-40% of the patients dead on both arms, appears identical.

Reviewer's Comments:
The lower bound of the HR is 0.7?0. Only 35% to 40% of the patients have died. This will b[illegible]d in the 4-month safety update, however, patients aft[illegible] 20 days will be receiving ADR-529 in both arms.

(10:1) 8.1.4.7 Quality [illegible]

Time to decrease in performance statu[illegible]s described in table 32B (attached). Occurence of performance status ≥ 3 while on study occurred sooner on the placebo arm (p = 0.029 by LR test). See also the attached KM plot. However, time to PS ≥ 3 at any time was not different. Median time to decreased body weight was slightly less sooner on the ADR-529 arm.

Reviewer's Comments:
I note that the number of events on which the data for the first and third analyses are experienced by ≤ 10% of patients. I don't consider the data meaningful. I don't understand the clinical meaning of the performance status of the small subgroup of patients on-study. I further see no rationale for the placebo group having a worse performance status unless doxorubicin toxicities are being ameliorated by ADR-529.

(10:1) 8.1.4.8 Patient disposition

Patient disposition is outlined in table 33B (attached). One notes that the 19% excess going off-study for cardiac toxicity on the placebo arm (28% vs 9%) is balanced by a 15% excess going off for progressive disease (51% vs 36%) on the ADR-529 arm. Other reasons seem balanced.

Attached table 34B displays the data in a revealing way, allowing one to note the time of removal from study for various reasons. This is important in evaluating the previously noted time-to-event analyses. The summation of these analyses over time, time to offstudy, is summarized in the attached KM plot from p 00618.

Looking at the top line of the table, we have the number of patients on study. At course 8 there are 57 on D and 72 on p. However, the a previous analysis showed that in the cardiac efficacy analysis which corresponded to this dose (400 mg/M^2) there were 33 D and 65 P patients.

Reviewer's Comments:

This is odd; ie assuming no doxorubicin dose reductions, 58% versus 90% who received this dose had cardiac data analyzed prior to the next course. At course 10, which should be 500 mg/M^2 of doxorubicin, 26 of 44 (60%) of D versus 34/45 (76%) of P were included in the cardiac analysis.

Reviewer's Comments:

The attached KM curve helps to put the findings of the study in perspective. Early in the study a few more patients go off-study on the ADR-529 arm. Late in the study, after the curves cross at about 225 days (about 500mg/M^2 of doxorubicin), more patients continue to receive doxorubicin on ADR-529 arm. The extent of the benefit from prolonging the ability to receive doxorubicin is in the area between the curves which I have shaded. Even in this analysis the log rank statistic suggests no significant difference wit p=0.38 (although I am not sure if the test is valid with curves which cross like these). From previous analyses we have not seen a difference in time to progression in this portion of the curve. So the benefit to these particular patients would seem to be in not having evidence of cardiac toxicity (clinical and LVEF) and being able to have more chemotherapy injected despite no observed additional benefit manifested in increased time to progression.

(10:1) 8.1.4.10 Toxicity

Clinical toxicities

Clinical toxicities DURING COURSE 1 are summarized in attached Table 12 B (08-00501). Nausea (70% vs 59%) was significantly more common ($p<0.05$) on the placebo arm. Fever (24% vs 14%, $p < 0.05$), neurotoxicity (8% vs 2%, $p<0.01$), and pain on injection (4% vs 1%, $p<0.05$) were significantly more common ($p<0.05$) on the ADR-529 arm. Sepsis (12% vs 7%) and infection (13% vs 7%) were more common in the ADR-529 arm, without reaching 'statistical significance.'

The most severe toxicities present during course 1 were compared and are presented in table 13B, found on pp 00508-005013 along with statistical comparisons (wilcoxon rank sum). The only additional information of note from this analysis was that there were 7 patients with pain on injection in the ADR-529 arm versus 1 on the placebo arm. The two with grade 3 or 4 pain were also on the ADR-529 arm (WRS p = 0.024).

Similarly these analyses were done over ALL COURSES. Attached Table 14B presents the comparative incidence. Significantly more common on the placebo arm were esophagitis (10% vs 4%, p<0.05), nausea (89% vs 79%, p <0.01), vomiting (77% vs 62%, p<0.01) and congestive heart failure (6% vs 1%). Significantly more common on the ADR-529 arm were fever (38% vs 33%, p<0.05 based on severity by WRS) and pain on injection (10% vs 3%, p<0.01). Mean severity scores were similar for clinical toxicities on the 2 arms.

The tables comparing most severe toxicity grades over all courses are on pp 00522-00527. Again these show statistical significant differences for nausea (p=0.048) and vomiting (p=0.041). The 15% excess of vomiting on the placebo arm was primarily grade I and II; the 10% excess of nausea was spread throughout grades I-IV. Overall, neurotoxicity was reported in 15% on ADR-529 vs 10% on ADR-529 (p=0.2). A presentation of grades of neurotoxicity was not done.

Adverse reactions reported DURING COURSE 1 are compared by COSTART terminology on pp 00533-00540. These were similar except for a higher incidence of asthenia on the placebo arm (19% vs 13%). The comparisons for incidence of adverse experiences for ALL COURSES are found on pp 00549-00559. These are generally similar on the 2 arms. Under Ner/CNS/B on p 00555(attached) there are no real differences to explain the 5% excess noted in incidence neurotoxicity in the analysis reported above. On both arms there were about 10% with anxiety, 8-9% with depression, and 5-8% with dizziness. Asthenia was slightly more common on the control arm (30% vs 25%). Peripheral edema was more common on the ADR-529 arm (14% vs 7%). On the ADR-529 arm there were 2 patients with diplopia, 1 with ptosis, and 1 with a visual field defect. Case report forms for these patients will be examined.

Reviewer's Comments:
The occurrence of less esophagitis, nausea and vomiting, and

asthenia on the ADR-529 could represent a protective effect from doxorubicin toxicity. More detail on the difference in reported neurotoxicity has been requested, and selected case report forms will be examined.

Laboratory tests

Renal and Hepatic

Renal and hepatic enzyme values after course 3 are compared in table 18B, pp 00562-563, for the 279 patients for whom data was available at this point. Comparison of grades by wilcoxon rank sum tests and comparisons of highest values showed no statistical differences. Comparison of most severe grade over all studies is presented in table 20B on pp 568-571. There was a trend toward more SGOT elevations (23% vs 16%, p=0.12) and creatinine elevations (4% vs 1%, p=0.14) on the placebo arm.

Myelosuppression

Data on nadir blood counts over the first 2 weeks of therapy are summarized in the table 23B (attached). The median is 360 in the ADR-529 arm vs 600 in the placebo arm (p<0.001) for the first course and 260 versus 350 (p=0.036) for all courses. Analyses of white counts were similar.

Data for platelets are summarized in table 24B (attached). Again the distributions are significantly different with a median of 168 on the ADR-529 arm versus 216 on the placebo arm (p<0.001) for first course and 133 vs 154 (p=0.019) for all courses.

Recovery counts on day 22 are summarized in table 26B (p 00579). By day 22 counts were similar on the 2 arms during course one, though there was a trend toward higher counts on the ADR-529 arm (p=0.10).

Similarly platelet counts at day 22 were analyzed for the first course and for all courses in table 27B (p 00583). One notes that the medians and means are 40,000 to 60,000 higher for both analyses for the ADR-529 arm with highly significant p values.

Reviewer's Comments:
The data show definitive evidence that ADR-529 even at the 10:1

ratio causes a lowering of granulocyte and platelet nadirs. The day 22 'rebound' platelet counts are convincingly higher on the ADR-529 arm.

(10:1) 8.1.4.11 Dosing intensity

Dose intensity expressed in mg/M2/course was not statistically different by WRS for the 2 arms (table 29B, attached). This table also demonstrates also that cumulative doses of the 3 agents were not statistically different in the 2 arms. Median dosing intensity was about 92-93% for doxorubicin and 86-90% for the other agents, with higher values on the placebo arm. The median courses of doxorubicin on the placebo arm was 6 compared to 5.5 on the ADR-529 arm, but the range extended further on the ADR-529 arm (p =0.12 by WRS).

The median cumulative doses of CTX and 5FU were slightly higher on the placebo arm (2930 vs 2500 mg/M^2) reflecting the higher median number of courses.

Attached table 31 B demonstrates no differences in number of patients with dose reductions or dose delays when compared by WRS (p=0.75). 55-57% of each arm had no reductions or delays. As opposed to findings in the 10:1 data, repeated delays dose-reductions were not more common on the ADR-529 arm.

8.1.5 Discussion and conclusions

8.1.5.1 Sponsor's discussion:

Cardioprotection

The following are from the sponsor's discussion of the study, with my comments inserted.

"In examining the results from this study the patient groups of this study were comparable with regard to both age and body weight, although there were a higher proportion of whites on the DZR arm. Pretreatment determined risk factors for cardiac disease were balanced in the 20:1 group except for more cases of lower MUGA scan values for the placebo (PLA) group. A similar balance was seen in the 10:1 group except for a 12% incidence of diabetes mellitus in the placebo group compared to 6% in the treatment (DZR) group.

With regard to the analysis of the time to untoward cardiac events, the 20:1 study demonstrated that patients in the control arm were over six times as likely to have a cardiac event at a given cumulative dose of Adriamycin compared to the DZR patients. This evidence of cardiac protection was most striking at the 400 mg/M^2 cumulative dose level and beyond, although marginal differences were noted at the earlier cumulative dose levels. The 10:1 group demonstrated that there was a 2.9 increased risk of developing a cardiac event in the placebo (PLA) group when compared to the treatment (DZR) group.

Reviewer's Comments:

Technically these points are correct, the point estimates of hazard ratio for time to cardiac event were about 6 at the 20:1 ratio and 2.9 at the 10:1 ratio. However, other points to consider:

1. *the lower bound of the 95% ci of the Hazard ratio of the 20:1 is 2.7 and that of the 10:1 is 1.46.*

2. *This aggregate endpoint, though composed of valid protocol-derived criteria for off-study, was not specified as an aggregate endpoint in the protocol.*

3. *The clinical significance of each elements composing the aggregate endpoint may be of varying clinical significance.*

"Congestive heart failure (CHF) was not observed in the 20:1 treatment arm (DZR), but was reported for two patients on the placebo (PLA) arm of the 20:1 group. In the 10:1 group, there were two (1%) patients with CHF on the treatment arm and ten (6%) patients with CHF on the control arm. There was over a ten-fold increased risk on the control arm (PLA) for CHF, per unit dose of doxorubicin, compared to the treatment arm (DZR). This result was statistically significant, p=0.005.

Reviewer's Comments:

Again the 10X risk of CHF has a lower bound of 95% ci of 1.36. Again to keep the effect in perspective for this patient group, one should keep in mind the overall 6% incidence and the assess the seriousness and reversibility of the events recorded. CRF's have been requested for this purpose.

"When the baseline values of the LVEF were compared to subsequent values obtained during the course of therapy, the mean drop in LVEF was found to become greater on the control arm with increasing cumulative doses of Adriamycin. These differences were consistent with previously published studies (63-65). In the 20:1 group, the control patients receiving at

least 300 mg/M^2 of Adriamycin had a significantly larger mean drop in LVEF scores compared to the DZR patients and this effect size appeared to increase with increasing cumulative doses of Adriamycin. At 300 mg/M^2, this difference between DZR and control was 6.4%, and at 550 mg/M^2, this difference had increased to 18.8%. A similar trend in increasing treatment differences with the higher cumulative doses of Adriamycin was also observed in the 10:1 group. At 400 mg/M^2, this difference (DZR minus PLA) was 6.7%, and at 550 mg/M^2, this difference had increased to 10.1%. The LVEF values for patients in the treatment (DZR) arms for both studies (20:1 and 10:1) remained at approximately the baseline values.

Reviewer's Comments:
The protocol called for an interim rule for stopping considering the LVEF at 500 mg/M^2 for p values of .00005, .003, .03, .027, and .042. According to my interpretation 5 looks at the efficacy data were taken at 3 month intervals before the 11/90 decision to stop was made. For the 60 patients making it to 500/M2 by the time the final cutoff for data collection was established, the difference (unadjusted) was 7.6%, p = 0.003. This was the protocol specified primary endpoint. It is difficult for me to know what boundary p value criterion should be applied here. The mean difference at 500/M2 was less than what had been specified in the protocol as being of clinical interest (10%). In addition, there are major asymmetries in the number of patients on study who underwent cardiac analyses at these points. ADRIA has been asked to address this point.

"Thus, cardioprotection activity has apparently been established for each of the endpoints at both the 20:1 and the 10:1 ratios. These data confirm the published report by Speyer and others (65,66).

Reviewer's Comments:
Despite all the caveats, there appears to be strong evidence of cardioprotective activity at both the 10:1 and 20:1 ratio. The major question is whether the protection afforded to the few that make it out to the tail of the time to event curves is clinically significant and whether it is worth potential toxicities and/or potential interference with anti-tumor activity to justify treatment of all patients.

Effect on anti-tumor activity

"In contrast to the consistent picture of positive DZR effects associated with the measures of cardioprotective activity, a puzzling set of results emerged for the antitumor findings. The difference in

the percentage of responders favored the control group for the 10:1 dosed patients. Although the individual group response rates fell within the expected range of results frequently associated with advanced diseased patients treated with FAC, the observed group differences are of some concern. Within the context of randomized trials, it is the difference between treatments that is strictly estimable. Assuming this negative outcome represented an interference of doxorubicin's antiproliferative activity by DZR, one would expect a similar effect for the 20:1 patients. On the contrary, about 6% more of the DZR patients responded compared to that observed on the control arm. Moreover, the results were so favorable in the small number of 20:1 "evaluable" patients that the lower bound of the 95% confidence interval was -9%. This result would suggest that, with reasonable likelihood, DZR effected no worse of an outcome than that occurring for the control in this subgroup of patients. Thus, within the same trial, exposing patients to a higher dose of DZR did not reproduce the negative findings associated with the lower dose ratio.

Reviewer's Comments:

It is difficult to discount the largest intact trial using the 10:1 ratio, the ratio recommended by the sponsor for approval. Further support is provided by more progressions (20% vs 10%) at first analysis for response in addition to less responders. This the trend toward earlier time to progression in the early months was reflected in a wilcoxon P value of 0.09. In addition, the imbalance in response rate was not corrected by a multivariate analysis of prognostic factors. The 20:1 data set was small, and the 95% ci difference in response (ADR-529 minus placebo) range from 22% to -14% whereas that of the 10:1 data set range from -28% to -4%. So, the 95% ci do overlap. Also, though perhaps not anticipated, it is possible that there is a range in which tumor protection is optimized. At least one animal study suggested this.

"Response rates by themselves are surrogate measures of patient benefit. Possibly stronger measures of activity include the rate of complete responses (CR) and the durability of a patient's remission. Evidence from either dose ratio suggested that the CR rate was approximately the same on both arms. Also, for the 10:1 patients, the duration of complete remission was relatively longer for the DZR patients. The overall remission durability, as measured by the time to disease progression, was comparable in both treatment groups. Apart from random variation, the point estimates of the hazard ratio were approximately unity. However, due to the early termination of the trial, a sufficient number of patients and/or events were not accrued to rigorously establish treatment equivalence for the time to disease progression and disease-free survival data. The mortality experience was no worse on the DZR arms (20:1 or 10:1) compared to the control patients.

Reviewer's Comments:
The CR rate in the 10:1 arm was 13% in the placebo arm and 9% in the ADR-529 arm. The notation of a longer CR duration on the ADR-529 arm seems cannot be a solid finding given these scanty data. In fact a lower response rate might be associated with a longer duration of response as a result of never getting responses in patients with the most aggressive, quick-to-regrow, tumors. The study did not have great power to assure equivalence of survival; the lower bound of the 95% ci for the hazard ratio for survival P:D is 0.72.

> "Thus, other indices of drug activity and/or benefit did not support the negative response differences observed for the 10:1 cohort of patients. It is not clear why this occurred. It is possible that response rates and measures like survival are very weakly associated in this disease setting. If this is truly the case, attention should be paid to those endpoints conferring greater benefit to the patient. That is, disease progression or survival. Also, the methods for rating response in this trial had the potential for resulting in a moderate degree of measurement error. Specifically, once a patient was initially rated as "responding", the protocol did not require that the response be present for some minimum period of time so as to rule out aberrations in the evaluation process. Commonly, a partial (PR) or complete response has to be present for a minimum of four weeks to be classified as a true response. The outcome of this reduced requirement on the current set of response results is unknown. However, in examining the duration of response for those patients with a CR or PR, the DZR patients were, on average, more likely not to relapse than the control patients. This finding was unrelated to the dose ratio used. All of the results from these analyses suggest that the summaries of response rates, as reported, could be misleading. At a minimum, one should not restrict their overall conclusions on antitumor effect solely on response rates.

Reviewer's Comments:
It is not inconceivable to me that interference with doxorubicin antitumor effect, ie lowering the effective dose of doxorubicin, might have this effect: lowering response rates but not affecting long-term time to progression or survival. If the sponsor claims that responses were not valid, then there should be an early progression excess on the placebo arm in the response duration analysis. The analysis of response duration, although commented upon, was not presented. In addition, even if such a "blip" was seen, it would not be inconsistent with valid response documentation. In other words, if the placebo arm were the more effective therapy, and was able to increase the response rate by 15%, it is possible that the tumors from this extra 15% of patients, responsive only to more effective doses of therapy,

might be expected to be more aggressive tumors and to progress sooner than more responsive tumors.

Toxicity

"Toxicity of the study drug was primarily that of myelosuppression and its sequelae including fever, infection, sepsis, and rarely (of the 20:1 dose ratio), death. In the 20:1 group, 25% of the patients on the treatment arm (DZR) had one or more episodes of sepsis during the first course of therapy compared to 8% of patients on the control arm. These same groups had an overall rate of sepsis of 38% of patients in the treatment arm compared to 17% of patients in the control arm, when all courses of therapy are considered. In the 10:1 group, 12% of patients on the treatment arm and 7% on the control arm had sepsis during the first course of therapy, and when all courses of therapy are considered for this same group, 18% of the treatment arm and 16% of the control arm were reported to have sepsis. These differences were not statistically significant. Thus, the 20:1 group treated with dexrazoxane had an unexpectedly high rate of sepsis. This risk was markedly reduced when the dose of dexrazoxane was decreased from 1000 mg/M^2 to 500 mg/M^2 and this reduction permitted eventual equalization of the rate of sepsis in the two arms when all courses of therapy at the 500 mg/M^2 dose of dexrazoxane are considered. The increased sepsis was due to myelosuppression and this experience was at variance with that of Speyer, *et al.* (65,68). Speyer used doses identical to those given in the 20:1 group and did not find an increased rate of sepsis, though an increase in myelosuppression was clearly present.

Reviewer's Comments:
The following points are from the 10:1 data:

During course 1, nausea was significantly more common on the placebo arm. Fever, neurotoxicity, and pain on injection were significantly more common on the ADR-529 arm. Sepsis (12% vs 7%) and infection (13% vs 7%) were more common on the ADR-529 arm although this did not reach statistical significance. In the analysis over all courses, esophagitis, nausea, vomiting, and congestive heart failure were significantly more common on the placebo arm, whereas fever and pain on injection were more common on the ADR-529 arm.

These data suggest that some of the other toxicities of doxorubicin might have been ameliorated.

"In the reported (non-solicited) toxicities, there was a decreased rate of asthenia in the 20:1 treatment group for the first course of therapy and over all courses. The difference between treatment arms with regard

to asthenia was not as great in the 10:1 group. Relative to the control group, more 20:1 DZR patients reported a rash over the course of treatment, and more 10:1 DZR patients reported peripheral edema. Otherwise, the rates of reported toxicities were about the same on both arms.

The nadir WBC, AGC, and platelet counts were lower in the treatment arm than in the control arm of the 10:1 group. The reduced nadir counts were seen after the first course and after all courses. Each of the differences was significant at a test size of 5%. As in the 20:1 group, each of the values had returned to normal by Day 22. The median hemoglobin drop from baseline was approximately 1.5 grams per deciliter in the first course and approximately 2.5 after all courses of therapy. The evaluation of other laboratory tests showed no apparent differences for any group of patients.

In the analysis of the serial assessment of the quality of life, the patients in the 20:1 group treated with dexrazoxane (DZR) showed an improvement in overall status when compared to the PLA patients. However, these improvements were not statistically significant. In the 10:1 study, the time to worsening of the performance status while on study was also prolonged on the DZR arm. The control group experienced a 2.5 increased risk for experiencing a reduced ambulatory stat compared to the DZR patients, a statistically significant ($p<0.05$) result. The DZR patients experienced a slightly greater tendency to lose weight during the time that treatment was being given.

Reviewer's Comments:

For reasons discussed earlier, I consider the analysis of time to deterioration of performance status ≥ 3 while on-study to be an exploratory, non-specified, subset analysis whose results are not explained in any presented rationale.

"Evaluation of the reasons that patients were removed from study (Table 33A) suggests some interesting phenomenon due to the nature of having two major end-points in this clinical trial. In the 20:1 study, of 119 treated patients, 73 were eventually removed from study for either progressive disease or cardiac toxicity. In addition, 19 came off study because of patient refusal, there were eight deaths, five were removed for protocol violations and four for adverse reactions. The deaths were predominantly on the treatment arm (seven of eight cases) and that was a major factor in reducing the dose of dexrazoxane from 1000 mg/M^2 to 500 mg/M^2 even though only three of these cases could be related to myelosuppression or infection. The other deaths were due to progressive disease (2), pulmonary embolus (1), respiratory failure with metabolic derangement (1), and intercurrent illness from sigmoid colon infarction (1).

Of the 19 patients removed from study because they refused further

therapy, nine were removed after they had received greater than ten courses of chemotherapy. Since it was not known whether the cardioprotective agent was being given to these patients, the fear of cardiotoxicity played a major role in the removal of these patients from study. This fear was generated from the health care workers, for the most part, and was ultimately passed on to the patient who declined further therapy. All nine of these cases that were removed from study after refusing further therapy, had been on the treatment (DZR) arm, and would probably have been protected from cardiac injury had further treatment been given.

Reviewer's Comments:
The claim that patients went offstudy because of fear generated from health care workers was not supported by any specific evidence. The sponsor will be asked to support this assertion and selected case report forms will be evaluated.

"There were 33 patients removed because of cardiac toxicity; nine patients had been on the treatment (DZR) arm and 24 on the unprotected control (PLA) arm. Of the nine on the treatment arm, six were removed as a result of the first follow-up LVEF test (Course 3, 150 mg/M^2 cumulative dose of Adriamycin). There were two patients on the control arm that were removed from the study arm during this same interval. Excluding these early failure cases, there were only two subsequent patients removed from study due to low LVEF from the treatment (DZR) arm but 22 patients from the control (PLA) arm.

In addition to these early losses due to toxicity and to low LVEF values, there was an increased failure rate early in the trial compared to later on due to progressive disease. For example, after Course 3, the time when the first follow-up tumor measurement evaluation was made, there were nine failures on the treatment arm and only six failures on the placebo arm. This imbalance of progressive disease between the two arms no longer existed after Course 6. By that time, the total number of cases off-study because of progressive disease had become approximately equal (11 patients on the DZR arm and 12 on the PLA arm).

As a result of the removal of the patients listed above from study, and the subsequent effectiveness of dexrazoxane (DZR) as a cardioprotective agent, proportionately more patients on the treatment (DZR) arm began to come off study at later dates because of progressive disease. Rather than being a reflection of an increase in the development of progressive disease on the treatment arm, this phenomenon represented the residue disposition of those cases not removed because of cardiac toxicity.

The evaluation of why the patients were removed from the 10:1 study (Table 34B) shows many similarities to the previous (20:1) study. One notable exception was that there were four deaths on the treatment (DZR) arm and six deaths on the placebo (PLA) arm. By the end of Course 3, 26 had been removed from the treatment arm, compared to only 13 from the control arm, for progressive disease. After Course 10, only 11 patients

had been removed from the treatment (DZR) arm due to cardiotoxicity compared to 37 from the control (PLA) arm. Other reasons for removal from study were approximately equal between the two treatment arms.

Reviewer's Comments:
The comment on early failures on the ADR-529 arm supports the suspicion that offstudy criteria were too cautious and that such time to event data includes some technically detectable LVEF changes of unknown clinical significance. Evaluation of LVEF after 3 courses of CAF is not common practice in the treatment of breast cancer.

8.1.5.2 Sponsor's conclusions

The following represent's the sponsor's conclusions with reviewer comments inserted.

"Dexrazoxane (DZR) has been shown to be cardioprotective against the chronic myocardial anthracycline effects of FAC chemotherapy in advanced breast cancer.

The cardioprotection activity was of statistical significance favoring the dexrazoxane arm in time to cardiotoxic events, and for the mean and median decline from baseline in the left ventricular ejection fraction. There was a higher incidence of congestive heart failure in the control group than in the patients receiving dexrazoxane. Cardioprotection was evident at both the 20:1 and 10:1 ratios of dexrazoxane to doxorubicin.

The response rate was statistically significantly lower in the dexrazoxane arm at the 10:1 ratio, but this was not the case at the 20:1 ratio. However, the addition of dexrazoxane to FAC chemotherapy did not result in significant differences in other measures of antitumor efficacy including time to progression, disease-free survival, and overall survival; and did not markedly alter the safety of the regimen. Statistical analyses revealed some differences between the treatment arms in safety that were not considered to be clinically relevant, except for a decrease in the median nadir granulocyte count and an increase in sepsis on the dexrazoxane arm

"The deaths were predominantly on the treatment arm (seven of eight cases) and that was a major factor in reducing the dose of dexrazoxane from 1000 mg/M^2 to 500 mg/M^2 even though only three of these cases could be related to myelosuppression or infection. The other deaths were due to progressive disease (2), pulmonary embolus (1), respiratory failure with metabolic derangement (1), and intercurrent illness from sigmoid colon infarction (1).

"Of the 19 patients removed from study because they refused further

therapy, nine were removed after they had received greater than ten courses of chemotherapy. Since it was not known whether the cardioprotective agent was being given to these patients, the fear of cardiotoxicity played a major role in the removal of these patients from study. This fear was generated from the health care workers, for the most part, and was ultimately passed on to the patient who declined further therapy. All nine of these cases that were removed from study after refusing further therapy, had been on the treatment (DZR) arm, and would probably have been protected from cardiac injury had further treatment been given.

There were 33 patients removed because of cardiac toxicity; nine patients had been on the treatment (DZR) arm and 24 on the unprotected control (PLA) arm. Of the nine on the treatment arm, six were removed as a result of the first follow-up LVEF test (Course 3, 150 mg/M^2 cumulative dose of Adriamycin). There were two patients on the control arm that were removed from the study arm during this same interval. Excluding these early failure cases, there were only two subsequent patients removed from study due to low LVEF from the treatment (DZR) arm but 22 patients from the control (PLA) arm.

8.1.5.3 Reviewer summary and conclusions, 20:1 data.

88001, a double-blind multicenter randomized comparison of FAC with placebo or ADR-529 in patients with advanced breast cancer and with measurable disease, was begun in November of 1987 using a 20:1 ratio of ADR-529 to doxorubicin. Accrual for the purposes of the 20:1 data was halted in November of 1988 when, due to an excess of deaths associated with myelosuppression on the ADR-529 arm of this study and the ADR529 arm of the concurrent study in lung cancer. Patients on 20:1 ADR-529 were switched at this time to the 10:1 ratio. The data presented as "20:1" data in this trial come from patients who, depending on time of entry and offstudy time, received varying amounts of 20:1 ADR-529 and 10:1 ADR-529. Although it is tempting to draw conclusions about the relative safety and efficacy of the 20:1 ratio to the 10:1 ratio based on this trial, it is important to remember that the data base is small and admixed with 10:1 data; moreover some of the toxicity detected initially leading to a dose ratio change may appear less after admixture of 10:1 data.

Differences in design of the initial aborted (20:1) trial from the later trial (10:1) included exclusion of non-measurable disease patients, no requirement for weekly CBC monitoring, and no requirement for repeat LVEF if going offstudy for LVEF change.

The data cutoff date was set for March 1991 in parallel with the other studies, so the data is mature. Key efficacy findings extracted from ADRIA's NDA analyses are presented in the following table :

Tabular summary of findings study 88001, 20:1 RATIO

Disease	Breast cancer		
Ratio of DZR	20:1 (at patient entry)		
	DZR	PLA	
# randomized			
total	67	54	
measurable	66	54	

Cardiac Effect

Time to "event" (34 events)	P:D	6.18	(2.70, 14.20)

LVEF decrease (mean difference, change from baseline, D-P)

Doxorubicin dose	Change from baseline (D minus P)	
500 mg/M² (D22, P8)[1]	10.6	p=0.002
550 mg/M² (D23, P5)	16.7	p=0.019

	DZR	PLA
CHF events	0	2

Tumor Effect

	DZR	PLA	95%ci
Response (120 pts)	38/66 (58%)	29/54 (54%)	(-14%, 22%)
Progression (121 events)	P:D 1.18		(0.80, 1.75)
Survival (95 deaths)	P:D 1.20		(0.80, 1.80)
Offstudy (120 events)	P:D 1.69		(1.13, 2.52)

[1]Numbers in parentheses represent number of patients analyzed.

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DEXRAZOXANE, (ADR-529, ICRF-187, ZINECARD®) PROTECTS AGAINST DOXORUBICIN INDUCED CHRONIC CARDIOTOXICITY: S.R. Weisberg, C.S. Rosenfeld, R.M. York, S.E. Jones, D.V. Spicer, A. Khojasteh, A.M. Desai, S. Wadler, A. Mittelman, K.B. Pendergrass, E. Velez-Garcia, J.O. Moore, N. Abramson, C.L. Vogel, S.M. Swain, G.H. Lyman, J.E. Feldmann, B.L. Tranum, C.J. Lusch, R.R. Joseph, P.L.C. Banks, D. Jones, K. Squillace, J. Winston, D. Scott, A. Kline, D.L. Hess, B. Hennebert, N. Curtis, R.D. Reynolds, A. Imondi, J. Filippi, P.K. Narang, N. Palepu, V. Verhoef, J. Bianchine, R.A. Gams. Hollywood FL; Atlanta; Dallas; Los Angeles; Philadelphia; New York; Valhalla; Kansas City; San Juan; Durham; Jacksonville; Miami; Washington DC; Tampa; Mobile; Little Rock; Reading; and Adria Laboratories, Columbus OH.

Dexrazoxane, a bispiperazinedione, has been shown to be an effective protector agent against the chronic cardiac changes of doxorubicin in animals as well as humans (Speyer *et al.*, *N Engl J Med* 1988; 319:745-52). To confirm this work, 121 advanced breast cancer patients who had received no previous chemotherapy for advanced breast disease were randomized to Dexrazoxane (1000 mg/M^2) (67 patients) or to the placebo control arm (54 patients) in this double blind study. All patients received chemotherapy every 3 weeks consisting of 500 mg/M^2 cyclophosphamide, 50 mg/M^2 Adriamycin®, and 500 mg/M^2 5-fluorouracil. Dexrazoxane/placebo was administered I.V. within 30 minutes preceding Adriamycin®. All patients had measurable disease and normal resting radionuclide left ventricular ejection fraction (LVEF/MUGA). MUGA scans were repeated after 3,6,8, and 10 courses, and after each subsequent course. Tumor measurements were made after every 3 courses of therapy. Patients receiving Dexrazoxane were found to be able to receive 4 to 5 times the cumulative Adriamycin® dose. None of the patients receiving Dexrazoxane and 2 of the control patients developed congestive heart failure. Patients receiving ≥300 mg/M^2 were protected from a drop in LVEF ($p<0.05$). At 550 mg/M^2, this difference was even greater (p-value 0.003). Twenty-three (34%) remain on study in the Dexrazoxane arm, compared to only 5 (9%) in the control arm. Response rates (CR+PR) were 57% in the Dexrazoxane arm and 54% in the placebo arm (intent to treat analysis). Dexrazoxane cardioprotective properties have been confirmed and should result in new applications of Adriamycin® therapy.

111 patients were randomized with a slight imbalance toward ADR-529. The difference in LVEF drop was 10.6 % at the protocol specified endpoint of 500 mg/M^2 of doxorubicin. There were 2 CHF events reported on the placebo arm. The retrospective time to cardiac event analysis strongly favors ADR-529.

The response data base includes 120 patients. The response rates, which likely reflect primarily 20:1 effect, are 58% for ADR-529 and 54% for placebo with 95% ci including a difference of no more that 14% against ADR529. Progression and survival which likely reflect effects of 20:1 and 10:1 ratios, both lean toward ADR-529 with 95% ci including a hazard ratio of no less that 0.80.

Toxicity data showed that sepsis was significantly more common (25% vs 8%) in course 1 in the ADR-529 arm, a course that was 20:1 for all patients in this data base. Mean severities of esophagitis and dysphagia were significantly less on the ADR-529 arm in course 1. In the mixed analysis of all courses, again sepsis, fever, and infection were more common on the ADR-529 arm. The adverse reaction of "asthenia" was more common on the placebo arm. Nadir counts were not done so myelosuppression was not directly assessed. Overall patients tended to stay onstudy significantly longer on the ADR-529 arm (LR p=0.009).

88001 Reviewer conclusions regarding 20:1 data

The trial provides supportive data that the 20:1 ratio of ADR-529 significantly lessens the LVEF decrease detected with repeated courses of FAC chemotherapy. The myelosuppressive toxicity of the 20:1 ratio was clearly shown and led to lethal consequences prior to alteration of the study. Differences in esophagitis and asthenia could suggest a protective effect of ADR-529 against other toxicities of the chemotherapy. Data on response, progression, and survival have limited power and include a mixture of 20:1 and 10:1 data.

8.1.5.3 Reviewer summary and conclusions, 10:1 data.

Historical background

Given the complexity of the data, it is helpful to review the time course of decisions leading to the structure of the 10:1 data which is the subject of review. The previous 20:1 study was basically restarted and the 10:1 ratio using the same protocol in early 1989. A few changes made within 2-3 months of restarting included exclusion of patients with performance status 2, inclusion of a non-measurable disease stratum, inclusion of an interim analysis plan and data monitoring committee, incorporation of weekly monitoring of blood counts, and requirement for repeat LVEF measurement in patients going offstudy for LVEF changes.

The monitoring committee met quarterly starting on January 6, 1989, to review safety data. The committee had been formed because of the concern about safety of the ADR529 regimen. Without consultation with the Agency a decision was made to include cardiac toxicity data in analyses beginning with the 4th meeting in October 1989. Since cardiac toxicity analyses included # of patients going offstudy for LVEF changes, the distinction between the analysis of toxicity and the analysis of efficacy may have become blurred by this decision. At the 8th meeting of the committee on November 5, 1990, the committee made the following recommendation:

> "Study 001 (breast) clearly shows that ADR-529 is cardioprotective at 500 mg/M^2. Therefore, this study should be stopped, as it is inappropriate to subject patients to increasing doses of DOX in the absence of ADR-529 (current clinical practice would suggest not treating the majority of patients beyond 350-450 mg/M^2). One now needs to resolve that prior to 500 mg/M^2, ADR-529 is not having a deleterious effect (i.e. on disease progression) in some patients, possibly to those at greater risk for myelosuppression."

At a December 6, 1990 meeting with the Agency, an analysis was presented suggesting that the boundary had been crossed for the comparison of LVEF at 500 mg/M^2 of doxorubicin for the 10:1 patients(in the table presented 13 patients were at 500 mg/M^2 in each arm, the LVEF difference was 9.8, O'Brien-Fleming Z

boundary for the 3rd analysis was 2.23, and calculated Z value was 2.85). (Clare Gnecco, PhD, the Agency statistician reviewing the NDA submission has been trying to get clarification of conditions surrounding this analysis to verify it. The actual data used to perform the analysis was reportedly purged from ADRIA's files as routine matter one year after it was performed.)
Reviewer's Comments:
It seems incredible that the data leading to stopping of the pivotal study would not have been frozen and saved.

It was agreed that ADRIA would collect response data which was outstanding and would meet again with the Agency in a few months regarding whether to submit the data for an NDA.

On January 14, 1991 the stopping of accrual for the trial of original design was formalized; patients onstudy were crossed over to ADR-529 at 350 mg/M^2 of doxorubicin. The existing structure of the multicenter trials was maintained and the trials were changed to a different design with a new cardiac endpoint, with the realization that comparative data on response might be useful in the future.

The Agency met with ADRIA and Craig Henderson, MD of ODAC on 3-27-91 to discuss whether an NDA should be filed. The following is an excerpt from my Medical Officer Review of 2-14-91 of data submitted to the Agency prior to this meeting (all data "in house" at ADRIA on 1-11-91); the response rate was noted to be significantly worse on the ADR-529 arm in the 10:1 data of the 88001 trial. In addition, concern was noted over progression data in several breast cancer trials at that time:

"88001 10:1 Response rate

Adr-529 42% (49/117)

No ADR-529 55% (71/129)

95% ci of difference: (-26%, -1%)"

"The following table is extracted from table 9 for the breast cancer trials only:

Study	Hazard Ratio, no Adr529:Adr529
88001 10:1	0.71 (0.49, 1.03)
88001 20:1	0.92 (0.59, 1.40)
88006 10:1	0.57 (0.30, 1.10)
NYU 20:1	0.73

The Kaplan Meier curves with statistical tests are attached. The difference in time to progression is statistically significant by the Wilcoxon test in the 10:1 patients (0.01) and in the combined 10:1 and 20:1 patients of 88001 (0.02). 88006 is nearly significant by the wilcoxon test (0.07).

These in aggregate strongly suggest that ADR-529 does have a negative impact on time to progression. If there is a quality of life advantage to not progressing which I suspect there is this argues against use of the drug in this clinical setting. In addition it raises concern for the use of the drug in any setting without strict proof of equivalence of clinical efficacy with and without adr-529."

At the 3-27-91 Agency concern over the antitumor data was expressed. ADRIA representatives felt that the NDA ought to be submitted. Dr. Gams of ADRIA expressed the view that cardiac efficacy had been demonstrated, and that an equivalence trial to test the effect on tumor would require a very long and very large trial which could be done in the Phase IV setting after approval. It was decided that the NDA would be submitted for ODAC's opinion. Accrual cutoff and data cutoff dates were set (accrual 1-14-91, data March 31, later extended to April for response.)

Data summary

Key efficacy findings extracted from analyses by ADRIA are presented in the following table :

Tabular summary of findings study 88001, 10:1 RATIO

Disease		Breast cancer	
Ratio of DZR		10:1	
		DZR	PLA
# randomized			
total		168	181
measurable		141	152
	Cardiac Effect		
Time to "event" (71 events)	P:D	2.88[1]	(1.65, 5.0)

LVEF decrease (mean difference, change from baseline, D-P):

Doxorubicin dose	Change from baseline (D minus P)	
500 mg/M^2 (D26, P34)[2]	7.6	p=0.003[1]
550 mg/M^2 (D21, P19)	10.1	p=0.036[1]

	DZR	PLA
CHF events	2	10

Tumor Effect

		DZR	PLA	95%ci
Response (293 pts)		67/141 (48%)	96/152 (63%)	(-27%, -4%)
Progression (185 events)	P:D	0.908		(0.68, 1.21)
Survival (127 deaths)	P:D	1.02		(0.72, 1.45)
Offstudy (301 events)	P:D	1.11		(0.88, 1.40)

[1]Data unadjusted for interim stopping effect.
[2]Numbers in parentheses represent number of patients analyzed.

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PREVENTION OF ADRIAMYCIN® CARDIOMYOPATHY WITH DEXRAZOXANE (ADR-529, ICRF-187). C.S. Rosenfeld, S.R. Weisberg, R.M. York, S.E. Jones, D.V. Spicer, A. Khojasteh, A.M. Desai, S. Wadler, A. Mittelman, K.B. Pendergrass, E. Velez-Garcia, J.O. Moore, N. Abramson, C.L. Vogel, S.M. Swain, G.H. Lyman, J.E. Feldmann, B.L. Tramm, C.J. Lusch, R.R. Joseph, P.L.C. Banks, D. Jones, K. Squillace, J. Winston, D. Scott, A. Kline, D.L. Hess, N. Curtis, R.D. Reynolds, P.K. Narang, J. Bianchine, R.A. Gams. Hollywood FL; Atlanta; Dallas; Los Angeles; Philadelphia; New York; Valhalla; Kansas City; San Juan; Durham; Jacksonville; Miami; Washington; Tampa; Mobile; Little Rock; Reading; and Adria Laboratories, Columbus, Ohio.

The prevention of the Adriamycin® induced chronic myocardiopathy could open the path for new approaches to the treatment of anthracycline sensitive malignancies. Dexrazoxane is an intracellular chelator which binds iron, after it complexes with Adriamycin®, resulting in the prevention of the formation of free radicals. 349 patients with advanced breast cancer, previously untreated with either anthracyclines or other chemotherapy for advanced disease, were entered from 35 institutions. All received 500 mg/M^2 5-FU, 50 mg/M^2 Adriamycin®, 500 mg/M^2 cyclophosphamide ± 500 mg/M^2 Dexrazoxane (DZR) q 3 wks. DZR was given I.V. not earlier than 30 minutes prior to Adriamycin®. 168 received DZR and 181 received blinded placebo. After 600 mg/M^2 cumulative Adriamycin®, 17 remained on DZR and only 8 on the placebo arm. Patients on the control arm had 2.5 times the risk for developing a cardiac event while on study (logrank $p<0.001$), while beneficial effect was noticed as early as 400 mg/M^2 on the DZR arm. Control patients had a 10-fold risk of developing congestive heart failure (CHF)(logrank $p=0.005$), with 1% (2 pts.) on DZR and 6% (10 pts.) on placebo developing CHF. After 400 mg/M^2 Adriamycin®, the mean resting LVEF/MUGA dropped 6.7% in the placebo group. The difference between the two groups was 10.1% at 550 mg/M^2. The disease-free survival was approximately equal in the two arms (hazard ratio = 0.918), and survival was approximately equal (logrank p-value = 0.90). Protection against Adriamycin® chronic cardiotoxicity, using reduced doses of Dexrazoxane (ADR-529, ICRF-187), had thus been demonstrated.

The data cutoff date was March 1991. One notes that since the analysis reviewed for the March 1991 meeting, about 50 more patients are included in the response analysis and 64 more progression events are reported.

Most patients (301/349) are offstudy. Only about half have had a documented progression and only one third have died. However, further data to be collected on these endpoints from these patients in the future will be confounded by crossover of placebo to ADR-529 after 6 courses (about 130 days).

349 patients were randomized to the 10:1 arm of this study. The difference in mean LVEF decrease from baseline was 7.6 at the protocol specified endpoint of 500 mg/M^2 of doxorubicin. There are now 60 patients in the 500 mg/M^2 analysis rather than the 36 at the time of the decision to stop. Although the appropriate statistical adjustment isn't totally clear, adjustment cause the estimate of effect to be slightly smaller and the p values would be closer to 0.05. There were 10 CHF events reported on the placebo arm and 2 on the ADR-529 arm. The retrospective time to cardiac event analysis strongly favors ADR-529; again the effect size may be somewhat overstated in the unadjusted analysis.

Response was statistically inferior (48% vs 63%, p=0.007) in the ADR-529 arm. Although the hazard ratios for progression and survival are 0.91 and 1.02, studies were not designed to prove equivalence of these endpoints; consequently the lower bound of the 95% confidence intervals do not offer a great deal of assurance that they indeed are equivalent (for progression 0.68 and for survival 0.72).

Toxicity was summarized in an earlier comment in this review: "During course 1, nausea was significantly more common on the placebo arm. Fever, neurotoxicity, and pain on injection were significantly more common on the ADR-529 arm. Sepsis (12% vs 7%) and infection (13% vs 7%) were more common on the ADR-529 arm although this did not reach statistical significance. In the analysis over all courses, esophagitis, nausea, vomiting, and congestive heart failure were significantly more common on the placebo arm, whereas fever and pain on injection were more common on the ADR-529 arm. "

88001 Reviewer conclusions regarding 10:1 data

The 10:1 data from the 88001 trial come from the only NDA trial done by ADRIA LABS which was completed according to design. Additional response data from update of ongoing trials having similarities in design to this trial may be available prior to the ODAC meeting. However, additional increments of information will not erase this finding of a highly statistically significant difference in the protocol-specified measurement of antitumor efficacy in the completed trial for the 10:1 ratio of ADR-529, the ratio proposed for approval. Prior assumptions one may have had about whether ADR-529 could interfere with the antitumor effect of FAC are in doubt. Only a very large body of data, representing the results of a trial specifically designed to prove equivalence in efficacy endpoints in breast cancer, could erase the significant doubt established by this trial. Other hints in the toxicity data support the possibility that ADR529 might exhibit a protective effect on normal tissues other than the heart.

As noted in the monitoring committee minutes, it is unclear that pushing FAC doses to a high cumulative dose of doxorubicin is standard practice. The time to offstudy curve clarifies how few women would actually continue to receive the additional FAC treatments because of ADR529 if all patients were given ADR-529 from the beginning of therapy.

The study did document clearly a protective biologic effect of ADR-529 as documented by lessening the rate of deterioration of LVEF. The clinical significance of this event for this population was less clearly established. A 5% incidence of reported CHF was reduced to 1%.

So the study seems to have demonstrated with high statistical significance 2 competing biological effects, one on response rates and the other on LVEF. The conflicting results in these 2 surrogate endpoints offers no convincing evidence for safety and efficacy in this population at this dose.

8.2 Study 88006

Primary FDA Reviewer, Alice Chen, MD (Staff Fellow)
Secondary Reviewer, Grant Williams, MD (Medical Officer)

TITLE: ADR-529 as a Cardioprotective in a Phase III Randomized Trial of FAC versus FAC + ADR-529 in the Treatment of Disseminated Carcinoma of the Breast

First patient randomized	May 3, 1989
Accrual Cutoff	January 14, 1991
Data Cutoff, cardiac data	March 31, 1991
Data Cutoff, tumor efficacy	April 30, 1991

Source of review: ADRIA Study report: V 1.74-1.76

INTRODUCTORY COMMENT:

Adria started their two Phase III trials, 088001 (breast cancer) and 088002 (small cell lung cancer), using 20:1 ratio. Safety monitoring revealed that there was an imbalance in the number of deaths on one of the study arms. After unblinding, 11 patients were found to have died on the ADR-529 arms vs. one patient on the placebo arms. Most deaths were from complications of prolonged myelosuppression. Adria recommended reducing the ratio to 10:1. In May 1989, this trial (88006), a second breast cancer trial, was initiated to better evaluate the safety and efficacy of this dose level. It was run parallel with the restarting the 88001 trial at the 10:1 ratio.

This study is identical to the ADRIA-sponsored 88001 study in breast cancer after the February 1989 amendment which changed the dose ratio to 10:1 and added an interim analysis plan. Refer to the review of 88001 for a discussion of protocol design details.

8.2.1 OBJECTIVES Same as 88001:

-To demonstrate cardioprotective effect of ADR-529.
-To determine if ADR-529 alters response rate to FAC.
-To assess the safety of doxorubicin plus ADR-529.

Study 88001

8.2.2 DESIGN Same as 88001:

This was a multi-center double-blind randomized Phase III two-arm controlled trial. Two arms consisted of CAF + either ADR-529 or placebo (PLA). Two stratification factors were cardiac risk factors versus no cardiac factors and measurable disease versus non-measurable disease.

8.2.3 PROTOCOL

8.2.3.1 ELIGIBILITY

Same as 88001. At the time this protocol was added PS 2 patients were no longer included. The April 1989 amendment applying to both protocols occurred before any patients were entered into this study. See initial page of 88001 review for details. This allowed the inclusion of patients without measurable disease.

8.2.3.2 RANDOMIZATION

As in trial 88001 (10:1 ratio), patients were stratified for cardiac vs. noncardiac risk factors and measurable vs. non-measurable disease before randomization. Patients were then assigned to treatment arm A or treatment arm B by a random code system for each stratum. Patients were randomized within each participating institution.

Cardiac risk factors are defined as in the 88001 review.

Table 6.3(attached) lists the numbers of patients participating in the various data summaries. One notes that only 35 patients (12 on DZR and 23 on PLA) have LVEF measurements at 500 mg/M^2 of doxorubicin.

8.2.3.3 TREATMENT

Administration of FAC with or without placebo was identical to that in trial 88001.

As in the 10:1 portion of study 88001, all patients received

ADR529 500 mg/m^2 after cumulative dose of 300 mg/m^2 of adriamycin.

8.2.3.4 EFFICACY

Efficacy variables were followed and defined as in 88001.

8.2.3.5 SAFETY

Safety monitoring and analysis was identical to that in 88001.

8.2.3.6 STATISTICS

The same statistical protocol design and the same analysis procedures were used in the 10:1 patients in 88001. Data Monitoring Committee reviewed data from this trial simultaneously.

8.2.3.7. PROTOCOL AMENDMENTS

Amendments 1 and 2 correspond to amendments 3 and 4 in the 88001 report.

8.2.4 RESULTS OF CLINICAL STUDY 88006

8.2.4.1 Center

A total of 185 patients were accrued by 52 centers. Though 52 centers accrued patients, only two sites accrued more than ten patients (Table 1, p31370). 41 of the 52 centers accrued less than 5 patients/center. The small number of patients accrued per center may defeat the purpose of block randomization of each center. This may account for the imbalance of patients in the arms, 81 on DZR and 104 on PLA.

Reviewer's Comments:
The imbalance in patient numbers is a common occurence in these studies. Although unsettling, I do not know of a way, with our systems of randomization and retrospective review, of verifying randomization or assuring that the patient list is complete.

8.2.4.2 Baseline Characteristics

The differences in baseline demographic characteristics and cardiac risk factors were nonsignificant except for the percent of black patients in the DZR vs. PLA arms (6 vs. 17%) with a p-value of 0.06 (table 2, p 3138). Cardiac risk factors are outlined in attached table 3). Of interest, > 1/3 of both groups of patients have LVEF </- 10% of the lower limit of normal for their institution.

Analysis of the baseline characteristics of the two groups showed no statistically significant difference. Analyzing the baseline cardiac risk factors, overall a greater number of PLA patients have age > 65, history of heart disease, prior radiation to the mediastinum, hypertension and diabetes. When number of cardiac risk factors were tabulated from the listings in appendix 7, table 3 of the NDA, the following results are shown.

Number of risk factors	0	1	2	3	4	5
DZR (#)	29	31	13	4	3	0
(%)	36	39	16	5	4	0
PLA (#)	29	37	31	6	1	1
(%)	28	35	30	6	1	1

If one assumes that the more cardiac risk factors patients have, the more predispose they are to cardiac toxicity, then the above data should be taken into consideration when interpreting the cardioprotective effects of ADR-529.

8.2.4.3 Baseline Disease Characteristics

These are compared in table 2 (p 3138). The DZR group of patients have 116 days longer median disease free interval (721 vs 605) defined as the time between initial disease diagnosis and first relapse, however, this was not significant by the log rank statistic method (p=0.74) The other baseline disease

characteristics are balanced.

8.2.4.4 Analysis of Cardioprotection

The analysis of time to cardiac event is described in Table 5 and Fig. 1 (attached). There were 22 events in the placebo arm versus 7 in the DZR arm. The hazard ratio favored the DZR arm (2.30). The p-value for the logrank test was 0.048 and the Wilcoxon p-value was 0.46. There were 3 CHF events in the control arm and none in the DZR arm. These occurred at 450 and 500 mg/M^2 as noted in figure 2. The difference was not statistically significant (p=0.17 by LR).

Table 7 describes the LVEF of cumulative doses of doxorubicin onstudy. At 500 mg/m^2 of doxorubicin, the original endpoint of the trial, there were 12 patients on DZR versus 23 patients on the control arm who had a LVEF measurement, with a mean drop of LVEF from baseline in the control arm of 10.0%. This was 6.2% greater than on the placebo arm (p=0.028). At 550 mg/M^2 the difference was 11.7% (p=0.001) with LVEF measurements done in 10 patients on the DZR arm and 17 on the control arm.

Reviewer's Comments:
It seems odd to have so many more patients on the control arm for this measurement. There must be a strong process encouraging dropout on the DZR arm to over balancing the cardiac-event driven dropout on the control arm. The number of patients with LVEF at 400/M2 is 21 for ADR-529 and 35 for placebo [note again decrease compared to 26 and 36 who were to receive a 9th course (450 mg/M^2)]. Like in the other studies, it would appear that a larger percentage on the placebo arm got a LVEF at this point. Using a similar selection process 12/12 on DZR compared to 17/25 appeared to have the measurement prior to proceeding to 550 mg/M^2. ADRIA LABS will be asked to address these apparent discrepancies.

8.2.4.5 Analysis of Antitumor Efficacy

Response data is presented in table 8 (attached). There were 54 patients with measurable disease in the DZR arm

and 69 in the PLA arm. The point estimate show that DZR vs placebo response rate is 57% vs. 52% (95% ci D minus P -13% to 23%). In the intent-to-treat analysis, 11% of the patients on the DZR arm achieved CR compared to 7% on the placebo arm. The percentage of patients that progressed despite therapy was similar on both arms. Approximately 81% vs 86% of the patients with measurable disease on DZR or placebo group, respectively, were considered by ADRIA to be evaluable for response. The reasons for excluding patients, listed in appendix VIII of the report, varied from wrong histology, prior chemotherapy to receiving less than 3 courses of therapy. As shown in table 8, the result of the analysis including only evaluable patients was similar in relative terms; there were slightly higher response rates in both arms. The median time to best response in both groups was reportedly 69 days.

Time to progression is outlined in table 9 and figure 3 (attached). 53% of the DZR and 49% of the placebo patients had progressed. The median failure times were 199 days on the DZR arm and 232 days on the PLA arm. The hazard ratio (P:D) was 0.949, logrank p-value = 0.80. Confidence intervals were very wide ((0.63, 1.43).

The disease free analysis is shown in Figure 4. Compared to the progression analysis, an additional 6 events were scored on the DZR arm and an additional 7 on the control arm. The point estimate of the hazard ratio (P:D) was slightly worse for DZR (0.832) and again the confidence intervals were quite wide.

Reviewer's Comments:
The causes of death and perhaps CRF's in these 13 patients dying prior to progression should be examined.

The survival analyses are presented in table 11 and figure 5. Only 24% of placebo arm and 37% of the DZR arm had died at the time of data cutoff. There was a strong trend toward inferior survival in the DZR arm. The median survival time was 420 days vs. 526 days with

logrank p-value = 0.06. The hazard ratio P:D was 0.604 (95% ci 0.355 to 1.03). One explanation given by the sponsor to account for this is that patients on the DZR arm were followed on the average 22 days longer, hence causing an imbalance in documentation of disease progression and death. The median follow up times were 326 days in the DZR group and 304 days in the PLA group.

Reviewer's Comments:
The sponsor's speculation is not likely. No analysis of the statistical significance of the finding is given. The difference of 22 days is minimal. Differential censoring would only affect this analysis if censoring was related to probability of death. Certainly, in view of the lack of corroboration of this survival trend by the 001 data and without a rationale such as a strong trend toward inferior time to progression or response, one would suspect chance fluctuation of data or problems with the randomization process. I am concerned about the latter with the imbalance in numbers randomized (81 versus 104).

If one is to discount this data, one should be prepared to discount the response data as well. However, I note that 1992 ASCO abstract #191 presents response analysis of evaluable patients and omits survival analysis. The first author was J.A. Maillaird; others include present or past ADRIA personnel P. Banks, D. Jones, R. Gams, and R.D. Reynolds. As I discuss elsewhere, the ASCO abstract reporting results of simultaneously stopped 001 trial include disease free survival and survival data, declaring them "approximately equal," but both the abstract and ASCO poster omitted any reference to unfavorable response rates in this larger data base (abstract #74). ADRIA authors are the same for the 2 abstracts. In the 001 study, there were 38% dead in the control arm versus 35% in the DZR arm. In this 006 study there were 37% dead in the DZR arm.

8.2.4.6 Analysis of Toxicity

Clinical Toxicity (Tables 12-15, p3150)

During course 1, more patients on the control arm experienced alopecia groups (table 12: 90% vs 77%, p<0.01). Table 14 displays the incidence of

toxicities over all courses. Significantly more common on the control arm at the p<0.01 level were stomatitis (52% vs 35%), dysphagia (10% vs 2%), and vomiting (78% vs 63%). Severity scores were higher on the control arm for fatigue and malaise (2.0 vs 1.7, p<0.01 by WRS). Fever, sepsis, and infection were not statistically different on the 2 arms for the first course of all courses combined.

Reviewer's Comments:
The decrease stomatitis, dysphagia, vomiting, and alopecia in the DZR group suggests some protection against these doxorubicin-associated toxicities.

Reported Toxicity (Tables 16 and 17, pp 3164-3180)

The most notable differences between the two groups was anxiety reported at the first course, 9% DZR arm vs. 0% PLA arm. In the summary of all courses, rhinitis(7% DZR, 20% PLA), cough (28% DZR, 40% PLA), infection (4% DZR, 12% PLA), depression (4% DZR, 11% PLA), headache (5% DZR, 12% PLA), and dyspnea (28% DZR, 35% PLA) were reported more by the PLA group while back pain (27% DZR, 20% PLA), anxiety (10% DZR, 4% PLA), fever (11% DZR, 8% PLA), and asthenia (27% DZR, 21% PLA) were reported more in the DZR group.

Laboratory Toxicity (table 18, pp3181-3193)

There were no significant differences between the two groups with respect to the selected hepatic and renal function tests (identical panel to that in study 88001).

Analyses of nadir blood counts are described in tables 22-28 (pp 3187-3193).Compared by WRS test, WBC and AGC nadir counts were not significantly different for course 1 or for all courses combined. As shown in table 24 (attached) nadir platelet counts were significantly lower on the

DZR arm during course 1 (p = 0.007). The sponsor considers the differences not to be of clinical significance, since the median nadir values were so high (DZR-170.5 x $10^3/mm^3$; PLA-208 x $10^3/mm^3$). However an analysis of the number of patients with clinically significant depressions was not done. The lower end of the range of values in the table includes clinically significant values down to 4,000 on the placebo and 21,000 on DZR. The median decrease of hemoglobin from baseline for the all courses analysis was 3.0 g/dl on DZR vs 2.8 g/dl on the control arm (p = 0.07).

Reviewer's Comments:
An analysis of numbers of patients experiencing myelosuppression to critical levels was requested.

8.2.4.7 Dosing Intensity

As shown in table 29 (attached) the DZR patients received a median of five courses of treatment compared to a median of six courses for the control patients, but the difference was not significant by WRS (p=0.46). The median and cumulative doses of cytoxan and 5FU were similar. 41% of the DZR and 48% of the control arm had dose reductions and 47% of the DZR versus 48% of the PLA arms had delay of therapy. None of these differences approached statistical significance. Similarly the median dose intensity index was not significantly different for doxorubicin (0.96 DZR vs 0.94 PLA) or the other cytotoxics (0.92 DZR and 0.87 PLA).

8.2.4.8 Measure of Quality of Life

Analyses of time to performance status ≥3 were performed and are presented in attached figure 7. Again there were few events on-study (about 10% in each arm). 17% to 27% of patients had events reported at any time. This analysis showed a trend in favor of placebo (hazard ratio p:d 0.62, LR p=0.13) but the lack of events past 350 days on the placebo arm is questionable. The absurdity of the analysis is

highlighted by realizing that the curves are superior to survival curves.

8.2.4.8 Patient Disposition, Time to Treatment Failure

Table 33 (attached) lists reasons patients went offstudy. This analysis is mature with 81%-85% offstudy. Overall, reasons for going offstudy show a similar distribution except for cardiac toxicity [8 events(12%) versus 22 events(26%)]. There were 4 onstudy deaths on the DZR arm versus 3 on PLA. Table 34 (attached) gives the distribution of events over by course number. Most of the deaths occurred in the first 3 courses. Many of the progressions (11 on each arm) occurred at the 3rd course when tumor measurements were first to be repeated. After 300 mg/M^2 of adriamycin, a total of 9 had gone off for cardiac toxicity on the placebo arm versus 4 on DZR. Most of the balance of the difference for this offstudy reason occurred at 10 or greater courses (11 on placebo versus 1 on DZR). The analysis for time to off-study is shown in figure 9 (attached). The curves were similar with median time to offstudy of about 150 days on each arm (p=0.46 by LR).

Reviewer's Comments:
This curve does not suggest any increase in ability to receive CAF in this patient population.

8.2.5 DISCUSSION AND CONCLUSIONS

8.2.5.1 Statistical considerations:

To date it has been difficult to get a clear description of what statistical analyses the monitoring committee relied upon at various points in the history of these studies to assess whether they should be stopped. See discussions of trial 88001 for most considerations. A quote from the discussion section of this study report is of interest:

> "An independent Data Monitoring Committee found the totality of results taken across these three studies compelling to the extent that patient accrual to a treatment arm lacking DZR would be inappropriate."

It is difficult to be definitive about how the cardiac data from this trial should be analyzed. If one considers that stopping was primarily due to data in the 001 trial, then one might not apply interim boundary standards to levels of significance. The sponsor has been asked for more details about the practices of the monitoring committee. Given the replication of the cardiac findings from all trials, I prefer not to get too tied up in these details for this trial. It would be desirable if investigators, regulators and statisticians would develop methods and guidelines for guiding decisions about stopping and analyzing trials when interim analyses involve more than one trial.

8.2.5.1 Sponsor's conclusions:

The sponsors discussion and conclusions are on pp 3127-3134. The sponsor concludes that the risk of a cardiac event was, on average, 2.3 higher, at any point in time, for the control arm. Changes in LVEF compared to baseline were significant at 500 mg/M^2, 500 mg/M^2, and 600 mg/M^2. Of the 69 patients on the treatment arm removed from study, eight (12%) were removed because of an abnormal LVEF compared to 22 (26%) of the 84 removed from the control arm. 12 of these were removed after course 9 (450 mg/M^2 of doxorubicin) on the control arm compared to only one case on the DZR arm. Safety was similar on the 2 arms except for lower median neutrophil and platelet counts on the DZR arm.

8.2.5.2 Medical Officer Discussion and Conclusions:

Initially one notes an imbalance in randomization, 81 on DZR arm and 104 on the placebo arm. Though 52 centers accrued patients, only two sites accrued more than ten patients (Table 1, p31370). 41 of the 52 centers accrued less than 5 patients/center. The small number of patients accrued per center defeated the purpose of blocked randomization of each center and explains how such an imbalance could have occurred. Unfortunately there are no definitive methods for verifying that valid randomization occurred. Since randomization occurred at ADRIA LABS, we can only retrospectively view the list, look for obvious inconsistencies, and trust that a valid method was used.

This study was planned with the primary objective of evaluating the cardioprotective property of ADR-529. The tumor response endpoint was secondary. The stopping point and sample size were all calculated using the CV endpoint. ADRIA calculates that had tumor response been a primary endpoint of the study, 775 patients would be required to have 80% power to detect a 10% difference between the DZR and PLA response rates.

Cardiac and tumor findings are summarized in the following table which was prepared from several analyses from the NDA.

Tabular summary of findings study 88006

Disease	Breast cancer				
Ratio of DXR	10:1				
	DXR		**PLA**		
# randomized					
total	81		104		
measurable	54		69		
Cardiac Effect					
	DXR		**PLA**		
Time to "event" (29 events)		P:D	2.30	p=0.05	(0.98, 5.40)
LVEF decrease (mean difference, change from baseline, D-P)					
500 mg/M² (12D, 23P)[1]			6.2	p=0.03	
550 mg/M² (10D, 17P)[1]			11.7	p=0.001	
CHF events	3		0		
Tumor Effect					
	DXR		**PLA**	**95%ci**	
Response (123 pts)	41/54 (57%)		36/69 (52%)	(-13%, 23%)	
Progression (94 events)	P:D	0.95		(0.63, 1.43)	
Survival (55 deaths)	P:D	0.60		(0.36, 1.03)	
Offstudy (153 events)	P:D	0.89		(0.65, 1.23)	

[1]Numbers in parentheses represent number of patients analyzed.

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PREVENTION OF CHRONIC ADRIAMYCIN® CARDIOTOXICITY WITH THE BISDIOXOPIPERAZINE DEXRAZOXANE (ICRF-187, ADR-529, ZINECARD®) IN PATIENTS WITH ADVANCED OR METASTATIC BREAST CANCER. L.A. Mailliard, J.L. Speyer, K. Hanson, B.S. Shaikh, A. Chang, K. Ryan, R. Navari, R.F. Berris, J.J. Schultz, J.B Craig, J. Garcia, P.S. Ritch, L.R. Laufman, R. Shildt, P. Danier, A. Kaufman, A. Weinberg, P.J. Flynn, W. Stein, Y.H. Pilch, M.J. Guarino, R.D. Reynolds, K. Squillace, A. Kline, D. Scott, P.L.C. Banks, D. Jones, J. Bianchine and R.A. Gams. Omaha, New York, Kansas City, Toledo, Rochester, Travis AFB, Birmingham, Denver, Newport News, Shreveport, Miami, Milwaukee, Columbus, Tulsa, Greenville, Sellersville, Providence, Minneapolis, Metairie, Chicago, Newark and Adria Laboratories, Columbus OH.

185 cases of chemotherapy naive (except non-anthracycline adjuvant therapy) advanced breast cancer were admitted to a study using standard chemotherapy, with or without the blinded study drug Dexrazoxane (DZR). All patients were treated with 500 mg/M^2 5-fluorouracil, 50 mg/M^2 Adriamycin® and 500 mg/M^2 cyclophosphamide I.V. every three weeks. Eighty-one (81) were randomized to receive DZR, given as a rapid I.V. infusion (500 mg/M^2) within 30 minutes prior to the administration of Adriamycin®. Mean age was 55.8 (DZR) vs 57.0. Median ECOG performance status was 0 in both arms. Adjuvant chemotherapy had been given to 35% of DZR vs 37% of controls, and hormonal therapy had been given to 54% (DZR) vs 48%. Toxicity was approximately equal except for slight increase in the treatment arm for granulocytopenia (median nadir WBC 1.3 vs 1.4), thrombocytopenia (median nadir 137.0 vs 151.5), infection (24% vs 17%) and sepsis (17% vs 15%). On the treatment arm, there were 6 (14%) CR plus 23 (52%) PR for CR+PR of 29/44 (66%) for evaluable cases; on the control arm, there were 5 (9%) CR plus 30 (52%) PR for CR+PR of 35/58 (60%). Cardiac evaluation showed 0 cases of CHF on the DZR arm and 3 on the control arm. There were 7 (9%) cardiac events on the DZR arm and 22 (26%) on the control arm. The resting MUGA/LVEF showed a mean fall of 7.9 (vs 4.1) after 400 mg/M^2, 10.0 after 500 mg/M^2 (vs 3.8), 12.4 after 550 mg/M^2 (vs 0.7) and 13.3 after 600 mg/M^2 (vs 2.0) cumulative Adriamycin®. Patients on the DZR arm did not develop clinical cardiotoxicity beyond 400 mg/M^2, while 12 of 22 (54%) of the cases on the control arm were removed after 400 mg/M^2. This study clearly demonstrates the cardioprotection effect against high cumulative doses of Adriamycin®.

Though the differences only approached statistical significance for survival (p=0.06), hazard ratios also slightly favored placebo for time-to-progression and " disease-free-survival (more precisely, "progression free survival"). The response rate slightly favored DZR. One possible reason for the lack of correlation of response and survival could be that survival was inferior only in the non-measurable disease group. This discrepancy may be account for by the fact that response rate is calculated from patients with measurable disease and the other data groups used all randomized patients, including the nonmeasurable patients. The offstudy pattern is remarkably different in this study, with no tendency for patients on the DZR arm to remain onstudy for a prolonged time.

This study was originally planned with 3 interim analyses and a final analysis. The number of formal interim analyses that occurred, which is unclear from the NDA, may determine which p-value should be used to determine significance of the data being presented. If we take the p-value that is appropriate for the number of interim analyses done, and consider this analysis an interim analysis, the interpretation of the current data would be different than if one used a nominal p value of 0.05. The only result of significance using this p-value may be the changes in LVEF over cumulative doses of 550 mg/m^2 of adriamycin. This study was stopped prematurely; however, primarily because of the results from 88001. More refined assessment of the statistical significance of results awaits clarification of monitoring practices by ADRIA and further consultation with Agency statisticians.

It should be noted that updated data past 130 days on study (300mg/M^2 of doxorubicin) for time to progression and survival will increasingly include patients on both arms who have received 10:1 DZR after crossover, and hence if any differences existed in hazards, they might tend to be obscured by crossover.

Clinical toxicity findings were summarized earlier:

> During course 1, more patients on the control arm experienced alopecia groups (table 12: 90% vs 77%, p<0.01). Over all courses, significantly more common on the control arm at the p<0.01 level were stomatitis (57% vs 35%), dysphagia (10% vs 2%), and vomiting (78% vs 63%). Severity

scores were higher on the control arm for fatigue and malaise (2.0 vs 1.7, $p<0.01$ by WRS).

There were few statistically significant findings of increased myelosuppression on the ADR-529 arm in this data base, except that nadir platelet counts were significantly lower on the DZR arm during course 1 ($p = 0.007$). The number of patients is only 60% that in the 10: 88001 study.

Regulatory conclusions:

This randomized controlled trial was stopped prematurely. Stopping was recommended by a monitoring board evaluating the results of the data from this study and the 88001 study of identical design. The study provides supportive data that ADR-529 at a 10:1 ratio given with FAC protects against cardiac effects of doxorubicin as manifested by changes in LVEF. Power to detect a potential effect on antitumor activity was relatively small. It provides no suggestion that patients can tolerate more prolonged treatment with multiple courses of FAC. Again some symptomatic toxicity findings suggest protection of non-cardiac tissues. Trends in this data should be examined in conjunction with data from 88001.

Prior to considering Agency review complete, the sponsor has been notified of many questions which need to be addressed. In addition the validity of selected data needs to be further verified by Agency review of tabulations and case report forms.

8.3 Trial #3, Clinical Study 88002 (small cell lung cancer)

From the ADRIA's report cover sheet(-02036):

TITLE: Evaluation of ADR-529 as a Cardioprotective Agent in a Randomized Double-Blind Phase III Trial of CAV+Placebo versus CAV+ADR-529 in the Treatment of Extensive Disease Small Cell Lung Cancer

CLINICAL STUDY NO.: 088002-999

PHASE OF STUDY: Phase III

PRINCIPAL INVESTIGATOR: Not Applicable - Multicenter Study

STUDY SITE: Multicenter

STUDY DATES:

First Patient Randomized:	February 12, 1988
Accrual Cutoff:	January 14, 1991
Data Cutoff:	March 31, 1991

CLINICAL MONITOR: Ralph D. Reynolds, M.D.
Adria Laboratories
(614) 764-8190

BIOSTATISTICIAN: Fredrick S. Whaley, Ph.D.
Adria Laboratories
(614) 764-8266

STATUS OF STUDY: Complete

REPORT DATE: January 23, 1992

" This report includes all patients entered prior to January 14, 1991. The data cutoff date is March 31, 1991, except for efficacy evaluations and analyses where data through April 30, 1991 were included."

Location of material reviewed:

Trial 88002

Medical Statistical Report:	V 72	
Protocol and amendments	V 72	p 2312
Randomization code		p 2434
Data listings	V73	p 2494
Other appendices	V74	

Reviewer's Introductory Comments:

The details of the history of this protocol and that of the simultaneous breast cancer protocol, 888001, are almost identical. Except for details of treatment (vincristine insted of 5FU and higher doses of Cyclophosphamide) and disease-specific criteria for entering and disease-monitoring schedules, the protocols and amendments are identical. See the description of protocol 88001 for details and comments on design considerations.

8.3.1 Objective:

From the protocol:

1. To demonstrate that ADR-529 administered intravenously has a cardioprotective effect when added to the CAV regimen in patients with extensive disease small cell lung cancer.

2. To determine if ADR-529 alters the response rate to CAV.

3. To assess the safety of the combination of CAV + ADR-529.

8.3.2 Design:

The design is well summarized by ADRIA:

"A prospectively randomized multicenter, parallel group, double-blind, placebo controlled trial was initiated in February 1988 to determine whether dexrazoxane (DZR) administered intravenously has a cardioprotective effect

when added to the cyclophosphamide, Adriamycin®, vincristine (CAV) regimen in patients with extensive disease small cell lung cancer (SCLC), to determine whether DZR altered the response rate to CAV and to assess the safety of the combination of CAV + DZR. The study was conducted utilizing computer-generated advice rules based on an expert system. A Data Monitoring Committee (DMC) met at intervals throughout the study to review the data."

8.3.3 Protocol

8.3.3.1 Details in Protocol Amendments:

Amendments to this protocol parallel those in the first breast cancer study, 88001. See the review of this protocol for details of the amendments.

The early amendments, #1 and #2 occurring in early 1989 included:

-A change to 10:1 ratio of ADR-529.

-Institition of a group sequential plan, and a data monitoring committee.

-Change in formulation.

-Addition of non-measurable disease patients for evaluation of toxicity and cardiac efficacy. Stratification procedure now was to include measurability in addition to cardiac risk.

-Weekly CBC was added.

The late amendment, #3, in January of 1991 called for addition of ADR-529 to both arms after 300 mg/M^2 of doxorubicin.

8.3.3.2 Eligibility:

Entry criteria are found on p 2334 and are well summarized by ADRIA:

"To be eligible patients had to have histologic or cytologic proof of small cell lung cancer with extensive disease

(Stage IIIB or IV), have received no prior chemotherapy and no radiotherapy (RT) other than local palliative RT, be 18 years of age or older, have a performance status (PS) of ≤2 on the ECOG scale, and have a life expectancy of ≥six months. In addition patients were to have a white cell count (WBC) ≥4000/mm^3 and/or an absolute granulocyte count of ≥1900/mm^3, a platelet count ≥100,000/mm^3, a bilirubin and serum creatinine ≤2 mg/dl, and a left ventricular ejection fraction (LVEF) at or above the lower limit of normal for the institution.

8.3.3.3 Treatment Plan:

From the protocol:

"Treatment Arm A (q 3 wks)

C - Cyclophosphamide 750 mg/M^2 I.V. Day 1
A - Adriamycin® 50 mg/M^2 I.V. Day 1
V - Vincristine 2.0 mg I.V. Day 1

Treatment Arm B (q 3 wks)

C - Cyclophosphamide 750 mg/M^2 I.V. Day 1
A - Adriamycin® 50 mg/M^2 I.V. Day 1
V - Vincristine 2.0 mg I.V. Day 1
A - ADR-529* 50 ml/M^2 I.V. Day 1

*50 ml/m^2 of ADR-529 properly reconstituted is equal to giving 500 mg/M^2."

The ADR-529 ratio was 20:1 prior to 11-2-88. On January 14, 1991 patients on both arms were given ADR-529 after 300 m of doxorubicin. The ADR-529 or placebo was given by slow IV push or rapid infusion and was followed within 30 minutes by the doxirubicin. Cyclophosphamide was given by IV push or infusion and the vincristine by IV push.

Dose modification:

The scheme was similar to that used in 88001 and 88006: Dose delay and dose-reduction of Cytoxan for granulocytopenic fever or

depressed day 22 blood counts with no dose reduction of doxorubicin. Vincristine was to be dose reduced to 1mg for grade 2 neurotoxicity and discontinued for > grade 3. The first cyclophosphamide reduction was 125 mg/M^2 and subsequent reductions were 50 mg/M^2.

Duration of therapy indications for early termination were identical to the 88001 protocol, ie termination for disease progression or for specifically indicated criteria for cardiac toxicity.

Patients could have irradiation of the primary pulmonary lesion if needed to relieve central pulmonary obstruction.

If the heart was irradiated, the patient would no longer be eligible for cardiotoxicity analysis. If other measurable disease was present the patient could remain on for response analysis.

8.3.3.4 Randomization

The 2 strata (after the 1989 amendment):

-Cardiac risk factors versus no cardiac risk factors
AND
-Measurable disease versus non-measurable disease only.

Cardiac risk factors consisted of one or more of the following:

" 1. Prior mediastinal irradiation
2. Age >65 years
3. History of heart disease (previous MI, significant arrhythmia, angina)
4. Hypertension requiring medical therapy
5. Diabetes mellitus requiring medical therapy
6. Baseline MUGA scan 0-10% above lower limit of normal for institution."

Schedule of evaluations:

A copy of the evaluation schedule is attached (from p 02072). It is identical to the 88001 schedule except for some disease-specific monitoring differences. Response is first assessed after

the third course of therapy. Cardiac monitoring is identical to 88001.

8.3.3.5 Efficacy criteria

The sections referring to cardioprotection assessment of efficacy is identical to 88001 (sections 11.1 and 11.2).

Details are provided for measuring tumor (p 02349). Measurements of bidimensional disease is standard. Unidimensional measurable disease was also accepted. ≥ 50% decrease in unidimensional measurement was deemed response for this group.

Reviewer's Comments:
Need to state when unidimensional disease was used.

Definitions of evaluability are similar: 3 courses of therapy for response, and at least one MUGA scan after baseline for cardiotoxicity, unless removed for a cardiac event.

As with 88001, the sponsor comments about the initial primary endpoint (p 02087):

> "The primary evaluation outlined in the protocol to establish cardioprotection was the difference between the two randomized groups in mean changes from baseline in the left ventricular ejection fractions (LVEF). This analysis, comprised essentially of patient subgroups, has the potential for selection bias, and should be interpreted with caution. To overcome this problem, an analysis was performed whereby all patients were assessed until the time of a cardiac event."

8.3.3.6 Toxicity

As with 88001, toxicity was assessed by ECOG/NCOG criteria, specifically "esophagitis, stomatitis, dysphagia, anorexia, nausea, vomiting, diarrhea, fatigue, malaise, fever, infection, sepsis, hemorrhage, alopecia, recall skin reaction, pain on injection, phlebitis, and neurotoxicity." Date of onset, date ending, and most severe grade were recorded.

Offstudy
As with 88001, the meaning of the following statement in the sponsor's report needs clarification:

> "To accommodate restrictions placed by the sponsor's data entry system, patients who had multiple reasons for going off-study had to be reclassified as having a single reason for withdrawal. For those few patients that were affected, the primary multiple failure category was progressive disease and cardiotoxicity. This outcome was reclassified as progressive disease."

8.3.3.7 Statistical considerations

Statistical considerations in protocol:

These sections are identical for the 88001 protocol and 88002 protocol as of the 3rd amendments.

A similar sample size was provided for this study:

> "Initially, enough patients will be entered to accrue 72 response evaluable patients per arm or 38 patients per arm who have received at least 500 mg/M^2 of Adriamycin®, whichever is greater. When these patients have been accrued, the assumptions on which sample size calculations for cardioprotective effect were made will be reassessed. Based on this reassessment, additional patients may be entered in order to achieve the desired statistical power, or to gain additional experience with ADR-529 as a cardioprotective agent."
>
> When the dose ratio was reduced, a goal of 60 patients achieving a dose of 500 m of doxorubicin was set.

8.3.4 Results

(20:1) 8.3.4 Results of 20:1 Drug Ratio

(20:1) 8.3.4.1 Investigators

See attached table 1A. 51 patients were entered at 22 institutions, ranging from 1-5 patients per institution.

(20:1) 8.3.4.2 Baseline characteristics

As compared in table 2 A (p 2149) the arms are comparable for age (median of 59 years on ADR-529 vs 63 years on placebo), sex (about 70% male) and race (about 90% white and 10% black). Cardiac risk factors are compared in attached table 3A were similar on the 2 arms for mediastinal radiation, history of heart disease, hypertention, and LVEF ≤ 10% above institutional lower limit. There were more patients over 65 years of age on the placebo arm (48% vs 27%). There were 4 patients with diabetes on the placebo arm compared to none on the ADR-529 arm.

Reviewer's Comments:
A quick analysis was done regarding this imbalnce. On the placebo arm, only one of 4 diabetes patients and 3 of 13 patients over 65 had cardiac events, so this is unlikely to have skewed the results.

Baseline disease status is compared in table 4A (p 2153). Time from diagnosis, # of disease sites, PS, prior therapy, and baseline blood counts were similar.

(20:1) 8.3.4.3 Data Sets Analyzed

These are summarized in a table from p 02105. LVEF groups are balanced in numbers until 500 mg/M^2, when there are 7 on the ADR-529 arm and 3 on the placebo arm undergoing analysis.

(20:1) 8.3.4.4 Cardiac effects

Time to cardiac event data is summarized in attached table 5A and Figure 1A. There were 4 events on the ADR-529 arm and 9 on the placebo arm with separation of the time to cardiotoxic event curves beginning at 400 mg/M^2. Only one CHF event occured, and it occured on the ADR-529 arm at 450 mg/M^2 of doxorubicin (pt 5201).

All of the 9 cardiac LVEF events on the placebo arm were substantial from my review of the data, whereas 2 of the ADR-529 events seemed minor (LVEF 50 to 43, and 55 to 48).

The difference is not statistically different, but the Hazard ratio favors ADR-529 (P:D 2.26 95% ci 0.67 to 7.64, p = 0.18 by LR).

The sponsor submitted another analysis of 20:1 patients with CHF on or off-study. This segments the results (see attached table 6.4.2.1). All 7 patients were in the PLA arm, all had low LVEF as offstudy reason

Change in LVEF is presented in attached table 7A. The effect is best characterized at the 400 mg/M^2 dose, where there are 16 patients and the mean change from baseline is -14.3 in the ADR-529 arm and 3.0 in the placebo arm, p = 0.004. At the protocol specified endpoint of 500 mg/M^2 there are only 3 patients in the placebo arm. The difference between the means is 12.1 (p = 0.04).

(20:1) 8.3.4.5 Antitumor Efficacy

Response results are presented in attached table 8A. 68% responded in the placebo arm compared to 58% in the ADR-529 arm (95% ci D minus P -36%, 16%, p = 0.45). There were 7 CRs in the ADR-529 arm versus 4 on the placebo arm.

Attached table 6.5.1.1 gives reasons for off-study for patients ADRIA used in their "evaluable" patients analysis. 4 of the ADR-529 patients died compared to on on the placebo arm.

Review of overview tabulations shows that PR's in the placebo arm were documented at 2-4 months:

1
2 XXXXXXXX
3 XX
4 XX
5 X

CR's were documented at 8, 5, 4, and 3 months.

Although the analysis was not submitted, the sponsor notes that on the average time to response was 65 to 66 days (the CR's alter this statistic from the one I am interested in, ie time to first response).
So it is likely that if dose intensity made a difference in response rates, it would be dose given before the 4th course of therapy, or dose reductions of the second and third courses.
, and in most patients at about 2 months. So, the main impact of

dose reduction on response would need to be from dose reductions of from courses #2 and #3.

Time to disease progression is presented in attached table 9A and figure 3A. There were 22 events in each arm. The hazard ratio was P:D was 0.908 (95% ci 0.50 to 1.66). Since there were more deaths on the ADR-529 arm, disease free survival trends worse for that arm as shown in table 10A and figure 4A (HR P:D 0.84, 95% ci 0.48 to 1.48, p = 0.55 by LR).

Reviewer's Comments:
Given the small number of patients, nothing conclusive can be stated about response, time to progression, or disease free survival at the 20:1 ratio.

(20:1) 8.3.4.6 Survival

Attached table 11A and Figure 5A summarize survival. With over 90% of the patients having died, Hazard ratio P:D was 0.91 (95% ci 0.52 to 1.61), p=0.75 by LR. Again the small size of the study limits conclusions.

(20:1) 8.3.4.7 Patient Disposition

Reasons for going off-study are presented in attached tables 33A, 34A, and 35A. Deaths (5 vs 1) and "other" (6 vs 1) were more common on the ADR-529 arm whereas cardiotoxicity (11 vs 4) was more common on the placebo arm.

Time to offstudy was similar on the 2 arms as shown in figure 9A (attached).

Reviewer's Comments:
Examination of tables 34A and 7A again raises questions.

	# onstudy at 8 400mg/M²	*# onstudy at 9*	*# LVEF at*
D arm	*10*	*10*	*7*
P arm	*5*	*5*	*9*

Again it is unclear why less onstudy patients had LVEF reported

in the cardiac analysis on the D arm at 400mg/M^2 (70%) than on the P arm (100%).

The sponsor's discussion of offstudy details is appropriate (p 2129):

> "Table 33A summarizes the patient disposition or primary reason for going off-study for the 20:1 patient cohort. All patients in this cohort are "off-study". On the DZR arm, 23% of patients went off-study because of disease progression, 15% because of cardiac events, 12% because of non-cardiac adverse events, and 8% because of refusal to continue on-study. In addition, 19% of the patients on this treatment arm expired while on-study, and six patients (23%) were withdrawn for reasons classified as "other". The cardiac events on the DZR arm were all declines in LVEF without clinically evident CHF. The final LVEF in two of the four patients was <40% (38% and 35%). The non-cardiac adverse events were related to the complications of myelosuppression. Of the five patients who expired on-study, three patients (Patient Nos. 5202, 17201, and 28201) expired of the complications of infection, probably secondary to myelosuppression. In two cases (Patient Nos. 5202 and 17201) the relationship to the study drug was considered to be "probable" while in Patient No. 28201 the relationship was categorized as "possible". In Patient Nos. 28101 and 48201 the most likely cause of death was progressive disease and resultant complications. These deaths were considered to be unrelated to the study drug. Of the six patients who were withdrawn from the study for reasons categorized as "other", two patients were withdrawn for deteriorating physical condition and four patients were withdrawn at the investigators' discretion for miscellaneous reasons.
>
> On the PLA arm 28% of patients were withdrawn because of disease progression, 44% were withdrawn because of cardiac events, and 12% had non-cardiac adverse events. In addition one patient each (4%) was withdrawn for the following reasons: refusal to continue on study, protocol violation, death, and "other". Of the 11 patients (44%) who were withdrawn because of a cardiac event, two patients were

withdrawn because of CHF (Patient No. 4201) or CHF in the presence of a low LVEF (Patient No. 5201). Five patients were withdrawn because of a low LVEF (<40 in two patients) while an additional four patients were withdrawn because of a low LVEF and subsequently developed CHF. In these four patients, three patients experienced further decline in LVEF (to <40%) after removal from the study prior to the development of CHF. The non-cardiac adverse events were related to myelosuppression in two patients and the third patient was withdrawn because of severe stomatitis. The protocol violation that resulted in withdrawal consisted of failure to administer the study drugs in accordance with protocol specifications. The cause of death in Patient No. 26201 was pneumonia which had commenced while the patient was leukopenic. This event is considered to be possibly related to the study medication. The patient withdrawn for "other" reasons had developed partial continuous epilepsy considered to be unrelated to the study drug."

(20:1) 8.3.4.8 **Toxicity**

Clinical:

At course one, clinical toxicities (compared in Table 12 A, p2173-2180) were not significantly different except that alopecia was more common on the placebo arm (84% vs 56%, p<0.05). Most severe grade of toxicities over all courses were compared in table 14A (pp 2188-2195). Neurotoxicity was more common in this study (48 to 56%) due to the use of vincristine in both arms. Although not statistically significant, sepsis and infection occured more frequently on the ADR-529 arm (sepsis 36% vs 24%, and infection 36% vs 20%). Adverse experiences are compared in tables 16A and 17A, pp 2202 to 2222). The listings are very similar except for 8 reports of anorexia on ADR-529 vs 3 on placebo (first course) and 3 reports of chest pain on ADR-529 versus none on placebo(all courses).

Laboratory:

Alkaline phosphatase, bilirubin, SGOT, LDH, and creatinine were comparable both at course 3 and over all courses (except for a higher median alkaline phosphatase at course 3 in the placebo arm, 115 vs 85, p=0.023).

There were insufficient data for a valid analysis of nadir counts since these were not systematically done for the 20:1 patients. Day 22 blood counts were similar on the 2 arms as shown in tables on pp 2261 to 2271.

(20:1) 8.3.4.9 Dosing intensity

Extent of on-study dosing is displayed in attached table 29A. None of the differences approach significance as demonstrated by the WRS test. Median dose per cycle is presented in table 30 A (attached). Deviations from dosing is displayed in table 31A. The patterns of reductions are similar as is dose intensity. The sponsor emphasizes that 5 patients on the ADR-529 arm versus one patient on the placebo arm had more than 5 dose reductions, however I suspect this is due to more patients being onstudy for prolonged therapy on the ADR-529 arm.

(10:1) 8.3.4 10:1 Drug Ratio Results

(10:1) 8.3.4.1 Investigators

See table 1B. 155 patients were entered at 52 institutions, ranging from 1 to 13 patients per institution.

(10:1) 8.3.4.2 Baseline characteristics

As compared in table 2 B (p 2150) the arms are comparable for age (median of 66 years on each arm), sex (70% male on ADR-529 versus 62% on placebo) and race (90%-95% white). Cardiac risk factors are compared in attached table 3B were similar on the 2 arms for mediastinal radiation, diabetes age greater than 65 years (51%), and history of heart disease. There were more with LVEF ≤ 10% above institutional lower limit on the ADR-529 arm (42% vs 29%, p=0.09)

Baseline disease status is compared in table 4B (p 2153). Time from diagnosis, # of disease sites, PS, prior therapy, and baseline blood counts were similar.

Reviewer's Comments:
These may not be optimal for assessment of extensive stage small cell cancer. An analysis to include sex, age, PS, and LDH was

requested.

(10:1) 8.3.4.3 Data Sets Analyzed

These are summarized in an attached table from p 02106. The number undergoing LVEF analysis at each dose are imbalanced in numbers; more in the placebo arm at 150 mg/M^2 and at 300 mg/M^2 , balanced at 400 mg/M^2, with more patients on ADR-529 at 500 mg/M^2 (12 vs 6). In terms of response analysis, one notes that 6 patients are missing from each arm even in the intent to treat analysis (There were 5 patients on each arm without measurable disease). In the evaluable patients analysis, only 43/67 on the ADR-529 arm are evaluable.

Reviewer's Comments:
With only 64% of the patients with measurable disease considered to be evaluable for response on the ADR-529 arm, one would have to be very suspicious of an analysis of response which excluded "inevaluble" patients.

(10:1) 8.3.4.4 Cardiac effects

Time to cardiac event data is summarized in attached table 5B and Figure 1B. There were 9 events on the ADR-529 arm and 24 on the placebo arm with separation of the time to cardiotoxic event curves beginning at 150 mg/M^2 but only definitively beginning at 400 gm/M2 (p = 0.029 by log rank). 7 CHF events occured onstudy, 5 on the placebo arm and 2 on the ADR-529 arm. These are not statistically different (see table 6B and figure 2B).

I have characterized the LVEF events in the following table:

ADR-529 arm		Placebo arm	
50	43	72	49
55	48	70	43
47	35	63	44
83	49	48	40
59	47	58	46
52	45	54	37
60	48	54	43
90	70	62	49
		57	43
		57	47
		72	48
		89	65
		60	49
		76	48
		72	49
		73	44
		77	43
		50	37
		84	36
		50	44
		52	45
		62	45

If one tries to define LVEF changes that are of more unquestionable significance, ie both a change of 10% and a final LVEF of less than 50%, one finds 4 such events on the ADR-529 arm compared to 17 on the placebo arm. This is even more suggestive of a cardioprotective effect than the 8 versus 22 LVEF events.

Change in LVEF for onstudy patients at various doses of doxorubicin is shown in table 7B. The magnitude of the difference between the arms of the mean was 5.6%. This analysis only involved 18 total patients. At 400 mg/M^2 of doxorubicin the difference in mean differences from baseline was 7.0% ($p = 0.028$, analysis involving 32 patients).

Again the sponsor did another analysis of 10:1 patients with CHF on or off-study and presents the results (see attached table 6.4.2.2).There are 8 versus 3 events listed. The table does a good job of displaying these events. Only 2 of these events on

the placebo arm occur at a reasonable dose of doxorubicin and do not have some sort of caveat (mediastinal RT, post MI, non-clinical findings).

(10:1) 8.3.4.5 Antitumor Efficacy

Response results are presented in attached table 8B. 59%% responded in the placebo arm compared to 45% in the ADR-529 arm ($p = 0.09$). There were 9% CRs in the ADR-529 arm versus 13% on the placebo arm. When the "inevaluable" patients, 24/67 on the ADR-529 arm and 14/76 on the placebo arm are excluded, the response rates become similar (67% and 68%).

Reviewer's Comments:
By the usual analysis, 95% ci cannot exclude a difference in response rates of 30%, whereas the evaluable patients analysis cannot exclude a response rate of 19%. Clearly one cannot exclude the possibility that ADR-529 might cause a large decrement in response rates. From a patient benefit standpoint, the intent-to-treat analysis identifies how many patients who started the study had documentation of the benefit we designate as "response." Whether ADR-529 causes inhibition of antitumor effect in small cell cancer seems to be hopelessly obscured by insufficient power and poor data quality.

The reasons for going off-study without response assessment are given in attached table 6.5.1.2. The categories of adverse experience (3), protocol violation (4), and "other" (3) account for an excess of 10 patients who did not have tumor measurement. These patients are described in the study appendix.

The narrative describing the categories of inevaluable patients is located on pp 2114-2115. The following tabulation is taken from that narrative:

PLACEBO ARM

14 were considered to be inevaluable for antitumor response:

-seven patients received only one course of therapy for the following reasons:

death	4
cardiac event	2
non-cardiac toxicity	1

-four patients received only *two courses* and were withdrawn for the following reasons:

progressive disease	2
investigator choice	1
cardiac toxicity	1

-One patient received three courses of therapy but the disease was not reassessed.

- Two patients received more than three courses of therapy but were considered inevaluable as one patient had limited rather than extensive disease and one patient had radiotherapy to the only measurable site of disease.

(Appendix VIII has individual patient information).

24 patients were considered to be inevaluable for the following reasons:

ADR529 ARM

-Two patients did not receive study drugs

-Twelve (12) patients received only one cycle of therapy:

ineligible	2
death	5
adverse event	1
disease progression	2
investigator error	1
protocol violation	1

-Eight patients received only two courses of therapy:

Progressive disease	2
ineligible	1
poor general status	1
adverse event	1
refused further therapy	1
death	1
withdrawn	1

-Two patients had more than three courses of therapy:

had limited disease	1
incomplete evaluation	1

Reviewer's Comments:
It is difficult to understand how one could consider 6 patients with progressive disease to be inevaluable (4 on D and 2 on P).

Review of overview tabulations shows that PR's in the placebo arm were documented at 2-4 months:

1
2 XXXXXXXX
3 XX
4 XX
5 X

CR's were documented at 8, 5, 4, and 3 months.

Although the analysis was not submitted, the sponsor notes that on the average time to response was 65 to 66 days (the CR's alter this statistic from the one I am interested in, ie time to first response).
So it is likely that if dose intensity made a difference in response rates, it would be dose given before the 4th course of therapy, or dose reductions of the second and third courses. So, the main impact of dose reduction on response would need to be from dose reductions of from courses #2 and #3.

Time to disease progression is presented in attached table 9B and figure 3B. There were 51 and 59 events on the ADR-529 and placebo arm respectively. The hazard ratio was P:D was 1.02 (95% ci 0.70 to 1.50). Disease free survival is shown in attached table 10B and figure 4B and demonstrates similar results.

(10:1) 8.3.4.6 Survival

Attached table 11B and Figure 5B summarize survival. With 60% and 66% having died, the hazard ratio P:D was 0.97 (95% ci 0.65 to 1.46) with p=0.75 by LR. The smaller p value by the Wilcoxon test (p=0.15) reflects a trend toward higher earlier mortality on the ADR-529 arm shown in Figure 5B. Again the small size of the study limits conclusions.

(10:1) 8.3.4.7 Patient disposition

Reasons for going offstudy and time to offstudy analyses are demonstrated in attached tables 33B, 34B, 35B, and Figure 9B.

Table 33B demonstrates that the imbalance in offstudy for cardiac toxicty (24% on placebo vs 11% on ADR-529) is balanced by more non-cardiac adverse reactions (7% vs 13%), patient refusal, and protocol violation. Table 34B shows that the offstudy events for cardiac reasons occured surprisingly early (an imbalance of 2 off ADR-529 versus 9 off placebo after 150 mg/M^2).

Reviewer's Comments:

This seems counter-intuitive. However, it may relate to the use of doxorubicin in older patients compared to the breast cancer trials.

The Wilcoxon p value (0.14) reflects a higher off-study hazard early (prior to 150 days) for the ADR-529 arm.

Because so many of the ADR-529 arm went off-study for atypical reasons, I have put in tabular form the sponsor's comments from the text on pp 2130-2131:

69 DZR patients who were off-study:

38% (26) were withdrawn because of disease progression,

11% (8) had cardiac events,

13% (9) were withdrawn because of a non-cardiac adverse event

related to myelosuppression : 4	
nausea and vomiting	3
vincristine neurotoxicity	1
adverse experience(?brain met)	1 (Patient No. 65103)

10% (7) refused further therapy

9% (6) were withdrawn because of a protocol violation

incorrect study drug	3
ineligible	3

9% (6) expired on-study

possible MI	1
sepsis	2
infection	2
sepsis and PE	1

10% (7) other were withdrawn because of reasons categorized as

"other".

ineligible (retrospective)	2
to receive alternate therapy	2
deterioration	2
cardiotoxicity (criteria not fulfilled)	1

Reviewer's Comments:
Examination of tables 34B and 7B again raises questions.

	# onstudy at 8	*# onstudy at 9*	*# LVEF at 400mg/M²*
D arm	*10*	*10*	*7*
P arm	*9*	*5*	*9*

Again it is unclear why less onstudy patients had LVEF reported in the cardiac analysis on the D arm at 400mg/M² (70%) than on the P arm (100%).

(10:1) 8.3.4.8 Toxicity

Clinical:

At course one, clinical toxicities (compared in Table 12B and 13B, p2174) were not significantly different except that again alopecia was more common on the placebo arm (75% vs 59%, $p<0.05$). Mean severity scores for the first course were higher in the ADR-529 arm for diarrhea (1.9 vs 1.1 with overal incidence of 10% in each arm) and for sepsis (3.6 vs 3.0) with overall incidence of 13% in ADR-529 arm vs 9% in the placebo arm. 5 patients had grade 2 or 3 diarrhea in the ADR-529 arm versus none in the placebo arm. 5 patients had grade 4 sepsis in the ADR-529 arm versus none in the placebo arm.

Most severe grade of toxicities over all courses were compared in tables 14B and 15B (pp 2189-2201). Fever occurred in a similar number of patients (35%) in each arm, but the severity score was significantly higher (2.2 vs 1.8) on the ADR-529 arm. Sepsis occurred in 23% on the ADR-529 arm and in 15% on the placebo arm, and the mean sepsis score was significantly higher on the ADR-529 arm (3.4 vs 3.1). Again, neurotoxicity was more common in this study (49% to 55%) due to the use of vincristine in both arms but there was no significant difference on between the arms.

Esophagitis, infrequent on both arms, occurred less frequently on the ADR-529 arm (1% vs 9%, see attached excerpt from table 15B). Streaking and erythema (7 patients versus 2 patients) and phlebitis (4 patients versus 1 patient) were non-significantly increased on the ADR-529 arm.

Adverse experiences are compared in tables 16B and 17B, pp 2207 to 2234). The listings are very similar for the 2 arms.

Laboratory:

Alkaline phosphatase, bilirubin, SGOT, LDH, and creatinine were comparable both at course 3 and over all courses.

Analyses of nadir counts are presented in tables 23B-27B, pp 22052 to 2270. Nadir median granulocyte counts were similar for course 1 (about 600, p=0.67) and were lower on both arms for the "all courses" analysis, trending lower on the ADR-529 arm (median 200 versus 300, p=0.08). Although not significantly different. Grade IV granulocytopenia (nadirs under 500) was slightly more on the ADR-529 arm (course 1: 48% vs 43%, all courses: 76% vs 66%). Nadir platelet counts were also lower on the ADR-529 arm (course 1: median 189 vs 240, p=0.011; all courses median 136 vs 162, p=0.19). The differences were of questionable significance, with very few on either arm having a platelet count under 25,000 (course 1: 5% vs 1%, all courses: 6% vs 1%). Again, day 22 platelet counts were significantly higher on the ADR-529 arm (course 1:median 490 vs 400, p=0.003; all courses: median 360 vs 294, p =0/06). Hemoglobin values were similar on the 2 arms.

Conclusions regarding toxicity, 10:1:

Toxicities were very similar on the 2 arms. Alopecia and esophagitis were less common on the ADR-529 arm, suggesting a protective effect. Myelosuppression and sepsis were more common on the ADR-529 arm.

(10:1) 8.3.4.9 Dosing intensity

3 attached tables explore dose of chemotherapy delived on each arm. Table 29B compares median # of courses and doses given. There is no significant difference. Table 31B compares deviations from intended dosing schedule. 71% on ADR-529 versus 61% on placebo had no dose reductions. At least 5 dose reductions

occured more often on the placebo arm(15% versus 5%). Dose intensity appeared similar except for a slight decrease in cytoxan dose intensity on the placebo arm.

Reviewer's Comments:
There is no hint of a lower dose intensity on the ADR-529 arm that might explain the trend toward lower response rates.

8.3.5 Discussion and conclusions

8.3.5.1 Sponsor's discussion:

Statistical issues:

There is a discussion on pp 02133-02135 with a lot kinds of estimation jargon that I don't understand. I agree that one shouldn't get to caught up in it for this trial, since its data was not the primary data leading to the stopping decision.

From discussion:
I have summarized the sponsor's discussion of this study and have inserted some quotes from it.

> The sponsor discusses the role of CAV in small cell carcinoma. A 1977 review by Broder is cited listing the response rate of smallcell cancer to single agent doxorubicin as 28% in 58 patients gathered from several series. A series of 45 patients with small cell carcinoma is cited Holoye with a 64% response rate, and 40% complete response. A review by Seifter and Ihde is quoted which states that CAV is standard therapy and that, although there is no consensus on duration of therapy, 4-6 months of therapy for responders is customary. The sponsor cites literature stating that cumulative doses of doxorubicin greater than 550 mg/M^2 are associated with a > 30% incidence of CHF.
>
> In the combined analyses (20:1 and 10:1) the sponsor notes that there wre 12 cases of documented CHF on the placebo arm versus none on the 20:1 arm of DZR and 2 on the 10:1 arm of DZR:
>
> "OF THE 12 CASES OF DEFINITE CHF, SEVEN PATIENTS HAD AN LVEF BELOW 40% AND ONE OF THE PATIENTS EXPIRED OF CONGESTIVE HEART FAILURE AFTER

RECEIVING A CUMULATIVE DOSE OF DOXORUBICIN OF 850 MG/M^2"

The difference in mean LVEF decrease was statistically significant as early as 150 mg/M^2 of doxorubicin at the 10:1 ratio and 300 mg/M^2 of doxorubicin at the 20:1 ratio.

No statistically significant differences in response rates were noted. The intent-to-treat analysis was thought to be misleading due to a high dropout rate for a variety of reasons. When evaluable patients are considered, response rates are almost equal. Although median valuefor survival was slightly shorter on the ADR-529 arm, the difference was not significant; and survival may not solely reflect study drugs since cff study therapy was not standardized.

Sponsor's discussion of dropouts:

"IN THIS STUDY THERE WAS A DISPROPORTIONATELY HIGHER NUMBER OF DROPOUTS ON THE DZR ARM AT THE 10:1 RATIO DURING THE FIRST THREE COURSES OF THERAPY. THE DROPOUTS OCCURRED FOR A VARIETY OF REASONS INCLUDING PATIENTS WHO WERE RANDOMIZED AND NOT TREATED, PROTOCOL VIOLATIONS, AND INVESTIGATOR DECISION. THIS MAY HAVE CONTRIBUTED TO THE FACT THAT THE MEDIAN NUMBER OF TREATMENT COURSES ADMINISTERED WAS LOWER ON THE DZR THAN ON THE CONTROL ARM. HOWEVER, A HIGHER PERCENTAGE OF THOSE PATIENTS WHO REMAINED ON-STUDY FOR AT LEAST THREE COURSES (14/50, 28% ON DZR; 10/71, 14% ON PLA) WERE ABLE TO REMAIN ON-STUDY FOR ≥TEN COURSES."

The sponsor's discussion of toxicity:

"AS WAS NOTED PREVIOUSLY, AT THE 20:1 RATIO THERE WERE MORE EARLY DEATHS ON THE DZR THAN ON THE CONTROL ARM, BUT AT THE 10:1 RATIO THE INCIDENCE OF DEATHS ON STUDY WAS SIMILAR ON THE TWO ARMS. "

"THERE WERE NO MAJOR STATISTICALLY SIGNIFICANT AND CLINICALLY RELEVANT DIFFERENCES BETWEEN THE TREATMENTS WITH RESPECT TO LABORATORY OR CLINICAL TOXICITIES WITH THE EXCEPTION OF AN INCREASED SEVERITY OF SEPSIS AND FEVER IN DZR PATIENTS AT THE 10:1 BUT NOT THE 20:1 RATIO AND A DECREASED NADIR AND RECOVERY PLATELET COUNTS ON THE DZR ARM AT THE 10:1 RATIO."

The sponsor's concluding discussion:

"THIS STUDY CONFIRMS THE DATA BY SPEYER, ET AL. THAT THE ADDITION OF DZR TO A CHEMOTHERAPEUTIC REGIMEN WHICH INCLUDES DOXORUBICIN RESULTS IN A LOWER INCIDENCE OF CARDIAC TOXICITY THAN IS OBSERVED IN THE CONTROL GROUP OF PATIENTS. ANALYSES OF THE SAFETY DATA DID NOT REVEAL MARKED DIFFERENCE BETWEEN THE TREATMENT ARMS IN THE INCIDENCE OR SEVERITY OF CLINICAL OR LABORATORY PARAMETERS. HOWEVER, THE NADIR PLATELET AND GRANULOCYTE COUNTS WERE LOWER ON THE DZR THAN THE CONTROL ARM AND PATIENTS ON THE DZR ARM EXPERIENCED A GREATER SEVERITY OF SEPTIC EPISODES. THE ASSESSMENT OF THE EFFECT OF DZR ON THE ANTITUMOR ACTIVITY IS SOMEWHAT OBSCURED BY THE HIGH DROPOUT RATE PRIOR TO COURSE 3 RESULTING IN A HIGH INEVALUABILITY RATE AND RELATIVELY LOW RESPONSE RATE IN THE INTENT-TO-TREAT ANALYSIS OF PATIENTS RECEIVING THE 10:1 RATIO. HOWEVER, AMONG EVALUABLE PATIENTS, THE RESPONSE (CR+PR) RATES WERE SIMILAR AT THE 10:1 RATIO AND THE CR RATE WAS HIGHER AT THE 20:1 RATIO IN DZR THAN CONTROL PATIENTS. AS THIS LATTER MEASURE IS CONSIDERED TO BE THE MOST USEFUL FOR ASSESSING CLINICAL UTILITY IN PATIENTS WITH SMALL CELL LUNG CANCER, IT SEEMS REASONABLE TO CONCLUDE THAT THE ANTITUMOR EFFICACY OF THE CAV REGIMEN IS NOT SIGNIFICANTLY IMPAIRED BY THE ADDITION OF DZR.

PATIENT ENTRY IN THIS TRIAL WAS PREMATURELY STOPPED. THE CONDUCT OF MULTIPLE ANALYSES FOR THE 10:1 PATIENTS HAD MINIMAL EFFECT ON THE CONCLUSIONS AS STATED. HOWEVER, EARLY STOPPING RESULTED IN AN INCREASED TYPE II ERROR FOR BOTH 20:1 AND 10:1 PATIENTS, RENDING APPARENT TREATMENT BENEFITS LESS RELIABLE THAN EXPECTED.

8.3.5.2 Sponsor's conclusions

The sponsor's conclusions in their entirety:

"UNDER THE CONDITIONS OF THIS STUDY AND IN THIS PATIENT POPULATION THE ADDITION OF DZR TO THE CAV REGIMEN EXERTED A CARDIOPROTECTIVE EFFECT WHEN COMPARED TO THE CONTROL GROUP. THERE WERE STATISTICALLY SIGNIFICANT DIFFERENCES IN FAVOR OF THE DZR GROUP IN THE TIME TO CARDIOTOXIC EVENTS AND THE MEAN AND MEDIAN DECLINES FROM BASELINE IN THE LEFT VENTRICULAR EJECTION FRACTION. THERE WAS A HIGHER INCIDENCE OF CONGESTIVE HEART FAILURE IN THE CONTROL GROUP THAN IN PATIENTS RECEIVING DZR BUT DUE TO THE SMALL NUMBER OF EVENTS, THIS DIFFERENCE WAS NOT STATISTICALLY SIGNIFICANT. CARDIOPROTECTION WAS EVIDENT AT BOTH THE 20:1 AND 10:1 RATIOS OF DZR TO DOX.

THE ADDITION OF DZR TO CAV DID NOT RESULT IN STATISTICALLY

SIGNIFICANT DIFFERENCES IN MEASUREMENTS OF ANTITUMOR EFFICACY INCLUDING RESPONSE RATES, TIME TO PROGRESSION, DISEASE FREE SURVIVAL AND OVERALL SURVIVAL. THERE WAS A 14% LOWER RESPONSE RATE IN DZR THAN IN PLA TREATED PATIENTS AT THE 10:1 RATIO IN THE INTENT-TO-TREAT ANALYSIS. THIS DIFFERENCE WAS OF BORLERLINE STATISTICAL SIGNIFICANCE. THE RESPONSE RATE IN THE EVALUABLE PATIENT COHORT AT THIS DOSE RATIO WAS ALMOST IDENTICAL ON THE TWO TREATMENT ARMS (67% AND 68% FOR DZR AND PLA, RESPECTIVELY).

THE ADDITION OF DZR TO CAV DID NOT MARKEDLY ALTER THE SAFETY OF THE REGIMEN. STATISTICAL ANALYSES REVEALED SOME DIFFERENCES BETWEEN THE TREATMENT ARMS THAT WERE NOT CONSIDERED TO BE CLINICALLY RELEVANT, EXCEPT FOR A DECREASE IN THE MEDIAN nADIR PLATELET AND GRANULOCYTE COUNTS ON THE DZR ARM AND AN INCREASE IN THE SEVERITY OF SEPSIS IN DZR PATIENTS EXPERIENCING THIS TOXICITY. AT THE 10:1 RATIO THE INCIDENCE OF DEATHS ON-STUDY WAS SIMILAR FOR THE TWO TREATMENT ARMS."

8.3.5.3 Reviewer summary and conclusions, 20:1 data.

88002, a double-blind multicenter randomized comparison of CAV with placebo or ADR-529 in patients with extensive stage small cell lung cancer with measurable disease, was begun in November of 1987 using a 20:1 ratio of ADR-529 to doxorubicin. Accrual for the purposes of the 20:1 ratio was halted in November of 1988 when, due to an excess of deaths associated with myelosuppression on the ADR-529 arm of this study and the ADR529 arm of the concurrent study in breast cancer. Patients on 20:1 ADR-529 were switched at this time to the 10:1 ratio. The data presented as "20:1" data in this trial come from patients who, depending on time of entry and offstudy time, received varying amounts of 20:1 ADR-529 and 10:1 ADR-529. Although it is tempting to draw conclusions about the relative safety and efficacy of the 20:1 ratio to the 10:1 ratio based on this trial, it is important to remember that the data base is small and admixed with 10:1 data; moreover some of the toxicity detected initially leading to a dose ratio change may appear less after admixture of 10:1 data.

Differences in design of the initial aborted (20:1) trial from the later (10:1) trial included exclusion of non-measurable disease patients, lack of a requirement for weekly CBC monitoring, and no requirement for repeat LVEF when going offstudy for LVEF change.

The data cutoff date was set for March 1991 in parallel with the other studies, so the data is mature. A multivariate analysis specific for small cell carcinoma has been requested.

Key efficacy findings extracted from ADRIA's NDA analyses are presented in the following table :

Tabular summary of findings study 88002, 20:1 RATIO

Disease	Small cell lung cancer			
Ratio of DZR	20:1 (at patient entry)			
	DZR		**PLA**	
# randomized				
total	26		25	
measurable	26		25	
Cardiac Effect				
Time to "event" (13 events)	P:D	2.26	(0.67, 7.6)	
LVEF decrease (mean difference, change from baseline, D-P)				
Doxorubicin dose	**Change from baseline (D minusP)**			
500 mg/M² (D7 , P3)[1]	12.1		p=0.04	
550 mg/M² (D3 , P1)	3.3		---	
	DZR		**PLA**	
CHF events	0		1 (plus 6 offstudy)	
Tumor Effect				
	DZR		**PLA**	**95%ci**
Response (51 pts)	15/26 (58%)		17/25 (68%)	(-36%, 18%)
Progression (44 events)	P:D	0.91		(0.50,1.66)
Survival (48 deaths)	P:D	0.91		(0.52,1.61)
Offstudy (51 events)	P:D	1.09		

[1]Numbers in parentheses represent number of patients analyzed.

51 patients were randomized at the 20:1 ratio. The difference in LVEF drop was 12.1% at the protocol specified endpoint of 500 mg/M^2 of doxorubicin in an analysis only including 10 patients (p=0.04). There was 1 onstudy CHF event reported on the placebo arm and 6 offstudy. The analysis of offstudy CHF was not done in the other ADRIA-sponsored studies and the quality of the data is uncertain. The retrospective time to cardiac event analysis based on 13 events favors ADR-529, but was not statistically significant.

The response data base includes 51 patients. The response rates, which likely reflect primarily 20:1 effect, are 58% for ADR-529 and 68% for placebo with large confidence intervals. Progression and survival which likely reflect effects of 20:1 and 10:1 ratios, both lean slightly toward ADR-529 (0.91) with wide confidence intervals.

There was a tendency to go offstudy earlier on the ADR529 arm for many reasons, including more deaths and adverse reactions.

Toxicities were similar except for significantly more alopecia in course one on the placebo arm and a non-significant increase in sepsis was seen on the ADR-529 arm (36% vs 24%). Nadir counts were not done so myelosuppression was not directly assessed.

88002 Reviewer conclusions regarding 20:1 data

The trial provides some non-definitive data that the 20:1 ratio of ADR-529 significantly lessens the LVEF decrease detected with repeated courses of CAV chemotherapy. An increase in sepsis and death early in the study led to a dose alteration. An decrease in alopecia in course one, observed in at least one other study is of note. Data on response, progression, and survival have limited power and include a mixture of 20:1 and 10:1 data. Early dropouts were more frequent on the ADR-529 arm.

8.3.5.3 Reviewer summary and conclusions, 10:1 data.

Historical background

See the same section of the 88001 trial for details of ADRIA-FDA interactions leading to the submission of the 10:1 data in this NDA. As with the breast cancer trial, the previous 20:1 study was basically restarted with the 10:1 ratio using the same protocol in early 1989. Changes in the new protocol included exclusion of patients with performance status 2, inclusion of a non-measurable disease stratum, inclusion of an interim analysis plan and data monitoring committee, incorporation of weekly monitoring of blood counts, and requirement for repeat LVEF measurement in patients going offstudy for LVEF changes.

At the 8th meeting of the committee on November 5, 1990, the committee made the following recommendation regarding the lung trial:

> "The lung study (002) should be stopped. The accruals are quite slow (CAV is not the regimen of choice for lung cancer anymore) and the response rates are comparable between arms A and B. The hearts in lung cancer patients are not different than those in breast cancer patients; if ADR-529 shows cardioprotection in breast cancer patients, it should have the potential to confer the same benefit regardless of tumor type. The FDA request for another tumor type should be answered by comparable response rates, to date, in the lung study."

The study was closed to accrual soon thereafter. A data cutoff date of 3-31-91 was set for NDA submission.

Key efficacy findings extracted from analyses by ADRIA are presented in the following table :

Tabular summary of findings study 88002, 10:1 RATIO

Disease	Small cell lung cancer		
Ratio of DZR	10:1		
	DZR	**PLA**	
# randomized			
total	73	82	
measurable	67	76	
	Cardiac Effect		
Time to "event" (33 events)	P:D 2.28		(1.06, 4.94)

LVEF decrease (mean difference, change from baseline, D-P)

Doxorubicin dose	**Change from baseline (D minus P)**	
500 mg/M² (D12, P6)[1]	5.6	p=0.24
550 mg/M² (D8, P4)	2.0	p=0.61

	DZR	**PLA**
CHF events	2 (+ 1 offstudy)	5 (+ 3 offstudy)

Tumor Effect

	DZR	**PLA**	**95%ci**
Response (143 pts)	30/67 (45%)	45/76 (59%)	(-30%, 2%) p=0.09
Progression (121 events)	P:D 1.02		(0.70,1.50)
Survival (97 deaths)	P:D 0.97		(0.65,1.46)
Offstudy (124vents)	P:D 0.96		

[1]Numbers in parentheses represent number of patients analyzed.

51 patients were randomized at the 20:1 ratio. The difference in LVEF drop was 12.1% at the protocol specified endpoint of 500 mg/M^2 of doxorubicin in an analysis only including 10 patients ($p=0.04$). There was 1 onstudy CHF event reported on the placebo arm and 6 offstudy. The analysis of offstudy CHF was not done in the other ADRIA-sponsored studies and the quality of the data is uncertain. The retrospective time to cardiac event analysis based on 13 events favors ADR-529, but was not statistically significant.

The response data base includes 51 patients. The response rates, which likely reflect primarily 20:1 effect, are 58% for ADR-529 and 68% for placebo with large confidence intervals. Progression and survival which likely reflect effects of 20:1 and 10:1 ratios, both lean slightly toward ADR-529 (0.91) with wide confidence intervals.

There was a tendency to go offstudy earlier on the ADR529 arm for many reasons, including more deaths and adverse reactions.

Toxicities were similar except for significantly more alopecia in course one on the placebo arm and a non-significant increase in sepsis was seen on the ADR-529 arm (36% vs 24%). Nadir counts were not done so myelosuppression was not directly assessed.

88002 Reviewer conclusions regarding 20:1 data

The trial provides some non-definitive data that the 20:1 ratio of ADR-529 significantly lessens the LVEF decrease detected with repeated courses of CAV chemotherapy. An increase in sepsis and death early in the study led to a dose alteration. An decrease in alopecia in course one, observed in at least one other study is of note. Data on response, progression, and survival have limited power and include a mixture of 20:1 and 10:1 data. Early dropouts were more frequent on the ADR-529 arm.

*993

ADVANCED SMALL CELL LUNG CANCER TREATED WITH CAV (CYCLOPHOSPHAMIDE+ADRIAMYCIN®+VINCRISTINE) CHEMOTHERAPY AND THE CARDIOPROTECTIVE AGENT DEXRAZOXANE (ADR-529, ICRF-187, ZINECARD®). J.E. Feldmann, S.E. Jones, S.R. Weisberg, D.R. Gandara, G.H.Lyman, R.M. York, J.A. Mailliard, D.M. Hayes, B. Tranum, M.B. Spaulding, B.S. Shaikh, A. Khojasteh, T. Wajima, R.R. Rivera, N. Abramson, W.L. Horvath, K.B. Pendergrass, R.D. Reynolds, M. Gerber, J. Winston, K. Squillace, A. Kline, B. Swearengin, D. Hess, D. Scott, P. Banks, G. Jones, D. Jones, and R.A. Gams. Adria Laboratories Cooperative Study, Columbus OH.

In 1988 Speyer (*N Engl J Med* 319:745) reported the cardioprotective properties of the intracellular chelating agent Dexrazoxane (DZR) in patients with breast cancer treated with Adriamycin® based chemotherapy. They used DZR doses of 1000 mg/M^2 with an Adriamycin® dose of 50 mg/M^2. In this study, 155 patients with advanced small cell lung cancer were entered on the study and treated with 750 mg/M^2 cyclophosphamide + 50 mg/M^2 Adriamycin® + 2 mg vincristine I.V. every 3 weeks, with or without the blinded study drug given as a 500 mg/M^2 I.V. bolus within 30 minutes prior to the administration of Adriamycin®. There were 43 evaluable patients on the DZR arm and 62 on the control (C) arm. Median age was 66.0 years on each arm. There were 70% (DZR) vs 62% (C) males, 90% (DZR) vs 95% (C) whites and a median ECOG score of 1 on each arm. Median nadir WBC was 1.4 (DZR) vs 1.5 (C), median AGC was 0.2 (DZR) vs 0.3 (C) and median platelets 135.5 (DZR) vs 162.5 (C). Median drop in hemoglobin was 3.4 (DZR) vs 3.1 (C). Other toxicities associated with DZR therapy included pain on injection (7% DZR vs 4% C), phlebitis (6% DZR vs 1% C), sepsis (23% DZR vs 15% C) and infection (27% DZR vs 23% C). There were 6 (DZR) vs 5 (C) deaths. The response rates were 5 (12%) CR + 24 (56%) PR for CR + PR (67%) for DZR, and 8 (13%) CR + 34 PR (55%) for CR + PR (68%) for C. The median time to failure was 183 days for each arm. CHF was observed in 2 (DZR) vs 5 (C) cases. There were 9 (12%) cardiac events (DZR) vs 24 (29%) cardiac events (C) (p=0.029). Of 14 patients removed from study for cardiac events after Course 5 (250 mg/M^2 cumulative dose of Adriamycin®), 10 (71%) were on the control arm. MUGA/LVEF serial measurements showed a mean drop after 150 mg/M^2 (1.4 DZR vs 6.8 C, p=0.003), after 300 mg/M^2 (2.0 DZR vs 7.5 C, p=0.07), after 400 mg/M^2 (0.1 DZR vs 7.1 C, p=0.028) and after 600 mg/M^2 Adriamycin® (1.5 DZR vs 13.5 C, p=0.09). Dexrazoxane has now been shown in several studies to be cardioprotective against the myocardial damage produced by the chronic administration of Adriamycin® based cancer chemotherapy.

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A total of 155 patients entered the 10:1 portion of the study. 124 are are offstudy, 121 have progressed and 97 have died.

Only 18 patients lasted to the 500 mg/M^2 dose of doxorubicin for the LVEF endpoint. The difference in mean LVEF decrease from baseline was 5.6 at the protocol specified endpoint of 500 mg/M^2 of doxorubicin, p=0.24. There were 5 onstudy CHF events reported on the placebo arm and 2 on the ADR-529 arm. However at least 3 of these patients on the placebo arm had recovery of LVEF to the normal range, suggesting that severe myocardial impairment was not always permanent. Additional CHF events (3 on placebo and one on ADR-529) were noted offstudy; analysis of offstudy CHF was not presented in the ADRIA-sponsored studies in breast cancer. The retrospective time to cardiac event analysis favored ADR-529 (p=0.03). 9 of the placebo cardiac offstudy events had occured by 150 mg/M^2, certainly an unexpected finding.

Response in the intent to treat analysis favored the placebo arm (59% vs 45%) p = 0.09, with confidence intervals including a 30% worse outcome for ADR-529. The sponsor's presentation of the evaluable disease response rates includes only 64% of patients with measurable disease. Hazard ratios for progression and survival were near unity, with lower bound of confidence intervals of 0.70 and 0.65.

Alopecia and esophagitis were less common on the ADR-529 arm, suggesting a protective effect. Diarrhea, 10% in each arm, was more severe on ADR529 arm, Myelosuppression and sepsis were more common on the ADR-529 arm.

88001 Reviewer conclusions regarding 10:1 data

This study was stopped prematurely. It offers some evidence that the 10:1 ratio slows deterioration of LVEF, especially in the retrospective time to event analysis. There was a higher incidence of events classified as "CHF" on the placebo arm. The quality of the data on such events occuring offstudy is not clear. A large number of early dropouts occured for various reasons on the ADR-529 arm. Whether ADR-529 causes inhibition of antitumor effect in small cell cancer seems to be hopelessly obscured by insufficient power and poor data quality for response.

Again, hints in the toxicity data support the possibility that ADR529 might exhibit a protective effect on normal tissues other than the heart.

8.4 Trial #4, Clinical Study 88011 (20:1, breast cancer, NYU)

See attached report cover sheet from study report.

Location of reviewed material:

Medical Statistical report, v 76-77.

NYU case report forms and missing tabulations in May 11 submissions from ADRIA LABS and

Figures for the study were submitted in an April 30, 1992 submission.

This trial was performed at New York University. The study report was prepared for ADRIA LABS .

8.4.1 Objective:

1. To determine if ADR529 "protects against doxorubicin cardiomyopathy; as assessed by clinical examination changes in the left ventricular ejection fraction...and pathologically by endomyocardial biopsy."

2. To determine if ADR529 modifies the toxicity and efficacy of a doxorubicin containing chemotherapy regimen.

8.4.2 Design:

This was an open randomized two-arm study comparing FAC to FAC plus ADR529 at a 20:1 ratio.

The protocol schema from p 03901 is attached.

8.4.3 Protocol

8.4.3.1 Details in Protocol Amendments:

There were 3 amendments (July 20, 1984, Dec 14 1984, and Aug 28, 1986). The following changes are according to

-July 20, 1984 (These can really be considered as part of the original protocol since it was dated one day after randomization of the first patient.):

Study 88011

-Removed the originally specified day 8 5FU.

-Non-measurable patients were to be included (previously measurable and "evaluable")

-December 14, 1984 (Again, these changes were filed at a time when 10 patients had been randomized.)

-Specified criteria for cardiac evaluation (MUGA scan at 0, 300, 450, each 100 mg/M^2 and at offstudy) and biopsy criteria.

-Stated there would be no doxorubicin dose adjustment for myelosuppression.

-August 28, 1986 (Occurred after 65 patients had been randomized.):

-Gave more specific criteria for dose adjustment. Allowed for doxorubicin dose decrease if myelosuppression occurred after 2 dose reductions of other drugs and also for grade 2 stomatitis.

-it qualified the offstudy cardiac criterion for fall in LVEF 20%. It added: "plus inability to increase LVEF by >50% with exercise" as a requirement.

-CEA's were to be performed every 6 weeks.

In addition I was able to find the original protocol (dated January 1984) in Agency files to help clarify earlier design issues. Synetron apparently only had access to a protocol which had incorporated most of the changes.

Reviewer's Comments:
The first 2 amendments apply to most of the data, since they were filed early in accrual. The last amendment might cause lower doses of doxorubicin on the more myelosuppressive arm and will be examined in the analysis. The added cardiac offstudy criterion attempted to prevent patients with a 20% LVEF drop but with normal cardiac function from going off study.

8.4.3.2 Eligibility:

Eligibility criteria are listed on p 08-4046. Most pertinent eligibility criteria:

-Metastatic or unresectable breast cancer.
-Measurable or unmeasurable disease.
-PS 0,1,2 or 3.
-Adjuvant therapy OK, previous hormonal therapy OK, but no previous doxorubicin.
-RT OK if less than 3000 rads to 50% of pelvic bone structures and lower spine.
-Adequate bonemarrow, hepatic (bilirubin 3.0 mg% and SGOT less < 60 iu/ml) unless due to metastatic disease to liver) and renal (creatinine < 2.0 mg%).
-excluded for heart disease(MI in past year, uncontrolled angina, history of congestive heart failure unless full recovery documented, symptomatic valvular heart disease).

Reviewer's Comments:
Technically the protocol criteria do not exclude patients who received chemotherapy for metastatic disease. However, all literature reports indicate that the only chemotherapy treated patients were those who had received it as adjuvant therapy.

8.4.3.4 Randomization

Two strata were utilized:

Prior adjuvant chemotherapy (Y or N)

Presence or absence of cardiac risk factors (prior thoracic and breast radiation, age greater than 65, hypertension, diabetes)

Reviewer's Comments:
From the attached randomization schema and from literature sources, it appears blocks of 10 were to be used at each center in each stratum.

8.4.3.5 Procedure

Treatment Plan

See attached schema. FAC (500/500/50) was to be given every 21

days, each administered over 5 minutes. ADR529 was to be given, at 20:1 ratio, within 30 minutes prior to chemotherapy.

Dose modifications:

Weekly counts were checked for the first 2 courses and doses were reduced according to the attached schema. From December 1984 to August 1986 the doxorubicin and ADR529 doses were not adjusted for hematologic toxicity, whereas after August 1986 they were dose-reduced for hematologic toxicity persisting past the second dose reduction. If counts were depressed (WBC <4000) then treatment was delayed one week. The initial dose of doxorubicin was dose reduced on basis of SGOT (iu) and bilirubin (mg%) if the SGOT was greater than 60 (according to magnitude of SGOT elevation and bilirubin level see page 4049).

8.4.3.6 Efficacy criteria

Response assessment

-Complete response was disappearance of "all clinical evidence of active tumor and symptoms" at 2 visits at least one month apart.

-PR was 50% reduction in size of all measurable tumor areas:
-products of length and width of bidimensional lesions.
-30% reduction in unidimensionally measured lesions.
-decrease of 50% in evaluable disease.
"these changes must be present in more than 50% of the involved organ sites. "
-bone lesions demonstrating healing on x-ray were considered an improvement.
-Third space fluids must decrease by 50% on 2 sequential visits separated by 4 weeks.
-PR was to be documented by 2 visits 4 weeks apart.

"Improved" was a classification for bone lesions:
-Bone lesions that remain static for 8 weeks or longer were considered an improvement if :
-there was a decrease of 1 point in analgesia requirements or decrease to no analgesia requirements or an increase of ECOG PS or a return to 100% performance. This had to occur for 2

visits 1 month apart.

Reviewer's Comments:
The response criteria include some criteria that might be subject to bias in an open trial. Inclusion of unidimensional lesions and evaluable disease are nonstandard and of questionable reproducibility. It is unclear whether even unmeasurable patients could have responses, such as CR. The response analysis of interest would be that of patients with traditional bidimensional measurable disease, realizing the hazard of trying to identify groups retrospectively.

I don't understand the initial statement that shrinkage should occur in all tumor areas, then the later statement that the changes had to occur in 50% of the involved organ sites.

-Progressive disease included any of the following:

-25% increase in size of any measurable lesion
-Appearance of any "significant" new lesion
-Significant deterioration in symptoms and weight (>10% weight loss)
-Decrease in performance status (2 or more grades).

Reviewer's Comments:
The last 2 are non-standard and could be inappropriately applied.

Survival

The protocol did specify that date and cause of death were to be recorded in all cases even after the patient went offstudy.

Cardiac endpoints:

Cardiac dysfunction is defined as any of the following:

-"Clinical heart failure"

-LVEF on exercise or resting MUGA less than 45%.

-Resting LVEF falling a total of 20% over 2 consecutive courses of therapy.

Reviewer's Comments:
Although did not list clinical heart failure as a reason for coming off study prior to the 1986 amendment, I found that it was present in the January 1984 amendment available in IND files.

There are multiple problems with the definition: Clinical heart failure is not defined and is subject to bias in this unblinded study. LVEF was not in inclusion criteria (although the literature variably stated 0.45 and 0.50 as a criterion), so presumably patients could already have LVEF near 45%. A difference from the ADRIA protocols is that either a change in resting or exercise MUGA scans suffice for coming off study, and the studies did not need to be repeated. The clause in the initial description of LVEF needing to fall "over 2 consecutive courses" is vague.

Evaluation schedule

See attached schedule from protocol.

Response:

If CXR was source of evaluable or measurable disease, it was to be performed every 6 weeks. Liver scans (?nuclear med or CT?) were to be performed every 8 weeks if used as measurable disease (5 cm lesion). Measurement of indicator lesions was to occur at 3 and 6 weeks. It is unclear from the schema what tumor measurements were to continue past 6 weeks. Measurement of CEA was to be every 6 weeks. One literature report stated that measurements of tumor were to occur every 3 courses, but this is not recorded in the protocol. The investigator, Dr. Speyer, told the medical officer that this was the usual practice.
Reviewer's Comments:
The schema only outlines tests for 6 weeks with special notations regarding cardiac tests, CXR, and liver scans. No mention is given for monitoring bone lesions. Specifications seem clear enough for response evaluation, however it is unclear what studies were to be done in monitoring time to progression. It is unclear from the schema and protocol whether non-cardiac toxicity was recorded in a systematic fashion.

Cardiac evaluation:

Baseline studies: Cardiac history and PE, EKG, CXR, rest and exercise MUGA scan.
These were repeated at 300 mg/M^2, 450 mg/M^2, and 100 mg/M^2 thereafter.

Endomyocardial biopsy was to be done if the patient agreed at 450 mg/M^2.

When patients went offstudy for either cardiac dysfunction or tumor progression these were to be repeated.

Reviewer's Comments:
If one arm were to have patients progressing sooner, more LVEF scans would be done at an earlier time in that arm.

Removal from study:

Patients were to be removed from study for tumor progression or cardiac dysfunction. Duration of therapy was not indicated, but according to the investigator, it was continued until one of these events occurred. A literature report noted that after 1987, investigators were advised to discontinue therapy after 1000 mg/M^2 of doxorubicin because of the incidence of patient fatigue.

8.4.3.7 Statistical considerations

Statistics:

The study was to have 80% power to detect a decrease from 60% to 20% in the % of patients getting more than 400 mg/M^2 of doxorubicin who had more than a 10% decrease in LVEF using a 2-tailed test at p = 0.05. 132 patients, 66 on each arm, would supply the estimated 33 evaluable patients past 400 mg/M^2 required. Similarly it was felt that a drop from 80% to 40% in the number of patients having a Billingham score of 2.0 on biopsy.

"To assess the effectiveness of ICRF-187 as a cardioprotector, we will test for differences in the proportions of patients experiencing cardiotoxicity, whether defined as a fall in LVEF or

as cardiac pathological changes. Each endpoint of cardiotoxicity will be examined separately." Logistic regression was to be used to adjust for prognostic factors. The endpoint for pathology was to be a score of 2.0. Each patient was to have a mean score calculated and these were be compared by chi square testing. Survival and DFS were to be compared by "life-table analysis" and Cox regression was to be used to adjust for prognostic factors.

"Graphical methods and contingency-table analysis (using chi-square tests) will be used to examine the association of biopsy score and percent fall in LVEF in the study and control groups (together and separately). To do this percent fall in LVEF will be categorized appropriately, eg 0-5%, 6-15%, etc."

Reviewer's Comments:
I am a bit confused at what the statistical endpoint was supposed to be for cardiac toxicity. The offstudy criteria were a drop of 20% or a drop to less that 45% or clinical CHF. The sample size seems to have been designed to identify patients over 400 mg/M^2 who had a drop of at least 10%.

Interim monitoring:

The principal investigator analyzed the data after 92 patients were entered and reported the results. He added 18 patients after the final 132 without another analysis, with permission of to get more data on endomyocardial biopsy.

Information from Agency IND files:

I reviewed the IND files of the IND for information related to the NYU study. I found one copy of the protocol in volume 3 (stamped January 1984) but I could find no amendments. The statistical section reveals the most striking difference. Target size was 192 patients rather than 132. The change appears to have occurred by 1985 (I found a sample size of 132 in a copy of a protocol in division files, attached to an export request).

Information on protocol and study conduct from literature:

1988 literature report (NEJM, 319: p 745)

In reviewing the literature references, the 1988 report on 92

patients mentions specifically that only adjuvant chemotherapy was allowed. It also uses "evaluable or measurable" disease but doesn't define it. It mentions that baseline LVEF had to be at least 0.45. It mentions that blocks of 10 were used per stratum. It also claims that cardiologist, nuclear medicine physicians, and pathologists were blinded to treatment group. In conclusions, it is stated that the study was not designed to test whether the drug affected antitumor effect, "a much larger trial would be required." "A therapeutic advantage would be expected only in patients who received a dose of doxorubicin that was greater than the usual dose for stopping treatment, 450 mg/M^2. ...larger trials are required to test for improved survival."

1992 literature report (JCO, January 1992, p117)

The 1992 report includes all 150 patients. It states that patients had to have a LVEF of at least 0.50. It states that patients receiving 1000 mg/M^2 in CR were advised to stop. No mention is made of requiring evaluable disease. This article states tumor measurements were determined at baseline and every 3 treatment cycles thereafter."

Another quote from this article:

> "The lack of improved disease-free survival suggests that the antitumor benefit conferred by FDC may be most significant in early treatment cycles. If such is the case, long-term treatment of patients with stable disease may only add additional toxicity regardless of whether there is cardiac protection. We did observe chronic fatigue in some patients receiving chemotherapy for more than 1 year. Therefor, starting in January 1987, we offered patients with no evidence of disease at 1000 m the optiom of discontinuing therapy. Future trials might include a quality-of-life evaluation to assess formally the overall impact of ICRF-187 on patients receiving doxorubicin-containing therapy."

Conclusions on protocol design from several sources:

(Study report, protocol included in NDA, literature, and Agency IND file, and conversations of medical officer with Dr. Speyer of NYU).

The study seems to have been well designed to collect extensive cardiac data. Offstudy criteria for cardiac dysfunction changed slightly over time. Clinical CHF was there from the start. A "a resting LVEF on exercise or resting gated pool scan less than 45% or a resting LVEF that falls a total of 20 percentage points over 2 consecutive courses of therapy" was changed to "a fall in resting ejection fraction of 0.20 or greater AND the inability to raise more than 0.50 with exercise in the August 1986 amendment. At this time the caveat that if a patient was borderline (eg had LVEF drop of 0.20 and was incapable of exercise) then the adriamycin would be held for a course, and subsequent treatment decision would be made by a team. The clinical definition of cardiac dysfunction to go offstudy was not detailed in the protocol, and the study was not blinded, so this endpoint seems more open to bias. Timing and details of cardiac exams were specified. Entry criteria made no note of minimum required LVEF in protocols that I reviewed, though literature reports specified 0.45 and 0.50.

Some details of tumor evaluation ,however, were not well specified. Non-measurable disease was included, however definitions of measurable, evaluable, and non-measurable were not given. In the analysis I would recommend identifying patients with bidimensionally measurable disease only for a response estimate, realizing that the situation is less than ideal without prospective identification.

Monitoring for time to progression was not well detailed. Protocol schema gave no detail past 6 weeks. One literature source and the investigator stated that tumor measurements were to occur every three courses. An early amendment called for CEA every 6 weeks. It is clear that response and progression data will be of poorer quality than the cardiac data. An examination of the listings might give an idea of how regularly tumor measurements were made, but these were had not been submitted as of May 11.

An expansion of the sample size to 150 patients was reportedly allowed by though I don't have documentation of this. An interim analysis on the initial 92 patients was unplanned.

8.4.4 Results

Randomization occurred between July 1984 and August 1989. All but one patient is offstudy.

Patient distribution:

76 patients were randomized to ADR-529 and 74 to placebo. About one third of the patients were at treated at hospitals associated with and the rest were treated at
The distribution of patients at the various centers is listed in attached table 1.

Baseline characteristics:

As shown in table 2 (p 3945) the groups were comparable in age (median 58 year). Race was recorded in only about half of the patients. Of these there were 5-8% Black.

Baseline cardiac risk factors which are compared in attached table 3 include age, mediastinal radiation, history of heart disease, hypertension, diabetes and LVEF $\leq$ 60%. There was no significant difference in any of these in the 2 arms. Only 16-19% were > 65 years.

Baseline disease status factors which are compared in table 4 (pp 3947-3953) included time from diagnosis, no. of disease sites, performance status, incidence of prior therapies (surgery, radiotherapy, chemotherapy, immunotherapy, and hormonal therapy), dominant disease site (visceral, bone soft tissue), and baseline blood counts, liver enzymes, and renal tests. The only significant difference was a difference in distribution of BUN (mean of 16 on ADR-529 and mean of 14 on placebo, p = 0.01). Although the median time from diagnosis was longer for ADR-529 (1002 vs 688 days), this was not statistically different (LR p=0.52). 30% on each arm had received chemotherapy and 43-46% had received hormonal therapy.

Reviewer's Comments:
The difference in BUN distribution is of doubtful significance; creatinine values were not significantly different.

The following is data on measurability from this table:

	ADR-529	Control
Total Number	76	74
Measurable only	20(26%)	19(26%)
Evaluable only	28 (37%)	33 (45%)
Both	28 (37%)	22 (30%)
At least measurable:	48 (65%)	41 (55%)

Reviewer's Comments:
The protocol did not define measurability. As retrospectively defined only 55-65% had measurable disease. Even if validly defined retrospectively, this study does not present a great deal of data on response.

Time to Cardiac Event:

From page 3917:

"An analysis was performed whereby all patients were assessed until time off-study or of last LVEF measurement up to 30 days after off-study. The protocol called for a decrease of 20% in LVEF as clinically significant. To remain compatible with the Adria-sponsored studies, for this analysis we defined an "event" as a) the occurence congestive heart failure (CHF), b) a drop in LVEF of greater than or equal to 10% from baseline as long as it was below NYU's lower limit of normal (50%), c) a greater than or equal to 20% decline in LVEF from baseline (the criteria of the protocol) or d) a drop in the ejection fraction greater than or equal to 5% below the institution's lower limit (50%)."

Reviewer's Comments:
One must be a bit suspect of this endpoint for 2 reasons. First, this analysis was never specified in the protocol. LVEF changes were to be separately analyzed. Second, the definition of an event has been altered retrospectively. An LVEF < 45%, clinical CHF, or a drop by 20% were the protocol criteria. The new definition would exclude some patients initially included: those with LVEF < 45% but with a change < 10% from baseline, and

patients with 20% decline to a point that was above 50%.

The sponsor will be asked to do the protocol-specified analysis.

The data for time to cardiac event is presented in attached table 5 and figure. I note that the denominator has excluded patients without any follow-up LVEF, 11 of 76 on ADR-529 and 13 of 74 on placebo. The events reported here include LVEF changes and investigator designation as clinical CHF.

Reviewer's Comments:
In the other studies, it is my impression that ADRIA censored these patients, and reasons for censoring were stated. ADRIA will be asked to address this issue.

The hazard ratio demonstrated a significantly higher risk of a cardiac event, as defined by ADRIA LABS, on the ADR-529 arm: Hazard ration (P:D) was 5.07 (95% ci 2.5 to 10.3) with $p < 0.01$ by LR.

Time to CHF is delineated in attached table 6 and figure. There were 7 events in the placebo arm and 2 in the ADR-529 arm, or in 3% of patients versus 9% ($p = 0.02$ by LR). The denominator for this analysis includes all patients.

Reviewer's Comments:
It is noted in the toxicity section of the report that most of the reported CHF events were grade 1. One must remember that this clinical endpoint was not defined and the investigators were not blinded.

Changes in LVEF are displayed in attached table 7. The mean drops in LVEF from baseline were greater on the placebo arm (11.4 at 450 mg/M^2 and 15.0 at 550 mg/M^2). It is notable that between 300 mg/M^2 and 450 mg/M^2 of doxorubicin, the sample size decreased by 28 patients (60 to 32) in the ADR-529 arm and decreased by 14 patients (56 to 42) in the placebo arm. In the next increment, there was only a drop of 1 patient in the ADR-529 arm versus 29 in the placebo arm.

Reviewer's Comments:
This is a similar pattern of dropouts seen in several other studies.

The following presents a comparison of the number in these analyses with LVEF measurements at 450 mg/M^2 of doxorubicin with those onstudy at course 10 (should be receiving 500 mg/M^2 if not dose reduced) from offstudy data of table 34:

	ADR529	PLACEBO
# with LVEF measurement at 450 mg/M^2	32	42
# on study at course 10	46	32
# on study at course 9	50	42

Reviewer's Comments:
It could be that some of the patients at course 10 had not yet reached 450 mg/M^2 due to dose reductions, however, there are still 33 on the ADR529 arm out to the 13th course (should have received 600 mg/M^2 if not dose reduced). The sponsor will be asked to explain this discrepancy. As presented, the LVEF differences in patients remaining onstudy impressively favors the ADR-529 arm.

The following summarizes data on Billingham scores described in the text of the review (p 3928):

	ADR529	Placebo
# patients with biopsies	16	17
# with adequate biopsies	16	15
Mean score	0.41	1.0
Median score	0	1.1
Range	0,1	0,2.5

p=0.06 by WRS test

Reviewer's Comments:
The report states that these data were not "tabulated." The

report does a poor job of evaluating this analysis. Who was biopsied after how much doxorubicin, reasons for biopsy, etc. Obviously not many patients reached the protocol specified endpoint of Billingham score of 2.0.

Offstudy analyses:

These analyses are displayed in attached tables 33, 34, and figure.

From the summary table 33, one can see that most on both arms went offstudy for either progression and/or cardiac toxicity. Going offstudy for other reasons were 21/76 (28%) on ADR-529 arm and 16/74 (22%). From table 34 one can see that 5/6 of the PI decisions on the ADR-529 arm occurred at some point later than 15 cycles. Clearly most of the difference in the curve occurs after about 250 days (corresponding to treatments of over 400 mg/M^2 of doxorubicin). Most of the difference in offstudy for progression appears to be due to later events when few are still on the placebo arm.

The overall analysis in the figure "TIME TO OFFSTUDY-ANY REASON" shows an impressive delay (Hazard ratio P:A 1.97, p<0.001 by LR) on the ADR-529 arm.

Reviewer's Comments:

Despite this impressive result in time onstudy receiving therapy, no improvement in antitumor efficacy or survival was demonstrated.

Antitumor efficacy

Response

Data on response is summarized in attached table 8. The response rate in measurable disease patients was 59% (24/41) in the placebo arm and 50% (24/48) in the ADR-529 arm with 95% ci (-12%, 30%) [in table this is labeled as D minus P, but must be P minus D].

Reviewer's Comments:

The presentation of all randomized patients is a meaningless presentation since it contains non-measurable patients and all kinds of undefined responses. The subgroup of measurable patients

needs close examination.

I note a comment on p 03929: "Five patients on the control arm and seven patients on the experimental arm were considered to be not evaluable for efficacy. One patient (No.95) was retrospectively determined to have non-small cell carcinoma of the lung; and, all the rest either received only one or two courses of therapy, or had too few data available for evaluation."

Reviewer's Comments:
The proper analysis for response would include all randomized patients with bidimensionally measurable disease. I will request this analysis.

Time to Progression

2 presentations of the data for time to progression are given. Time to progression onstudy is presented in table 9. For this analysis censoring occurred when patients went offstudy for other reasons, such as cardiac toxicity.

Reviewer's Comments:
Given the much large number of patients censored for cardiac toxicity on the placebo arm, this causes a very uneven censoring pattern; and I question its validity.

The Hazard ratio is 1.06 with 95% ci P:D[0.66, 1.7] and p = 0.8 by LR.

The second presentation, time to progression, is given in table 9 and attached figure. Patients were censored if they had not progressed at last known followup.
Reviewer's Comments:
This would be the usual analysis. The sponsor raised an issue of questionable data quality of information on progression collected after going offstudy, and hence added the various onstudy efficacy analyses. Given the lack of detail about followup for this endpoint in the protocol, it is certainly possible that this data is of poor quality or that the analysis might be biased against ADR-529. This would occur if the reporting of progression offstudy was systematically delayed relative to the reporting of onstudy progression.

74% of the ADR-529 arm versus 65% of the control arm had events observed. On the ADR-529 arm 85% (48/56) of these had been observed onstudy. On the placebo arm 65% (32/48) of the events had been observed on study. The hazard ratio favored placebo (P:D 0.81, 95% ci [0.55, 1.20]) but this did not approach statistical significance.

Disease free survival

Similar types of analysis were done for time to progression or death, which I label disease free survival. The first analysis presented in table 9 and figure labeled "TIME TO DISEASE PROGRESSION OR DEATH ON STUDY" censors for other off-study reasons. For this analysis the hazard ratio P:D is 1.18 with 95% ci [0.75, 1.85] and p = 0.47 by LR.

The preferable analyses, assuming good data quality, does not censor for other offstudy reasons, and is presented in table 10A and attached figure. The Hazard ratio P:D is 0.93 with 95% ci [0.66, 1.31] and LR p=0.69.

Survival

One analysis of survival was done for "on study" deaths This demonstrates the absurdity of this approach since there were only 8 onstudy deaths (3 on ADR-529 and 5 on placebo).

The survival analysis with censoring at last known-alive date for patients not known to be dead is shown in attached table 11A and attached figure. 68% of the patients in ADR-529 arm and 77% in placebo arm had reported events. Hazard ratio P:D was 1.05 with 95% ci [0.72, 1.54] and p =0.80 by LR.

Reviewer's Comments:
There is no obvious trend in the survival data, with large confidence intervals.

Toxicity

Onstudy

Toxicity information was only collected while the patient was onstudy. The apparent practice of the investigator was only to

report positive toxicities.

The incidence of toxicities in course 1 are given in tables 12 and 12A (p3964). It is difficult to judge the completeness of data, since the largest entry for the denominator is 22, with most being less than 10. This indicates that most events were not entered unless positive, but does not indicate how thoroughly such events were entered. Incidents of events were similar. Several toxicity categories of interest are selected for display, number of events is given:

Diarrhea	10	2
Sepsis	3	1
Infection	7	10

The severity grades are compared in tables 13 and 13A. These add nothing notable to the analysis except that most of the Diarrhea was grade I.

Tables 14 and 14A present this for analyses of events on all courses on-study. Again I have extracted a few entries:

Diarrhea	22	16
phlebitis	6	2
sepsis	7	5
infection	34	41

The comparison by grade and with WRS test is not notable except for the following analyses of CHF. 2 different analyses were done:

Grade	ADR-529	placebo	
0	73	66	p = 0.09 by WRS
1	3	6	
2	0	1	
3	0	1	

Grade	ADR-529	placebo	
0	38	40	p = 0.16 by WRS
1	3	6	
2	0	1	
3	0	1	

In the first analysis patients with no reports were given a grade of 0, in the second they were omitted. Either way, one notes an excess of 6 events on the ADR-529 arm. The following were ECOG definitions for Cardiac toxicity (function):

I - asymptomatic decline of LVEF by less than 20% of baseline value

II asymptomatic decline by more than 20% of baseline

III mild chf responsive to therapy

IV severe or refractory chf.

So there is really only 1 event of symptomatic quality recorded.

Adverse events

These are compared in table 17 (pp 03988-03996). There are no differences of note.

Laboratory

Compared were alkaline phosphatase, bilirubin, SGOT, LDH, BUN, creatinine. Table 18 (p 03998) presents the comparison at after course 3, a comparison with data from 40-50 patients on each arm (there were 66 and 68 patients who got course #4.) When compared over all courses by grade of elevation and WRS, there were no significant differences. When compared by non categorical tests, alkaline phosphatase (median 149 versus 120.5, p = 0.05) and creatinine (median 1.1 vs 1.0, p = 0.05) were significantly higher on the ADR-529 arm.

Reviewer's Comments:

From the distribution of toxicity grade it is obvious that if these differences are real, they are due to slight elevations which are not likely to be of clinical importance. It is quite possible that there is biased ascertainment, with more measurements on the ADR-529 arm from more time on study.

Data on nadir counts is presented in attached tables 24, 25, and 26. The distribution of nadir granulocyte counts over the first 2 weeks of course 1 was similar (median 560 vs 760, p=0.69 by wrs). Over all courses, the nadir was lower on the ADR-529 arm (260 vs 470, p=0.10). Day 22 granulocyte counts were similar on the 2 arms. Nadir platelet counts were lower on the ADR-529 arm (median 185 vs 252, p=0.01), but the number with clinically significant depressions is not given. Day 22 platelet counts were similar on the 2 arms. The drop in hemoglobin values after course 1 were slightly more on the ADR-529 arm (median 1.4 g/dl versus 1.2, p=0.02). There was no difference over all courses (2.7 versus 2.6 g/dl).

Doses delivered

Attached table 29 compares dose intensity on the 2 arms. More courses of therapy were given on the ADR-529 arm (median of 11 vs 9). The median doses of CTX and 5FU were lower, by about 50 mg/M^2, on the ADR-529 arm as were the cumulative doses of these drugs. Part of table 30 is also attached and demonstrates that differences in dosing were observable starting with the third course, medians for cytoxan being different by about 70 to 120

mg/M2/course in this range. Thereafter differences per course are smaller.

Table 31 demonstrates results of a dose intensity index: the numerator was divided by the dose that would have been expected over the total treatment period if no delays or reductions had occurred. When analyzed in this way the dose intensities were similar in both arms. The doxorubicin value was 0.66 (ADR-529) vs 0.63 (placebo), p =0.18). In the analysis above one notes that there were 28 patients with dose reductions in the ADR-529 arm and 17 in the placebo arm (p=0.08).

Reviewer's Comments:
It is obvious that more dose reductions occurred in the ADR-529 arm. This is probably due to more patients continuing longer on study but may also be due to dose reduction due to myelosuppression, which was allowed after 2 dose reductions of the other drugs. An analysis of doxorubicin dose reductions over time would be of interest. An analysis of deviations from schedule over time would also be of interest.

It is unlikely that dose reductions had a major effect on response rates, since most dose reductions seemed to occur after the second dose. However, in the intermediate period, dose reductions of cytoxan and 5FU might have had some effect on time to progression. Certainly one would like to make sure that there was not an early big difference in doxorubicin dose intensity, since this might have had an effect on cardiotoxicity.

The text of the report gives further details:

> "In the experimental group, 28 patients underwent a dose reduction of doxorubicin during the course of therapy, 24 for hematologic toxicity and four for other reasons (one for extravasation, two by protocol error, and one for intercurrent illness). Twenty (20) of the patients had their doxorubicin dose reduced to 51-75% of the prescribed initial dose and eight were reduced below 51%. in all but two of these 28 patients the dose of 5-FU and cyclophosphamide were reduced first followed one or more courses later by a dose reduction in doxorubicin. In the control group, 17 patients underwent a dose reduction of doxorubicin, 14 for either hematologic toxicity or stomatitis and three for other

reasons. Ten of these patients had their doxorubicin dose reduced to between 51-75% of the prescribed initial dose and seven below 51%. The number of dose reductions below 51% were comparable within the two groups while the number of dose reductions to 51-75% were twice as frequent in the experimental group. The clinical practice ("pattern") of dose reduction of doxorubicin dose appeared to differ between the two groups. In the exp[illegible] group the doxorubicin dose was generally re[illegible] or more courses after the dose of 5-Fu and cyclop[illegible] as reduced while in the control group the do[illegible]cin was decreased concurrently with 5FU and [illegible]amide in nine of 17 patients."

Reviewer's Comments:
This suggests that lack of blind may have had some effect on behavior, leading to earlier dose reduction of doxorubicin in the placebo arm.

Quality of Life

The measures of quality of life, onstudy incidence of Performance Status > 3 on-study and >15% decrease in body weight on study (presented in Table 32, p04016) occurred in so few patients that no meaningful comparison be made.

Statistical considerations

The study report notes that the sample size was increased from 132 to 150 "to obtain additional endomyocardial biopsy data and was done without benefit of an analysis performed at that stage." It is also noted that no adjustment was made in the final analysis for the unplanned interim analysis.

Reviewer's Comments:
The communication with regarding sample size adjustment will be verified.

Medical Officer evaluation of case report forms and tabulations

Sources for review:

Synertron evaluation forms and case report forms:

May 11 submission from ADRIA, with 4-page evaluation form" for all patients and CRF's for patients 20, 40, etc. ...140.

Investigator flow sheets (CRF's) and tabulations

- The May 11 submission from includes investigator case report forms on corresponding patients 20, 40, etc.

Questions addressed in review are in bold.

1. **Are cardiac data recorded on source form?**

Synertron CRF: has a cardiac data sheet.

Source CRF:

For patient #60, a summary sheet is a cardiac flow sheet is present and the entries for this are similar on flow sheet, flow sheet and in cardiac tabulation.

For patient #40, I could verify easily one of 12 MUGA scans in the tabulations. No summary data sheet was in the source form. So obviously there was another source. These were on the CRF from but the source was not noted.

Reviewer's Comments:
Obviously a more complete source of LVEF data was used. I will request documentation of this source for cases 20,40, etc.

2. **In what way was tumor response data analyzed?**

From evaluation form, measurable disease at entry is specified but not the definition which was used. On the CRF, attempts were made to list disease sites with measurements. The source CRF did not have adequately organized documentation of response. There was not a systemic determination of indicator lesions with baseline measurements. attempted to do this retrospectively, but there is no way for the reviewer to

systematically examine the quality of this process.

Reviewer's Comments:
will be asked to clarify criteria it used to determine measurability and response and how rigidly these were followed.

3. Points of interest from data tabulations and data listings:

Missing data listings are supplied in the May 11 submission from . Points to note from examination of these listings:

In baseline characteristics listing one notes 4 LVEF's under 50 (49, 49, 46, and 49); 3 of these are on control arm).

The tabulations have present no good way of evaluating the quality of the CHF events.

Response tabulations simply list PR, NC, Improved, CR, with no measurements. This was an individual judgment made by on examination of CRF. The IMPROVED entries were not in patients with measurable disease as implied in another table. These 6 patients with evaluable disease consisted of 4 ADR-529 patients and 3 placebo patients.

From examination of the Survival and Progression tabulations I find patients alive with followup which ranges from 1985 to 1991. More recently randomized patients had more current followup. Because of tabulation arrangement, it was difficult to make an assessment whether ascertainment was equal on the 2 arms.

On patient 120, I don't find death date on source CRF. I find it on followup form: "dead 4-4-89 as per s visit 3-7-91" So for survival also, more information is on the CRF. It should be noted that censoring for survival occurred at last followup date rather than at data cutoff date, so the lack of

complete followup is of less concern.

There is really no way for the medical reviewer to analyze quality of followup for progression (on or offstudy) from tabulations.

The dose reduction tabulations allow an assessment of % dose decrease by date, but not by course number, so one cannot ascertain when in the courses the Adriamycin was dose reduced.

8.4.5.1 Sponsor's discussion:

I have abstracted major points from this discussion (p 03938):

1. "Whether or not ADR-529 protected against doxorubicin cardiomyopathy as assessed by clinical examination could not be conclusively determined." The sponsor claims that clinical exam data, CXR and EKG were not regularly recorded.

2. LVEF data were very complete (95%) and unequivocally demonstrates that ADR-529 protects against doxorubicin cardiotoxicity as demonstrated in table 7.

3. Endomyocardial biopsies were obtained in 31 patients, 20%, or about one third of the patients who received 450 mg/M^2 of doxorubicin and strongly suggest a protective effect of ADR-529. The biopsies were reviewed independently and in a blinded fashion by an NIH pathologist.

4. No "significant added toxicity" was noted, however, since there were many unmentioned toxicities, the data quality is questionable. There was a statistically greater degree of platelet suppression in the ADR-529 arm in the first course. There was a "lower cumulative dose of 5FU and cyclophosphamide per course" on the ADR-529 arm. There were no adverse clinical findings associated with this difference.

5. There was no difference in the efficacy of FAC in the two groups despite the fact that patients in the ADR-529 arm received less cytotoxic therapy on a per course basis than did the control group.

8.4.5.2 Sponsor's conclusions

"In summary, ADR-529 has been shown to have a clinically and statistically significant cardioprotective effect against the cardiomyopathy induced by doxorubicin. This cardioprotective effect was obtained at no detrimental cost. There was no clinically significant added toxicity and no loss of antitumor efficacy as a result of the addition of ADR-529 to the three-drug cytotoxic regimen, FAC. Based on the results of this study, ADR-529 can be said to have significant protective activity and no detrimental effect and warrants further evaluation and potential use as an agent capable of protecting against the cardiotoxic effect of doxorubicin. The ability to administer a cumulative dose up to 450 mg/M^2 of doxorubicin has been shown to have significant antitumor effect in a number of situations and the ability to protect against its most significant adverse functional toxicity, i.e., the development of cardiomyopathy, is of significant clinical importance."

8.4.5.3 Reviewer summary and discussion:

The presentation of this study had some deficiencies compared to the usual NDA quality expected. Tabulations of response and cardiac endpoints were not submitted until May 11. There was significant delay in supplying the data to the statistician. The data for the NDA was taken from ECOG flow sheets supplied by to a data processing company, but additional data was independently obtained by audits of the clinical site.

Since this trial had design differences from the ADRIA-sponsored trials I have abstracted some points from different parts of this review:

Significant differences from ADRIA protocols:

The following are differences of note from the ADRIA-sponsored protocols:

-It included non-measurable disease from the start.

-It included ECOG 2 and 3.

-It was unblinded, so clinical cardiac events might be less trustworthy.

-There were different strata: in addition to cardiac risk, the other stratum was based on whether adjuvant chemotherapy had been received instead of on measurability of disease).

-The dose of C and F were reduced on basis of nadir counts instead of only being reduced for neutropenic fever or for delays in treatment. This might have altered the degree of morbidity noted from myelosuppression.

-Doxorubicin was dose reduced for initial LFT abnormalities.

-Study report was analyzed retrospectively with ADRIA criteria for cardiac toxicity rather than those specified in the protocol.

-There was no requirement for repeat MUGA scan if going offstudy for LVEF decrease (this was similar to the 20:1 data in the other trials whereas after the 10:1 change a repeat LVEF was required.)

-Patients were not prospectively stratified as having measurable disease, therefore determining the group in which to examine this question was retrospective to some degree.

-Did not include MUGA scan at 150 mg/M^2, so patients were less likely to go offstudy for real or spurious LVEF changes at this stage.

Summary of Design issues

This was a randomized open study of CAF with or without ADR529 at a 20:1 ratio in patients with advanced breast cancer.

The IND was done under an IND. The IND contains little information on amendments or any details of study conduct, so the conduct of the study is primarily described by

The main protocol amendment of note is one occurring about halfway through the study which allowed doxorubicin dose

adjustments in some circumstances.

The cardiac objective of the study included clinical, MUGA, and biopsy endpoints. Offstudy criteria also included these. However the clinical cardiac events anticipated were not defined and no analysis of clinical events was specified.

The details of offstudy criteria on the basis of LVEF were somewhat different from the other ADRIA sponsored studies. Entry criteria did not specify a lower limit of LVEF for entry. A drop to 45% or a drop of 20% (qualified by other contingencies) were required. No duplicate scan was required.

Synertron had this comment regarding the quality of response documentation:

> "Tumor measurements were occasionally to frequently not recorded and evaluations of anti-tumor efficacy were, therefore, frequently made on the basis of "evaluable" rather than measurable disease."

Response and recurrence monitoring was not well detailed in the protocol after 6 weeks and there was no way for the reviewer to assess the actual quality of followup.

Cardiac and tumor findings are summarized in the following table which was prepared from selected analyses presented in the NDA:

Tabular summary of findings study 88011

Disease	Breast cancer				
Ratio of DZR	20:1				
	DZR		**PLA**		
# randomized					
total	76		74		
measurable	48		41		
	Cardiac Effect				
	DZR		**PLA**		
Time to "event" (29 events)		P:D 5.07		p<0.01	(2.5, 10.3)
LVEF decrease (mean difference, change from baseline, D-P)					
450 mg/M² (32D, 42P)[1]		11.4		p<0.001	
550 mg/M² (31D, 13P)[1]		15.0		p=<.001	
CHF events	2		7		
	Tumor Effect				
	DZR		**PLA**		**95%ci**
Response (89 pts)	24/48 (50%)		24/41 (59%)		(-12%, 30%)
Progression (104 events)		P:D 0.81			(0.55, 1.20)
Survival (109 deaths)		P:D 1.05			(0.72, 1.54)
Offstudy (149 events)		P:D 1.97	(P<0.001)		(1.39, 2.81)

[1]Numbers in parentheses represent number of patients analyzed.

The time to cardiac event analysis highly favored ADR-529. The hazard ratio (P:D) was 5.07 (95% ci 2.5 to 10.3) with p < 0.01 by LR. There were 7 placebo versus 2 ADR-529 CHF events. The clinical significance of these events was not elaborated, but the reports in the toxicity section indicated most to be Grade I. The mean drops in LVEF from baseline were greater on the placebo arm (11.4 at 450 mg/M^2 and 15.0 at 550 mg/M^2). The mean Billingham score on 33 patients was 0.41 on the ADR-529 arm versus 1.0 on the placebo arm (p=0.06). However, the protocol specified endpoint was number of patients with a score of 2.0.

The response rates in the intent to treat analysis in the application and overviews are misleading; the intent to treat analysis as presented in this study includes patients without measurable disease, whereas the intent to treat analysis of ADRIA studies only includes patients prospectively stratified with measurable disease. In this study, 48 (65%) in the ADR-529 arm and 41 (55%) in the placebo are were determined retrospectively by to have measurable disease. In the analysis of patients determined retrospectively by to have measurable disease, the response rate was 59% (24/41) in the placebo arm and 50% (24/48) in the ADR-529 arm with 95% ci (-12%, 30%). No proper analysis of response is submitted. The "measurable disease" analysis apparently excludes 12 patients deemed "inevaluable".

The time to off-study analysis shows a highly significant difference; the placebo patients tended to come offstudy first, driven by cardiac toxicity (p<0.001). However, as Dr. Speyer notes in the 1992 JCO account of the trial, this difference did not translate into prolongation of response duration or any tangible patient benefit. One can only speculate whether the lack of detectable benefit is due to an absence of underlying benefit or to an inability detect the benefit because of deficiencies in study design, study power, or documentation.

The analysis of time to progression appears hopelessly tainted by uncertainty. There is a question of quality of data given the lack of a followup schedule in the protocol so that the normal analysis uncensored for other offstudy events such as cardiac events, may be incomplete or biased (The Hazard ratio P:D is 0.93 with 95% ci [0.66, 1.31] and LR p=0.69). The onstudy analysis, censoring at time of offstudy for any reason (including cardiac

toxicity), presents an asymmetric censoring pattern yielding results of questionable validity.

The survival appears similar with wide confidence intervals. This analysis gives a hazard ratio P:D of 1.05 with 95% ci [0.72, 1.54] and p =0.80 by LR.

The quality of the toxicity analysis is uncertain since in most cases, lack of toxicity was not recorded.

8.4.5.3 Reviewer conclusions:

Assuming answers to questions are obtained verify the validity of LVEF data presented, this study demonstrates that a 20:1 ratio of ADR-529 has a statistically significant protective effect on doxorubicin-induced cardiotoxicity as represented by changes in LVEF. The clinical significance of this effect in this population was not clearly delineated. CHF events were not defined and were noted by unblinded observers. The study provides little reliable information about whether ADR-529 at this ratio protects against the anti-tumor effect of FAC or alters its toxicity profile.

Additional questions on this study to be communicated to and ADRIA LABS.

Regarding the 88011 study

1. Which patients had bidimensional measurable disease?

2. Which patients had only unidimensional measurable disease?

3. What criteria did use for determining response in the patients with measurable disease? Were all of these based on documented measurements? Was the one-month-duration documentation requirement enforced?

4. Present analysis of response in all patients who at onstudy time had bidi- -sionally measurable disease. Include "IMP" from this anal- is.

6. "Five patients on the control arm and seven patients on the experimental arm were considered to be not evaluable for

efficacy. One patient (No.95) was retrospectively determined to have non-small cell carcinoma of the lung; and, all the rest either received only one or two courses of therapy, or had too few data available for evaluation."

The 12 patients referred to should be individually listed and it should be stated whether they had "measurable disease" and what their individual reasons for non-evaluability were.

7. A comparison is made from table 7 and table 34:

	ADR529	PLACEBO
# with LVEF measurement at 450 mg/M^2	32	42
# on study at course 10	46	32
# on study at course 9	50	42

Although 8 more patients on the ADR-529 arm received course 9 (which would have resulted in 450 mg/M^2 of doxorubicin if there had been no doxorubicin dose-reduction) 10 more placebo patients had a LVEF analyzed at 450 mg/M^2. Please explain.

8. What was the source for the cardiac biopsy data. Present a representative copy of a source document.

9. Present dosing over time for doxorubicin in the same way it was presented for cyclophosphamide in table 30.

10. Address the primary analysis specified in the protocol. Was the protocol specified analysis ever done? The actual analysis described was to compare the number of patients receiving at least 400 mg/M^2 with a LVEF drop of at least 10%.

11. 11 patients on ADR-529 arm and 13 patients on the placebo arm had were not included in time to cardiac event analyses because of no LVEF measurement, which were to start at 300 mg/M^2 of doxorubicin. List these patients and their off-study cumulative doxorubicin dose.

12. What was the source of the LVEF data. Some NYU flow sheets did not have complete data. Please supply copies of the source data from patients 20, 40, ...140.

13. Explain why response, cardiac, etc. tabulations were omitted from the original submission to the Agency.

Regarding ADRIA sponsored studies:

14. It is noted that in the NYU analysis, cardiac event times were censored at the last LVEF measurements rather than at offstudy times, and patients without at least one LVEF measurement were not included. Is this what occurred in analyses of ADRIA-sponsored studies? If not, discuss the implications of such the different analyses.

9 Chemoprotectors for Cancer Chemotherapy:

This section is composed of discussions of various issues of a regulatory and theoretical nature related to this NDA.

General regulatory and theoretical considerations:

There are different approaches one could take to considering criteria for approval of an agent intended to ameliorate toxicities of chemotherapy. If prior information and rationale lead one to strongly believe that tumor protection by the agent is not in the realm of possibility, one may only require proof of symptom amelioration related to the proposed protectant effect. This would appear to me to be the Agency approach to anti-emetics.

However, when one understands with less certainty the mechanisms of anti-tumor effect versus mechanisms of host protection, one may need to be more cautious. A recent study of the use of pyridoxine intended to decrease neurotoxicity of chemotherapy for ovarian cancer suggested it had a negative effect on duration of response (Can Inv, 10:pp1-9, 1992).

There are two regulatory/theoretical concepts I would like to discuss: specificity and overall benefit.

Proving specificity (amelioration of patient toxicity without affecting anti-tumor activity)

Ideally one would like firm proof that protection from toxicities is limited to host but not does not protect tumor. A recent abstract from ASCO provides the kind of dose response background data that might allow such proof. In that abstract the following are response rates for various doses of Epirubicin:

Response rate	Epirubicin dose(mg/M^2)
21%	40
25%	60
47%	90
35%	135

At 90 mg/M^2, one could be sure that one was near the steep portion of the dose response curve for response; if one showed significant amelioration of toxicity at 90 mg/M^2 of Epirubicin with no detrimental effect on tumor response rates, one could feel confident that no large decrement in dose intensity had been

"seen" by the tumor. However, at 135 mg/M^2, an agent could non-selectively neutralize at least 45 mg/M^2 of Epirubicin, with I am sure a detectable decrease in toxicity, without negatively affecting tumor response rates.

Such proof of selectivity would be valuable. It might allow one to generalize to other tumor types or use in other regimens. Without proof or assumption of specificity, generalizing from results with CAF in breast cancer to situations where the dose intensity of adriamycin might be critically important (perhaps in regimens for aggressive lymphomas) could result in serious harm.

I don't think that we know the importance of dose intensity of adriamycin at the 50mg/M^2 level of doxorubicin in CAF in advanced breast cancer. For instance, how certain are we that decreasing the doxorubicin dose by one third in the FAC regimen would lead to a detectable decrease in response rates, progression, or survival? If we don't know, then it is conceivable that one third of the dose of doxorubicin could be non-selectively neutralized, and the threshold of doxorubicin toxicity might not occur until an apparent dose of 750 mg/M^2 of doxorubicin rather than the usual 500 mg/M^2. Consequently, I don't think trials of the design included in the NDA (ie in multi-drug regimens where the contribution and dose-response characteristics of individual agents are unknown) have the potential to prove selectivity even if tumor outcomes were absolutely equivalent.

Proving net clinical benefit:

If there is doubt about the selectivity of an agent to be used in combination with a chemotherapeutic regimen, and that regimen is assumed to confer clinical benefit, then one needs to be confident that net risk/benefit considerations with addition of the new agent are positive. In this case, one would want to make sure that whatever clinical benefit is ascribed to CAF has not been affected negatively to such a degree as to outweigh the patient-benefit attributed to the protector, ADR-529. One means of doing this would be to prove equivalence of clinical efficacy of CAF with or without ADR-529 in addition to proving cardio-protective efficacy of ADR-529. In addition, one would not want the toxicity of ADR-529 to outweigh its cardioprotective benefit.

Alternatively, one might show that net outcome for a critical endpoint was superior for CAF plus ADR-529 (For instance one might show that the ability to give more doxorubicin because of cardioprotection led to superior survival, etc.).

I think that the clinical data in this application have led to enough doubt about specificity that one of these two criteria for approval should now be required. The considerations are similar to considerations in first line therapy of ovarian cancer. New regimens are required to show proof of equivalence to standard regimens in critical efficacy outcomes. The situation we face with ADR-529 and CAF is now no different in my mind; we have a new combination regimen with an altered toxicity profile and with uncertain relative efficacy.

Benefit is shown only for extended CAF use:

Another serious concern relates to when, in the course of therapy with CAF, the cardioprotective benefit of ADR-529 becomes manifest. The cardiotoxicity of doxorubicin has been considered to be clinically significant and dose-limiting primarily after a cumulative dose of 450mg/M^2 to 550 mg/M^2 of doxorubicin. It is unclear to me that additional therapy beyond this point in the treatment of patients with advanced breast cancer confers patient benefit. So, in a population, all of whom eventually die of breast cancer, approval for this indication would suggest that all patients initially receiving CAF for advanced breast cancer take ADR-529, an agent with documented myelosuppressive potential, to protect against a toxicity primarily occurring at a point where continued therapy with CAF is of uncertain benefit. It was not at all clear from the studies in aggregate that substantially more CAF would be delivered, despite LVEF monitoring which is more aggressive than customary and which accentuated the number of patients going offstudy on the placebo arm. There was certainly no hint in the data that survival or time to progression were favorably influenced by prolonged administration of CAF in the presence of ADR-529.

Comments on potential danger of off-label use:

The oncology community uses many drugs for off-label indications. Information on appropriateness of use for individuals is often obtained from the literature. Recent literature communications to oncologists on ADR-529 have not contained many cautionary remarks regarding the possibility of tumor protection. As discussed elsewhere in this review, the recent 1992 ASCO abstract and poster referring to the 10:1 data from the 88001 trial selectively omitted the worrisome response data (with p=0.007 against ADR-529); a concurrent abstract describing the smaller 88006 data base included the more favorable response data. One would certainly be concerned that approval of ADR-529 for some limited indication would lead to inappropriate and perhaps

harmful use in potentially curative settings because of bias introduced into the community through these channels. Requests to the Agency for compassionate use in many clinical settings, including lymphoma, have been received since the publication of the abstracts. In addition, requests for use in patients with low baseline LVEF (excluded from all studies) or whom have already experienced doxorubicin induced toxicity (which was an offstudy criterion for all studies) are also being received. I know of no studies of ADR-529 in these settings.

I there any conceivable rationale for 10:1 ratio being more tumor-protective than the 20:1 ratio?

The human data are insufficient to draw a conclusion on whether, if there is a protective effect of ADR-529 on anti-tumor efficacy of CAF, tumor protection is greater at the 10:1 ratio. It would seem counter-intuitive that the lower dose might be more protective. However, if it were established that the 10:1 ratio protected tumor and the 20:1 ratio did not, a reasonable rationale could be constructed. ADR-529 is not simply an inert chelating agent. In animal models it has activity with doxorubicin against some tumors, especially leukemia cell lines. In humans it obviously has detectable activity against bone marrow stem cells in the presence of CAF leading to additional myelosuppression. Although little activity was seen in human tumors even at high doses, it is conceivable that there could be increased in the presence of CAF. If this activity was only detectable at the higher dose of ADR-529 (20:1), obviously a dose with more impact on human bone marrow, then such an effect might negate the detectable inhibiting effect on direct CAF activity. Certainly, this is highly speculative, but could explain a seemingly untenable finding.

The pharmacology section of this review refers to an animal study of a human breast tumor model transplanted beneath the renal capsule in nude mice. Several ratios (5:1, 10:1, 15:1, and 20:1) of ADR-529 were tested with doxorubicin. All combinations showed equal initial tumor shrinkage in response to doxorubicin; the 10:1 ratio showed a significantly faster tumor regrowth. Certainly there are other animal data failing to show tumor inhibition in many tumor models, but the intrinsic activity of ADR-529 itself in some of these models could have hidden a protective effect on doxorubicin activity. Although the data in this study could be in error, they do raise a red flag and offer some support that the findings of a lesser response rate in trial 001 are valid.

Clinical toxicity data suggesting lack of tissue specificity

Several of the studies included subtle suggestions of protection of other tissues beside the heart from chemotherapy. Esophagitis was noted to be less frequent on the ADR-529 arm in several studies. Ultimately alopecia was nearly uniform on both study arms, but alopecia during course 1 was noted to be more frequent in several studies on the placebo arm. I do not know enough about the physiology of these tissues to know whether the purported mechanism of toxicity is similar to chemotherapy effect on tumor or the toxic effect on the heart. Again, although not definitive, these findings suggest that we reexamine assumptions of selectivity.

10 Overall summary and conclusions on pivotal trials (preliminary)

Again conclusions are preliminary, review of the data is still ongoing. Conclusions assume the general accuracy of analyses presented by ADRIA LABS.

Refer to summaries and "the tabular summaries of data" in respective sections of the review, located through the table of contents.

The focus of the decision on approval should be on the 10:1 data in breast cancer, the ratio proposed and the clinical setting with the most data (refer to tabular summaries of this data on pp 47 and 63). The evidence for the presence of cardioprotection as manifested in a decreased rate of LVEF deterioration is consistent through all studies. However, the design of the studies did not focus on the clinical importance of this protection; rather on whether the protection from LVEF deterioration was detectable. For instance, emphasis was not on documenting and describing symptomatic CHF events, the only kind of cardio-toxic events likely to be of import to this population with incurable disease. The studies were designed to follow patients very closely for LVEF deterioration, starting at 150 mg/M^2. Patients were removed from study for what could, in some cases be rather minor LVEF changes. Only patients remaining onstudy through all these testing hurdles would remain for the primary endpoint measurement, determination of LVEF at 500 mg/M^2. In retrospect this technique would select out, prior to the primary analysis, those patients most likely to demonstrate cardiotoxicity and deterioration in LVEF.

The analysis of "time to cardiac event" performed retrospectively by the sponsor is logical. However, the event criteria were intended to guide offstudy decisions, and were not necessarily examined to serve as equal partners in an aggregate efficacy endpoint.

As a result we have cardiac data showing indisputable protective biologic effect of ADR-529 using both 10:1 and 20:1 ratios. However, the conditions of the studies were somewhat artificial for estimating clinical benefit. Most practitioners do not start monitoring LVEF at 150 mg/M^2 of doxorubicin and take patients off study for rigid LVEF criteria based on change from baseline. Consequently, the true clinical impact of ADR-529 used with CAF in a more customary fashion may have been obscured. More patients went off the placebo arm earlier and hence got less CAF than they

would have, altering both their ultimate clinical cardiotoxic manifestations and perhaps also their tumor associated outcomes.

The offstudy curve of the largest 10:1 study (88001) (attachment to p 28) puts in perspective how few patients actually remained onstudy to receive whatever benefits continued CAF might impart. After treating all patients from the start of therapy, only a few remain in the tail of the curve to receive potential, but unproven, net benefit.

The evidence regarding tumor effect from the 2 trials using the 10:1 ratio are summarized below. From the 88001 trial officially completed on the basis of an interim analysis:

	Tumor Effect		
	DZR	PLA	95%ci
Response (293 pts)	67/141 (48%)	96/152 (63%)	(-27%, -4%)
Progression (185 events)	P:D 0.908		(0.68, 1.21)
Survival (127 deaths)	P:D 1.02		(0.72, 1.45)

The evidence from the 88006 trial, officially terminated on the basis of the interim analysis of the 88001 trial:

	Tumor Effect		
	DZR	PLA	95%ci
Response (123 pts)	41/54 (57%)	36/69 (52%)	(-13%, 23%)
Progression (94 events)	P:D 0.95		(0.63, 1.43)
Survival (55 deaths)	P:D 0.60		(0.36, 1.03)

The 88001 trial, the largest intact database from one trial strongly suggests (p=0.007) that response is inferior on the ADR-529 arm. Response was the protocol-specified primary measurement of antitumor activity. The lower bound of the 95% confidence intervals for progression and survival (0. 8 and 0.72) do not offer great assurance that these parameters are not affected. The response rates in the 006 trial, which has only 42% as many patients with measurable disease, does not support this finding.

However, the results do not counter balance those in 001. The lower bound of the confidence intervals for progression (0.72 and 0.36) in trial 88006 are certainly no proof of equivalence, in the fact the survival data in 006 shows a strong trend (p=0.06) against ADR-529. Further updates of survival and progression data from both trials will be confounded by crossover.

When making a decision about the potential of the 10:1 ratio to protect tumor, it is not clear what one should do with the 20:1 data; however I will present it here.

From the 20:1 data in the 001 trial:

Tumor Effect

	DZR	PLA	95%ci-
Response (120 pts)	38/66 (58%)	29/54 (54%)	(-14%, 22%)
Progression (121 events)	P:D 1.18		(0.80, 1.75)
Survival (95 deaths)	P:D 1.20		(0.80, 1.80)

The following is from the 20:1 data in the study. As noted in the review, the quality of response data is less well documented. Even the following analysis in patients with measurable disease presented by excludes some patients as "inevaluable." However, data presented as "intent to treat" analysis from this trial in the overviews is not valid, including patients without measurable disease.

From the 88011 trial :

Tumor Effect

	DZR	PLA	95%ci
Response (89 pts)	24/48 (50%)	24/41 (59%)	(-12%, 30%)
Progression (104 events)	P:D 0.81		(0.55, 1.20)
Survival (109 deaths)	P:D 1.05		(0.72, 1.54)

The results in these small numbers of patients offer no strong support in either direction.

The results from the patients entered into the 20:1 and 10:1 portions of the lung cancer study (88002) can be found on pp 92 and 95 respectively. Only 50 patients were entered into the 20:1 portion of the study. The intent to treat analysis of the 10:1 portion offers no comfort, despite all the caveats about dropouts, with a 59% response rate in the placebo arm and 45% in the ADR-529 arm (p=0.09).

Conclusions

1. Several trials demonstrate evidence that ADR-529, given prior to CAF, slows the rate of LVEF deterioration seen with increasing cumulative doses of doxorubicin. Evidence is convincing with both 10:1 and 20:1 ratios.

2. Events described as CHF by monitors and investigators were uncommon on both arms of studies, but occurred less frequently on ADR-529 arms. The overall clinical impact of the cardioprotection in the setting of treatment of advanced breast cancer has not been clearly demonstrated.

3. Lethalities noted by ADRIA LABS, associated with the 20:1 ratio of ADR-529 given with CAF and CAV, necessitated discontinuation of studies using this ratio.

4. An increase in myelosuppression was detectable in studies using the 10:1 ratio.
5. The only completed trial using the 10:1 ratio with CAF showed a highly significant difference in response rates, inferior in the ADR-529 arm. Data from other studies is inconclusive.

6. The clinical benefit from CAF given beyond 6-7 cycles (350 mg/M^2 of doxorubicin) is questionable. It is only beyond this point that the protective effect of ADR-529 is likely to be of relevance.

Recommendations (preliminary)

The above results have introduced highly significant doubt regarding the selectivity of the protective effect of ADR-529. Additional response data will be viewed in the update, but are unlikely to erase the significant doubt. Approval of ADR-529 for a limited indication or for widespread compassionate use would seem not to be indicated given the doubt regarding selectivity. The biased information available to the community is also a

significant concern.

Given this doubt, large comparative studies should be performed with the goal of demonstrating the net clinical benefit (or alternatively of proving cardiac benefit plus demonstrating equivalence of anti-tumor efficacy) of chemotherapy regimens with ADR-529 compared to the same regimens without ADR-529.

Trials to demonstrate specificity of action, performed with single agents having known dose-response characteristics, should also be considered. In addition, trials are needed in patients with impaired myocardium.

11 Results reported in 1992 Proceedings of American Society of Clinical Oncology

The reviewer attended the 1992 meeting of ASCO and noted 4 abstracts describing data in the NDA. It was noted that the abstract and poster from the 10:1 data in the 88001 trial in breast cancer omitted the unfavorable information on response. The poster presentation did mention response rate determination as an objective, but included no results. When the ADRIA representative at the poster, the study Monitor, was questioned, he said the data were not available when it was put together. He noted that few viewers had questioned him on the response rates. I think this omission should be of serious concern from a regulatory and from an academic standpoint.

Copies of the abstracts relating to the NDA data in the 1992 abstracts have been placed behind each of the corresponding summary tables in the review (pp 42, 47, 63, and 95).

The presentation of the abstract from the 10:1 data in the 001 trial, while not presenting response, does quote survival and progression hazard ratios and describes them as approximately equal on the two arms. The placebo arm is said to be at a 10 fold risk of developing CHF. There is no mention of the data on increased myelosuppression in the ADR-529 arm.

In contrast the presentation of the data from the 88006 breast cancer trial (10:1 ratio), which was stopped at the same time with the same data cutoff date for response (4-31-91) as the 88001 trial, gives response data from the evaluable patients analysis, with the same number of responses noted (29 and 35) as reported in the NDA. Here, the hazards for progression and more unfavorable survival tendency (p=0.06) are not included. The situation surrounding what appears to be a highly biased presentation of data on these 2 concurrent trials should be evaluated.

The presentation of the 10:1 data from the small cell trial (88002) gives the evaluable analysis of response (67% D vs 68% C) and omits the less favorable intent to treat analysis (45% D vs 59% P).

In the presentation of the 20:1 data from 88001, it is stated that patients are able to receive "4 to 5 times" the cumulative does of doxorubicin. The fact that the ratio was altered and accrual terminated because of lethalities on ADR-529 arms at the 20:1 ratio was not mentioned.

The Division in the Agency which is responsible for evaluating advertising will be notified of these abstracts to assess whether they should be interpreted as misleading pre-marketing advertising. ODAC may want to consider how the bias which has been introduced into the Oncology Community can best be corrected.

Grant A. Williams, MD

Alice Chen, MD
Staff fellow (primary reviewer for study 88006)

NDA 20-212
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HFD-150/GWilliams
HFD-150/ECutler
HFD-150/JJohnson
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ATTACHMENTS FOR NDA 20-212 MOR #1

MOR #1
Attachments

Attachments

3.7.2 Study Schema II - Amended Protocol (Effective Date November 2, 1988)

RANDOMIZATION ASSIGNMENT	
TREATMENT A	**TREATMENT B**
F 500 mg/M² I.V. A 50 mg/M² I.V. C 500 mg/M² I.V.	F 500 mg/M² I.V. A 50 mg/M² I.V. C 500 mg/M² I.V.
LM Control - 50 ml/M² I.V.	DZR-500 mg/M² I.V.
↓	↓
Repeat every three weeks as recovery from toxicity allowed	Repeat every three weeks as recovery from toxicity allowed
↓	↓
Off-study if PD, cardiac event, unacceptable toxicity, patient refusal	Off-study if PD, cardiac event, unacceptable toxicity, patient refusal
↓	↓
Follow-up every three months until death	Follow-up every three months until death

3.7.3 Study Schema III - Current Protocol (Effective Date January 14, 1991)

RANDOMIZATION ASSIGNMENT	
TREATMENT A	**TREATMENT B**
F 500 mg/M^2 I.V. A 50 mg/M^2 I.V. C 500 mg/M^2 I.V.	F 500 mg/M^2 I.V. A 50 mg/M^2 I.V. C 500 mg/M^2 I.V.
LM Control - 50 ml/M^2 I.V.	DZR-500 mg/M^2 I.V.
↓	↓
Repeat every three weeks - six cycles*, as recovery from toxicity allowed.	Repeat every three weeks as recovery from toxicity allowed.
↓	↓
Cumulative dose of A 300 mg/M^2, delete LM Control, add DZR 500 mg/M^2	Off-study if PD, cardiac event, unacceptable toxicity, patient refusal
↓	↓
Repeat every three weeks as recovery from toxicity allowed.	Follow-up every three months until death
↓	
Off-study if PD, cardiac event, unacceptable toxicity, patient refusal	
↓	
Follow-up every three months until death	

*Patients were to be discontinued prior to six cycles for PD, cardiac event, refusal, toxicity.

5B

9.0 SCHEDULE OF EVALUATION

	Baseline (l)	Weekly for 1st Four Courses	After Every Course (a)	After Every Third Course	After Adriamycin® Cumulative Dose of: 150 mg/M², 300 mg/M², 400 mg/M², 500 mg/M² and then After Every 50 mg/M² (g)	End of Treatment	Follow-Up q 3 Months Death
General Medical History	X						
Cardiac History	X						
Complete Physical Examination	X					X	
Cardiac Signs and Symptoms	X				X (g)	X	X
BP, Pulse, Weight	X		X			X	
BSA	X						
Performance Status (ECOG)	X		X				
Signs/Symptoms (b)	X		X			X	
Toxicities			X			X	
Physical Examination Measurements (c)	X			X		X	X (h)
Tc Bone Scan and Other X-Ray or Scans Necessary to Assess Disease (c)	X			X (f)		X (f,k)	X (f,h)
Chest X-Ray	X				X (d)	X (k)	
EKG	X					X (k)	
MUGA Scan (Resting LVEF)	X				X (g)	X (k)	X (i)
Hemoglobin, Hematocrit, WBC, Differential, Platelets (l)	X	X	X			X	
Chemistries	X			X		X	
Follow-Up Form							X

TABLE 6.3.1
20:1 Patients

Number of Patients Participating in Data Summaries

	DZR	PLA
No. Randomized	67	54
Cardiotoxicity Efficacy Time to Event (Drops in LVEF, CHF)	67	54
Changes in LVEF Baseline 150 mg/M^2 300 mg/M^2 400 mg/M^2 500 mg/M^2 550 mg/M^2 600 mg/M^2	 67 50 34 28 22 23 19	 54 49 34 21 8 5 1
Antitumor Efficacy Response Rates Intent-to-Treat[a] Evaluable[a,b]	 66 52	 54 47
Disease Progression	67	54
Disease-Free Survival	67	54
Survival	67	54
Safety Course 1 *(Clinical Toxicities, Hematologies)*[c] Course 3 *(Lab Toxicities)* All Courses	 65 50 65	 53 47 53
Quality of Life Changes in Performance Status Changes in Body Weight	 67 67	 54 54

[a]Randomized with bidimensional, measurable disease.
[b]≥3 courses of therapy with objective assessment of disease status and not in gross violation of the protocol.
[c]Maximum number of patients participating in at least one analysis.

TABLE 2A

BASELINE DEMOGRAPHIC CHARACTERISTICS

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
Age (years)			WRS	X^2=0.38 P=0.54
Mean	54.4	55.7		
Median	55.0	58.0		
St. Dev.	13.0	10.7		
(min., max.)	29, 81	35, 77		
n	67	54		
Weight (kg)			WRS	X^2=0.84 P=0.35
Mean	67.9	70.5		
Median	65.3	69.1		
St. Dev.	14.4	16.2		
(min., max.)	40.3, 117	37, 117		
n	67	54		
Sex				
Female (%)	67 (100%)	54 (100%)		
Race			FXT	P=0.045
White (%)	59 (88%)	41 (76%)		
Black (%)	8 (12%)	9 (17%)		
Other (%)	0 (0%)	4 (7%)		

[a]WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square.
FXT=Fisher's Exact Test

TABLE 3A

BASELINE CARDIAC RISK FACTORS
20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
Age >65 years			PCS	X^2<0.01 P=0.94
Yes (%)	14 (21%)	11 (20%)		
No (%)	53 (79%)	43 (80%)		
Prior Radiation to Mediastinum			PCS	X^2=1.69 P=0.19
Yes (%)	5 (7%)	8 (15%)		
No (%)	62 (93%)	46 (85%)		
History of Heart Disease[b]			PCS	X^2=2.08 P=0.15
Yes (%)	9 (13%)	3 (6%)		
No (%)	58 (87%)	51 (94%)		
Hypertension[c]			PCS	X^2=2.42 P=0.12
Yes (%)	15 (22%)	19 (35%)		
No (%)	52 (78%)	35 (65%)		
Diabetes Mellitus[c]			FXT	P=0.13
Yes (%)	6 (9%)	1 (2%)		
No (%)	61 (91%)	53 (98%)		
LVEF ≤10% above Lower Limit of Normal			PCS	X^2=0.17 P=0.68
Yes (%)	20 (30%)	18 (33%)		
No (%)	47 (70%)	36 (67%)		

[a]FXT=Fisher's Exact test; PCS=Pearson Chi Square.
[b]Previous myocardial infarction, significant arrhythmia, angina.
[c]Requiring medical therapy.

TABLE 4A *(cont'd)*

BASELINE DISEASE STATUS

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Immunotherapy Yes (%) No (%)	 1 (1%) 66 (99%)	 0 (0%) 54 (100%)	FXT	P=1.00
Hormonal Therapy Yes (%) No (%)	 24 (36%) 43 (64%)	 29 (54%) 25 (46%)	PCS	X^2=3.89 P=0.049
Dominant Disease Site			WRS	X^2=1.51 P=0.22
Visceral (%)	54 (81%)	39 (72%)		
Bone (%)	9 (13%)	7 (13%)		
Soft Tissue (%)	4 (6%)	8 (15%)		
Number of Estrogen Receptors			WRS	X^2=0.28 P=0.60
0-10 (%)	29 (50%)	17 (41%)		
10-100 (%)	19 (33%)	18 (44%)		
>100 (%)	10 (17%)	6 (15%)		
Number of Progesterone Receptors			WRS	X^2=1.25 P=0.26
0-10 (%)	31 (56%)	27 (69%)		
10-100 (%)	14 (25%)	6 (15%)		
>100 (%)	10 (18%)	6 (15%)		
Disease Measurability Status			FXT	P=0.11
Measurable (%)	10 (15%)	15 (28%)		
Non-Measurable (%)	1 (1%)	0 (0%)		
Both (%)	56 (84%)	39 (72%)		

[a]FXT=Fisher's Exact test; WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square; LR=Logrank.
[b]Time between initial disease diagnosis and first relapse.

TABLE 4A

BASELINE DISEASE STATUS

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
Disease Free Interval[b]			LR	X^2=3.79 P=0.052
Median	439.5	672.5		
No. Disease Sites			WRS	X^2=0.04 P=0.85
Mean	3.9	3.9		
Median	4.0	3.0		
St. Dev.	1.9	2.2		
(min., max.)	1, 10	1, 9		
n	67	54		
Performance Status			WRS	X^2=0.10 P=0.75
0	31 (46%)	26 (48%)		
1	25 (37%)	14 (26%)		
2	10 (15%)	14 (26%)		
3	0 (0%)	0 (0%)		
4	1 (2%)	0 (0%)		
Incidence of Prior Therapies				
Surgery			PCS	X^2=1.85 P=0.17
Yes (%)	55 (82%)	49 (91%)		
No (%)	12 (18%)	5 (9%)		
Radiotherapy			PCS	X^2=1.18 P=0.28
Yes (%)	13 (19%)	15 (28%)		
No (%)	54 (81%)	39 (72%)		
Chemotherapy			PCS	X^2=0.43 P=0.51
Yes (%)	21 (31%)	20 (37%)		
No (%)	46 (69%)	34 (63%)		

[a]FXT=Fisher's Exact test; WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square; LR=Logrank.
[b]Time between initial disease diagnosis and first relapse.

TABLE 5A

TIME TO CARDIAC EVENT

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
No. Events (%)	9 (13%)	25 (46%)		
Median Event Time (mg/M^2 of DOX[b])	—[c]	450		
Hazard Ratio (P:D)	6.177		LR	X^2=23.25 P<0.001
95% C.I. of (P:D)	(2.692, 14.174)		GW	X^2=4.49 P=0.034

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin
[c]The median is unestimable.

Stephens Computations

Figure 1A Time to Cardiotoxic Event
201 Patients

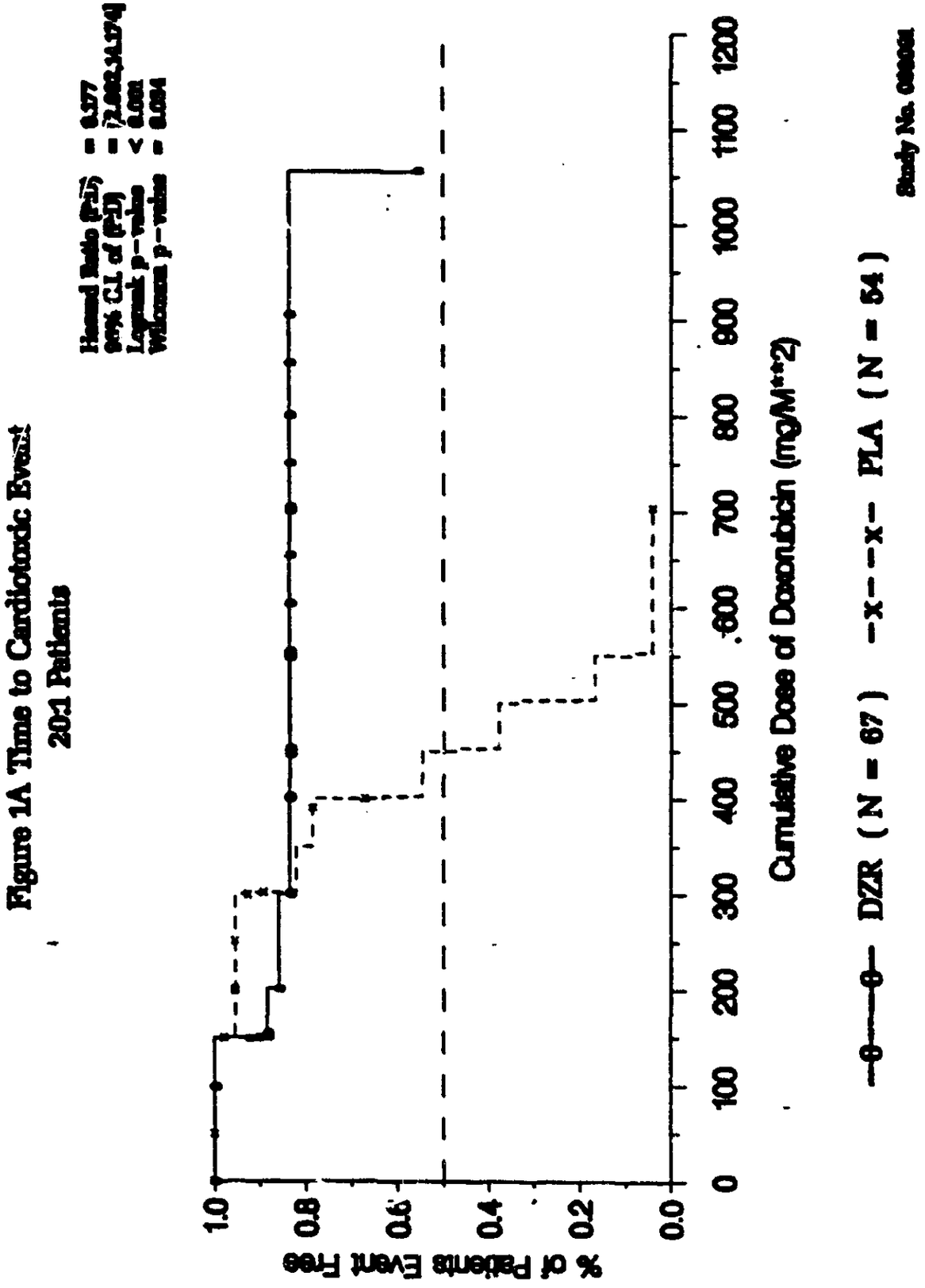

TABLE 6A

TIME TO CONGESTIVE HEART FAILURE

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
No. Events (%)	0 (0%)	2 (4%)		
Median Event Time (mg/M^2 of DOX[b])	–[c]	–[c]		
Hazard Ratio (P:D)	79.653		LR	X^2=6.71 P=0.010
95% C.I. of (P:D)	(2.903, >100)		GW	X^2=6.40 P=0.011

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin
[c]The median is unestimable.

TABLE 7A

CHANGES IN LVEF (%) OVER CUMULATIVE DOSE OF DOXORUBICIN
20:1 Patients

	Cumulative Dose of Doxorubicin (mg/M²)													
	Baseline		150		300		400		500		550		600	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Mean	65.0	62.1	61.4	57.8	64.3	56.1	64.6	54.4	63.2	49.4	63.9	47.2	64.1	60.0
Median	65.0	61.0	62.5	58.7	62.0	57.5	63.5	54.0	63.0	47.5	62.0	43.0	62.0	60.0
Std. Dev.	9.0	8.8	10.9	8.8	10.9	10.1	8.7	7.3	6.5	8.8	8.6	13.1	8.9	--
n	67	54	50	49	34	34	28	21	22	8	23	5	19	1
Estimate of Effect[a]	2.9		3.6		8.2		10.2		13.8		16.7		4.1	
p-value[b]	0.08		0.045		0.003		<0.001		0.002		0.019		--	
Change from Baseline														
Mean			-3.8	-4.4	0.0	-6.4	-0.2	-9.9	-2.3	-12.9	-2.0	-20.8	-2.3	-13.0
Median			-3.0	-4.0	1.0	-6.5	2.0	-8.0	-1.0	-13.0	-1.0	-22.0	-1.0	-13.0
Std. Dev.			10.8	5.4	8.6	6.6	7.9	7.8	8.6	5.3	8.4	11.1	7.6	--
n			50	49	34	34	28	21	22	8	23	5	19	1
Estimate of Effect[a]			0.6		6.4		9.7		10.6		18.8		10.7	
p-value[b]			0.18		0.001		<0.001		0.003		0.003		--	

[a]Mean difference between treatment group means: D minus P.
[b]Wilcoxon rank sum test.

Figure 2A Time to Congestive Heart Failure
20:1 Patients

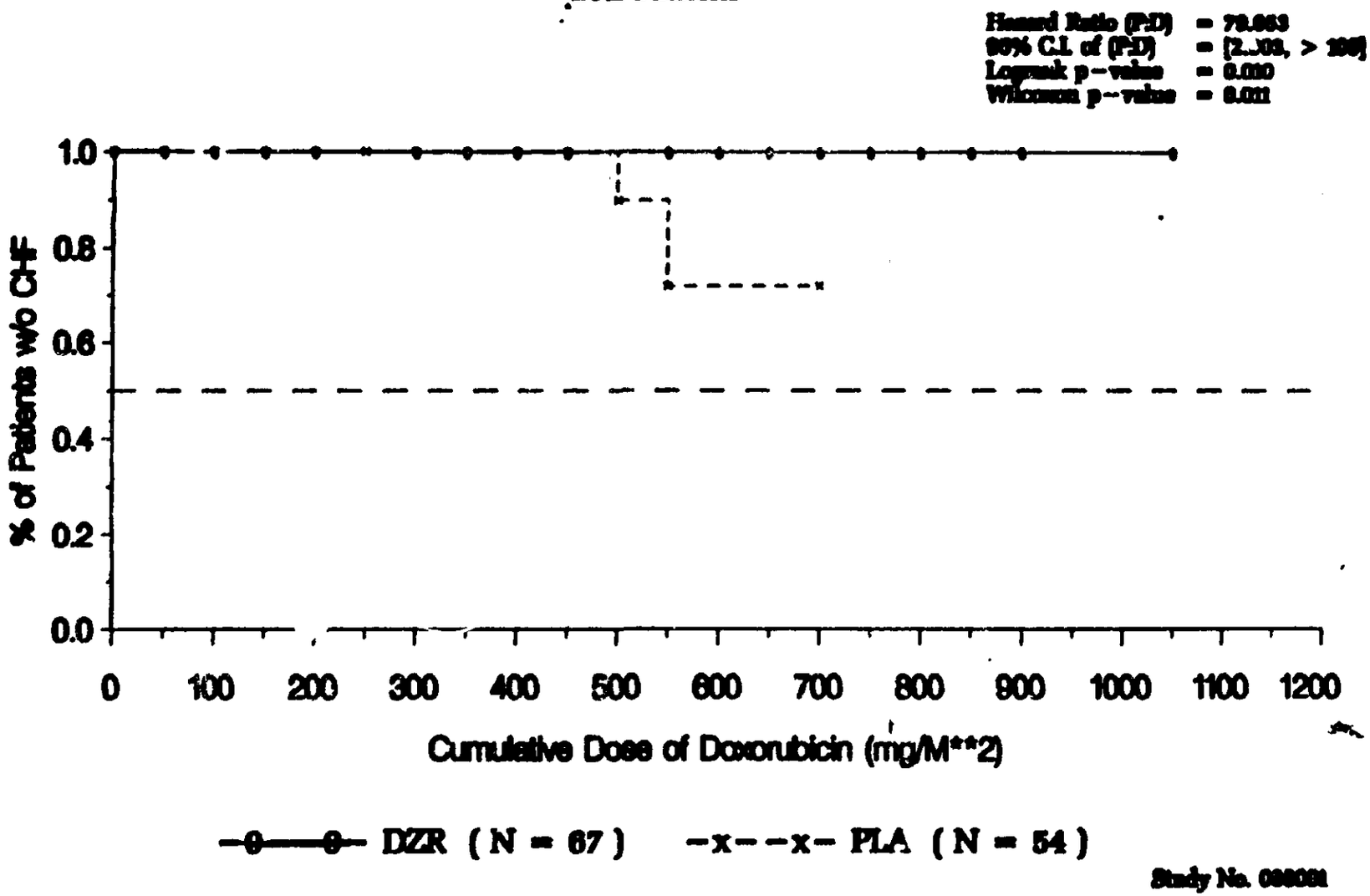

TABLE 6.5.1.1
20:1 Patients

Number of Patients Included in Response Rate Summaries

	DZR	PLA
No. of Randomized Patients	67	54
No. of Patients with Measurable Disease[a]	66	54
No. of Intent-to-Treat Patients	66	54
No. of Patients with Tumor Assessment	59	50
No. of Off-Study Patients without Tumor Assessment[b]	7	4
Reasons Off-Study[c]		
Progressive Disease	0	0
Cardiotoxicity	0	0
Adverse Reaction	1	0
Patient Refusal	1	1
Protocol Violation	0	1
Death	4	0
Lost to Follow-Up	1	0
Randomized, Not Treated	0	1
Other	0	1
No. of Evaluable Patients[d]	52	47

[a]Patients with nonmeasurable disease were excluded from the response rate analyses.
[b]These patients were categorized as treatment failures in the intent-to-treat analysis.
[c]Patients lacking objective determination of tumor status while on-study.
[d]Patients receiving ≥three courses of treatment, had objective measurement of their disease, and were not in gross violation of the protocol.

The point estimates in Table 8A suggest that there were no notable differences in response rates between the two treatment arms at the 20:1 ratio. Approximately 4% and 10% more of the DZR patients responded compared to the control patients for the intent-to-treat and evaluable cases analyses, respectively. Due to the limited sample size, these data were not

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TABLE 8A

RESPONSE RATES
20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Randomized Patients				
No. Patients	66	54		
Best Response				
CR (%)	8 (12%)	7 (13%)		
PR (%)	30 (45%)	22 (41%)		
SD (%)	11 (17%)	12 (22%)		
PD (%)	10 (15%)	9 (17%)		
NA (%)[b]	7 (11%)	4 (7%)		
Response Rate[c]	38/66 (58%)	29/54 (54%)		
Estimate of Effect[c,d]	4%		PCS	X^2=0.18 P=0.67
95% C.I. of Effect[c,d]	(-14%, 22%)			
Evaluable Patients				
No. Patients	52	47		
Best Response				
CR (%)	8 (15%)	7 (15%)		
PR (%)	27 (52%)	20 (43%)		
SD (%)	11 (21%)	12 (26%)		
PD (%)	6 (12%)	8 (17%)		
Response Rate[c]	35/52 (67%)	27/47 (57%)		
Estimate of Effect[c,d]	10%		PCS	X^2=1.03 P=0.31
95% C.I. of Effect[c,d]	(-9%, 29%)			

[a]PCS=Pearson chi square (comparing proportion of responders to nonresponders).
[b]NA=Not assessed while on-study; categorized as treatment failures.
[c]Response=CR+PR.
[d]Difference between treatment groups in response rates: D minus P.

TABLE 9A

TIME TO DISEASE PROGRESSION

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
No. Events (%)	52 (78%)	49 (91%)		
Median Failure Time (days)	231	225		
Hazard Ratio (P:D)	1.179		LR	X^2=0.68 P=0.41
95% C.I. of (P:D)	(0.795, 1.749)		GW	X^2=0.12 P=0.73

[a]LR=Logrank; GW=Generalized Wilcoxon.

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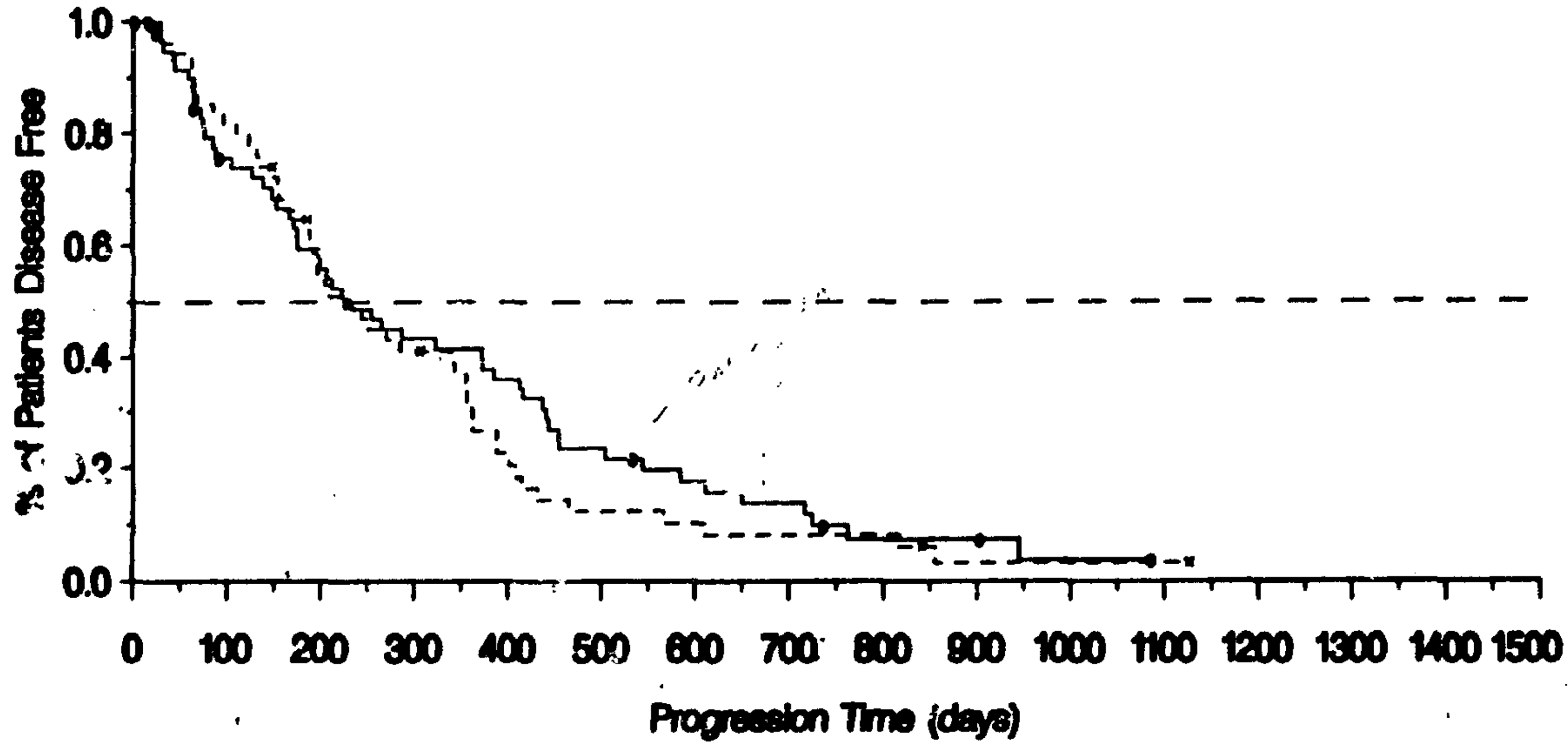
Figure 3A Time to Disease Progression
20:1 Patients

Hazard Ratio (P:D) = 1.179
95% C.I. of (P:D) = [0.794,1.749]
Logrank p-value = 0.41
Wilcoxon p-value = 0.73



-0--0- DZR (N = 67) -x--x- PLA (N = 54)

Study No. 000001

08-00605

TABLE 11A

SURVIVAL

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
No. Dead (%)	50 (75%)	45 (83%)		
Median Survival (days)	517	502		
Hazard Ratio (P:D)	1.197		LR	X^2=0.76 P=0.38
95% C.I. of (P:D)	(0.798, 1.796)		GW	X^2=0.13 P=0.71

[a]LR=Logrank; GW=Generalized Wilcoxon.

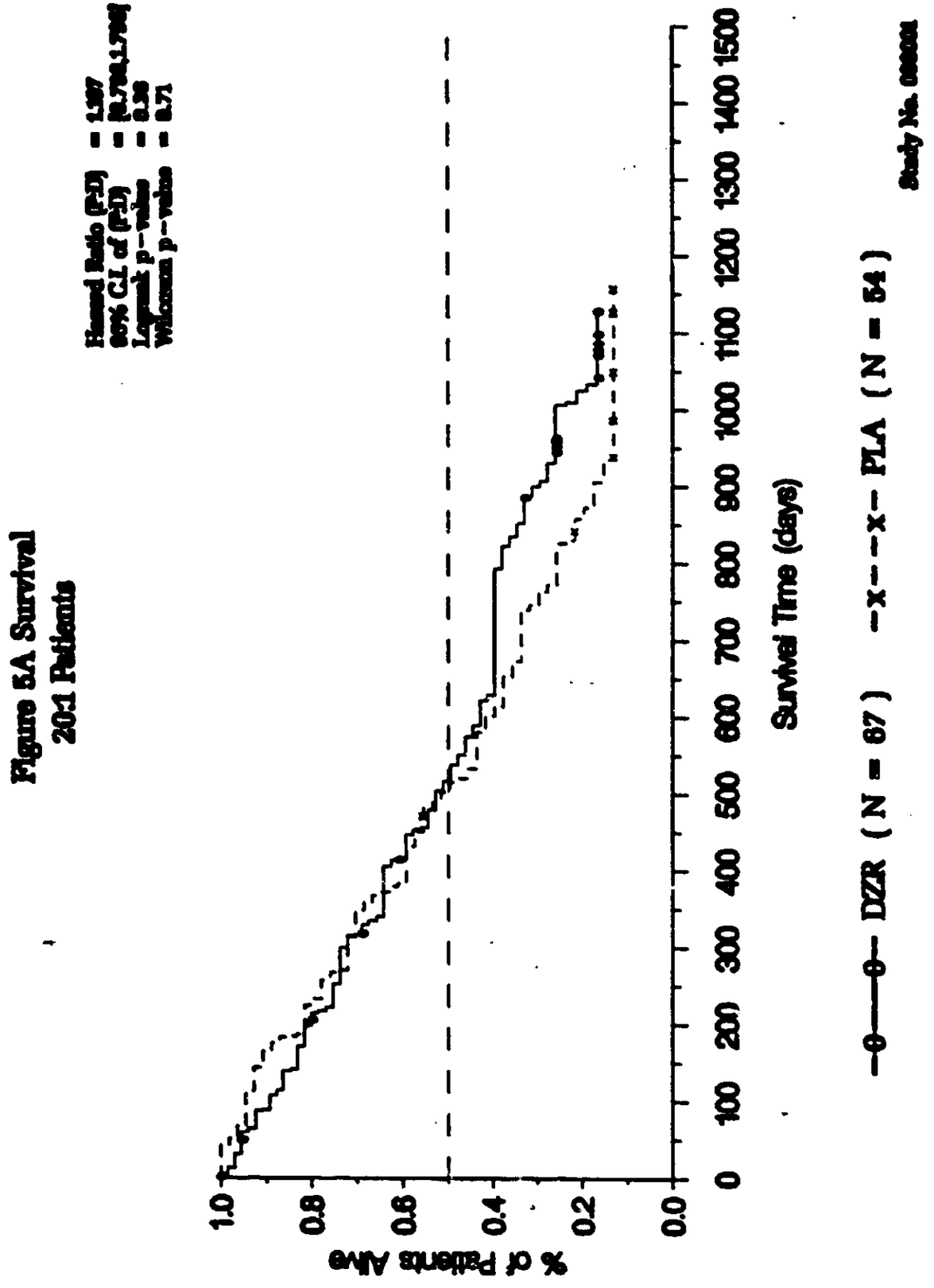


Figure 8A Survival
20:1 Patients
Hazard Ratio (P:D) = 1.397
95% C.I. of (P:D) = [0.776,2.516]
Logrank p-value = 0.26
Wilcoxon p-value = 0.71
% of Patients Alive
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
Survival Time (days)
—0— DZR (N = 67) -x--x- PLA (N = 64)
Study No. 000000

TABLE 32A

QUALITY OF LIFE MEASURES

TIME TO DECREASED STATUS

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54	[redacted]	[redacted]
Performance Status ≥3 (On-Study)				
No. Events (%)	6 (9%)	6 (11%)	[redacted]	[redacted]
Median Time (days)	--[c]	--[c]	[redacted]	[redacted]
Hazard Ratio (P:D)	1.080		LR	X^2=0.02 P=0.89
95% C.I. of (P:D)	(0.318, 3.353)		GW	X^2<0.01 P=0.94
Performance Status ≥3 (Any Time)				
No. Events (%)	26 (40%)	31 (47%)	[redacted]	[redacted]
Median Time (days)	840	590	[redacted]	[redacted]
Hazard Ratio (P:D)	1.383		LR	X^2=1.48 P=0.22
95% C.I. of (P:D)	(0.818, 2.339)		GW	X^2=0.92 P=0.34
≥15% Decrease in Body Weight[b]				
No. Events (%)	5 (7%)	7 (13%)	[redacted]	[redacted]
Median Time (days)	--[c]	--[c]	[redacted]	[redacted]
Hazard Ratio (P:D)	1.930		LR	X^2=1.17 P=0.28
95% C.I. of (P:D)	(0.574, 6.496)		GW	X^2=0.25 P=0.62

[a]LR=Logrank; GW=Generalized Wilcoxon.
[b]Relative to baseline.
[c]The median is unestimated.

TABLE 10A

DISEASE FREE SURVIVAL

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54	[redacted]	[redacted]
No. Events (%)	61 (91%)	50 (93%)	[redacted]	[redacted]
Median Failure Time (days)	227	225	[redacted]	[redacted]
Hazard Ratio (P:D)	1.087		LR	X^2=0.19 P=0.66
95% C.I. of (P:D)	(0.747, 1.584)		GW	X^2<0.01 P=0.98

[a]LR=Logrank; GW=Generalized Wilcoxon.

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Figure 4A Disease Free Survival
201 Patients

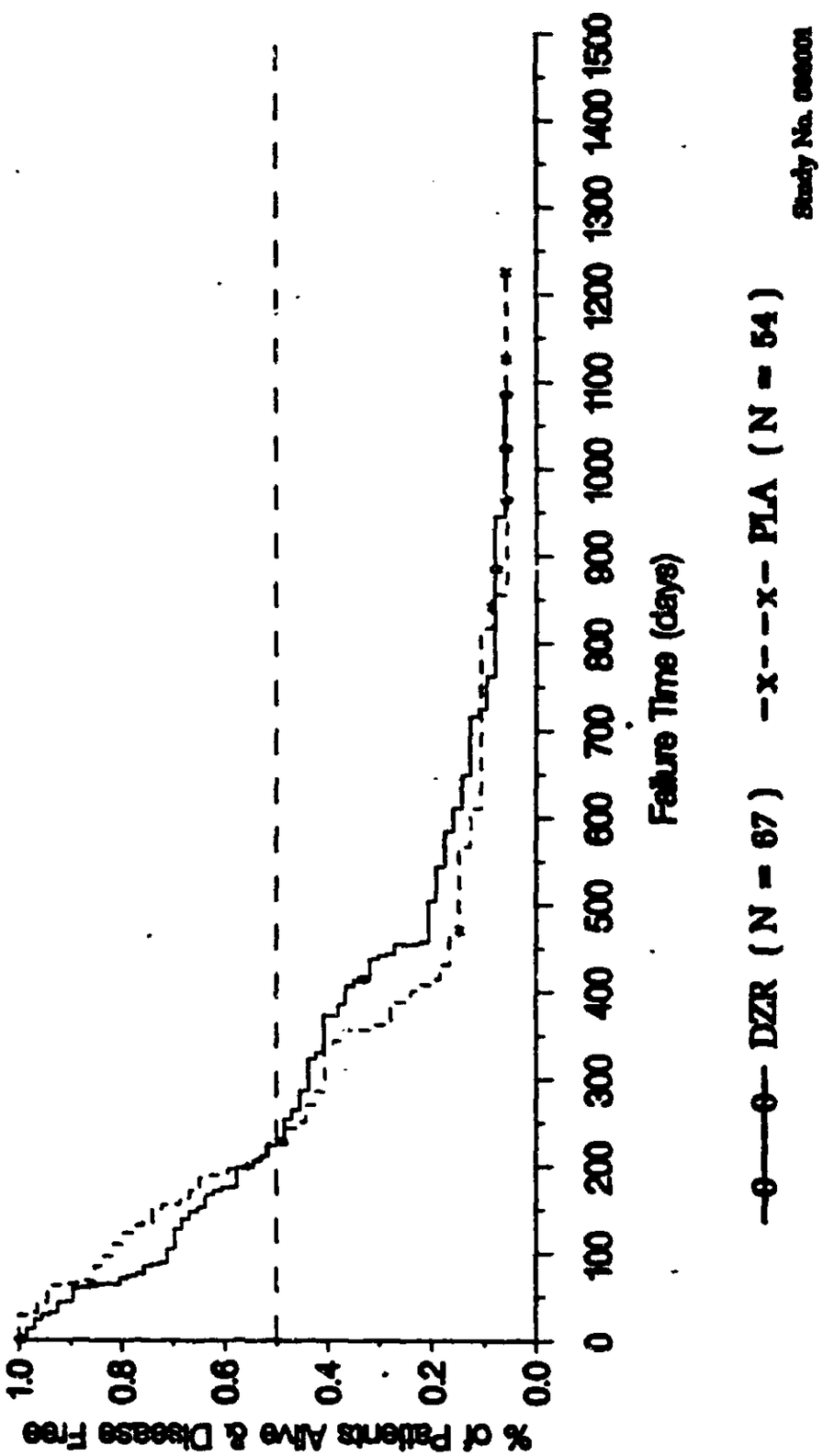

TABLE 15A *(cont'd)*

CLINICAL TOXICITIES

DISTRIBUTION OF MOST SEVERE TOXICITY GRADES OVER ALL COURSES

20:1 Patients

Variable	DZR (%)	PLA (%)	Statistical Test	
			Statistic[a]	Result
Sepsis			WRS	X^2=6.93 P=0.008
0	40 (62%)	44 (83%)		
3	22 (34%)	9 (17%)		
4	3 (5%)	0 (0%)		
Infection			PCS	X^2=5.00 P=0.025
Present	23 (35%)	9 (17%)		
Absent	42 (65%)	44 (83%)		
Neurotoxicity			PCS	X^2=0.01 P=0.92
Present	9 (14%)	7 (13%)		
Absent	56 (86%)	46 (87%)		

[a]WRS=Wilcoxon rank sum; PCS=Pearson chi square; FXT=Fisher's exact test. These tests are based on all scores, including zero.

TABLE 17A *(cont'd)*

REPORTED ADVERSE EXPERIENCES - ALL COURSES

20:1 Patients

Adverse Experience	DZR (%)	PLA (%)
NER/CNS/B		
Agitation	1 (1%)	0 (0%)
Amnesia	1 (1%)	0 (0%)
Anxiety	5 (7%)	1 (2%)
Ataxia	1 (1%)	2 (4%)
Bucco-glossal Syndrome	0 (0%)	1 (2%)
Confusion	1 (1%)	0 (0%)
Convulsion	1 (1%)	0 (0%)
Depression	6 (9%)	5 (9%)
Dizziness	5 (7%)	3 (6%)
Dystonia	0 (0%)	0 (0%)
Hyperkinesia	1 (1%)	0 (0%)
Hypertonia	2 (3%)	2 (4%)
Hypokinesia	1 (1%)	0 (0%)
Incoordination	2 (3%)	0 (0%)
Insomnia	3 (4%)	2 (4%)
Nervousness	5 (7%)	2 (4%)
Somnolence	1 (1%)	0 (0%)
Speech Disorder	0 (0%)	0 (0%)
Thinking Abnormal	1 (1%)	0 (0%)
Tremor	1 (1%)	0 (0%)
Vertigo	1 (1%)	0 (0%)
NER/CNS/SC	(34)	(17)
Paraplegia	0 (0%)	0 (0%)
NER/GEN		
Abnormal Gait	0 (0%)	0 (0%)
Hypersthesia	0 (0%)	0 (0%)
NER/PNS		
Paresthesia	3 (4%)	5 (9%)
NER/PNS/SN		
Foot Drop	0 (0%)	0 (0%)
RES/BRON		
Asthma	1 (1%)	0 (0%)
Bronchitis	0 (0%)	0 (0%)
RES/DPRM		
Hiccup	0 (0%)	0 (0%)

TABLE 29A

EXTENT OF ON-STUDY DOSING

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic*	Result
No. Randomized	67	54		
No. Courses Given			WRS	X^2=0.15 P=0.70
Median	6	6		
Range (min.-max.)	0, 35	0, 14		
CTX/Course (mg/M²)			WRS	X^2=0.10 P=0.75
Median	487.5	493.7		
Range (min.-max.)	0, 515.4	0, 500		
5-FU/Course (mg/M²)			WRS	X^2=0.10 P=0.75
Median	487.5	493.7		
(min.-max.)	0, 515.4	0, 500		
Cumulative CTX (mg/M²)			WRS	X^2=0.02 P=0.88
Median	2507.7	2900		
Range (min.-max.)	0, 12906.9	0, 7000		
Cumulative 5-FU (mg/M²)			WRS	X^2=0.02 P=0.88
Median	2507.7	2900		
Range (min.-max.)	0, 12906.9	0, 7000		

*WRS=Wilcoxon rank sum.
CTX=Cyclophosphamide; 5-FU=5-Fluorouracil.

TABLE 31A

DEVIATIONS FROM INTENDED DOSING SCHEDULE
20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	67	54		
Median No. of Courses	6	6	WRS	X^2=0.15 P=0.70
No. of Patients with Dose Reductions			WRS	X^2=0.21 *P*=0.65
0	32 (48%)	27 (50%)		
1	7 (10%)	8 (15%)		
2	6 (9%)	3 (6%)		
3	5 (7%)	2 (4%)		
4	2 (3%)	5 (9%)		
≥5	15 (22%)	9 (17%)		
No. of Patients with Dose Delays			WRS	X^2=1.19 P=0.27
0	30 (45%)	27 (50%)		
1	16 (24%)	8 (15%)		
2	6 (9%)	12 (22%		
3	3 (4%)	3 (6%)		
4	2 (3%)	2 (4%)		
≥5	10 (15%)	2 (4%)		
Median Dosing Intensity				
DOX	0.94	0.93	WRS	X^2=0.10 P=0.75
CTX/5-FU	0.89	0.91	WRS	X^2<0.01 P=0.97

[a]WRS=Wilcoxon rank sum.
CTX, 5-FU (CTX=Cyclophosphamide, 5-FU=5-Fluorouracil).
DOX (DOX=Doxorubicin).

TABLE 33A

PATIENT DISPOSITION

20:1 Patients

	DZR	PLA
No. Randomized	67	54
No. On-Study (%)	1 (1%)	0 (0%)
No. Off-Study (%)	66 (99%)	54 (100%)
Primary Reason Off-Study (%)		
Progressive Disease	25 (38%)	15 (28%)
Cardiac Toxicity[a]	9 (14%)	24 (44%)
Adverse Reaction[b]	3 (5%)	1 (2%)
Patient Refusal	13 (20%)	6 (11%)
Protocol Violation	3 (5%)	2 (4%)
Death	7 (11%)	1 (2%)
Lost to Follow-Up	1 (2%)	0 (0%)
Other	4 (6%)	4 (7%)
Randomized, Not Treated	1 (2%)	1 (2%)

[a]Includes drop in LVEF, CHF, and other cardiac related toxicities.
[b]Non-cardiac related reasons.

TABLE 34A

PATIENT DISPOSITION BY COURSE

20:1 Patients

	Course																					
	1		2		3		4		5		6		7		8		9		10		>10	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P
No. Patients On-Study	66[a]	53[a]	60	50	54	49	40	42	35	39	35	34	32	24	31	24	27	14	24	9	23	5
No. Patients Off-Study	6	3	6	1	14	7	5	3	0	5	3	10	1	0	4	10	3	5	1	4	23	5
Reason Off-Study:																						
Progressive Disease	0	1	3	1	6	4	1	2	0	3	1	1	1	0	3	2	3	1	0	0	7	0
Cardiac Toxicity[b]	0	0	0	0	6	2	1	0	0	0	0	5	0	0	0	5	0	4	1	3	1	5
Adverse Reaction[c]	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0
Patient Refusal	2	1	0	0	0	0	0	0	0	0	2	3	0	0	0	2	0	0	0	0	9	0
Protocol Violation	0	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Death	2	0	1	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lost to Follow-Up	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	1	1	0	0	1	0	1	0	0	1	1	0	0	0	0	2	0
Censored	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

[a]Excludes a patient randomized, not treated.
[b]Includes drop in LVEF, CHF, and other cardiac related toxicities.
[c]Non-cardiac related reasons.

TABLE 35A

TIME TO OFF-STUDY

ANY REASON

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic*	Result
No. Randomized	67	54	[redacted]	[redacted]
No. Off-Study (%)	66 (99%)	54 (100%)	[redacted]	[redacted]
Median Time (days)	153	150	[redacted]	[redacted]
Hazard Ratio (P:D)	1.689		LR	X^2=6.79 P=0.009
95% C.I. of (P:D)	(1.132, 2.520)		GW	X^2=0.35 P=0.55

*LR=Logrank; GW=Generalized Wilcoxon.

Figure 9A Time to Off–Study
201 Patients

[redacted] = 1.000
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]

TABLE 2B

BASELINE DEMOGRAPHIC CHARACTERISTICS

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
Age (years)			WRS	X^2=0.87 P=0.35
Mean	56.4	55.4		
Median	58.0	56		
St. Dev.	12.2	10.9		
(min.-max.)	26, 84	25, 82		
n	168	181		
Weight (kg)			WRS	X^2=2.41 P=0.12
Mean	70.3	67.7		
Median	67.0	66		
St. Dev.	14.9	13.5		
(min.-max.)	39, 119	45.8, 119.5		
n	168	181		
Sex				
Female (%)	168 (100%)	181 (100%)		
Race			PCS	X^2=1.01 P=0.60
White (%)	124 (74%)	125 (69%)		
Black (%)	30 (18%)	37 (20%)		
Other (%)	14 (8%)	19 (11%)		

[a]WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square.
FXT=Fisher's Exact Test

21B

TABLE 3B

BASELINE CARDIAC RISK FACTORS
10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
Age >65 years			PCS	X^2=1.63 P=0.20
Yes (%)	41 (24%)	34 (19%)		
No (%)	127 (76%)	147 (81%)		
Prior Radiation to Mediastinum			PCS	X^2=1.72 P=0.19
Yes (%)	20 (12%)	14 (8%)		
No (%)	148 (88%)	167 (92%)		
History of Heart Disease[b]			PCS	X^2=0.04 P=0.84
Yes (%)	14 (8%)	14 (8%)		
No (%)	154 (92%)	167 (92%)		
Hypertension[c]			PCS	X^2=0.29 P=0.59
Yes (%)	46 (27%)	45 (25%)		
No (%)	122 (73%)	136 (75%)		
Diabetes Mellitus[c]			PCS	X^2=4.02 P=0.045
Yes (%)	10 (6%)	22 (12%)		
No (%)	158 (94%)	159 (88%)		
LVEF ≤10% above Lower Limit of Normal			PCS	X^2=3.03 P=0.08
Yes (%)	62 (37%)	51 (28%)		
No (%)	106 (63%)	130 (72%)		

[a]PCS=Pearson Chi Square.
[b]Previous myocardial infarction, significant arrhythmia, angina.
[c]Requiring medical therapy.

21 C

TABLE 4B *(cont'd)*

BASELINE DISEASE STATUS

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Immunotherapy Yes (%) No (%)	0 (0%) 168 (100%)	0 (0%) 181 (100%)	PCS	
Hormonal Therapy Yes (%) No (%)	89 (53%) 79 (47%)	85 (47%) 96 (53%)	PCS	X^2=1.26 P=0.26
Dominant Disease Site			WRS	X^2<0.01 P=0.97
Visceral (%)	126 (75%)	138 (76%)		
Bone (%)	31 (19%)	28 (15%)		
Soft Tissue (%)	10 (6%)	15 (8%)		
Number of Estrogen Receptors			WRS	X^2=2.09 P=0.15
0-10 (fmol/mg)	58 (45%)	78 (55%)		
10-100 (fmol/mg)	48 (38%)	43 (30%)		
>100 (fmol/mg)	22 (17%)	21 (15%)		
Number of Progesterone Receptors			WRS	X^2=0.40 P=0.53
0-10 (fmol/mg)	70 (57%)	88 (63%)		
10-100 (fmol/mg)	39 (32%)	34 (24%)		
>100 (fmol/mg)	13 (11%)	18 (13%)		
Disease Measurability Status			PCS	X^2=0.05 P=0.98
Measurable (%)	30 (18%)	21 (17%)		
Non-Measurable (%)	26 (16%)	29 (16%)		
Both (%)	111 (66%)	121 (67%)		

ONLY

[a]FXT=Fisher's Exact test; WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square; LR=Logrank.
[b]Time between initial disease diagnosis and first relapse.

21 D

TABLE 4B *(cont'd)*

BASELINE DISEASE STATUS

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
WBC ($x10^3/mm^3$)			WRS	X^2=2.76 P=0.10
Mean	7.3	7.7		
Median	6.7	7.2		
St. Dev.	2.9	2.8		
(min., max.)	3.3, 25.6	2.8, 18.3		
n	168	181		
Absolute Granulocytes ($x10^3/mm^3$)			WRS	X^2=4.11 P=0.043
Mean	5.098	5.525		
Median	4.583	4.884		
St. Dev.	2.770	2.533		
(min., max.)	1.47, 24.576	1.554, 16.104		
n	168	180		
Platelets ($x10^3/mm^3$)			WRS	X^2=0.42 P=0.52
Mean	324.0	326.2		
Median	300.0	314.0		
St. Dev.	111.3	99.1		
(min., max.)	111, 964	121, 633		
n	168	180		
Hemoglobin (g/dl)			WRS	X^2=0.17 P=0.68
Mean	12.5	12.8		
Median	12.7	12.6		
St. Dev.	1.6	1.7		
(min., max.)	8.1, 17.4	8.2, 16.4		
n	167	177		

[a]FXT=Fisher's Exact test; WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square; LR=Logrank.
[b]Time between initial disease diagnosis and first relapse.

TABLE 4B

BASELINE DISEASE STATUS

10:1 Patients

Variable	DZR	FLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
Disease Free Interval[b]			LR	X^2=2.54 P=0.11
Median	681.5	606.0		
No. Disease Sites			WRS	X^2=0.43 P=0.51
Mean	3.5	3.6		
Median	3.0	3.0		
St. Dev.	2.2	2.1		
(min.-max.)	0, 13	1, 11		
n	168	181		
Performance Status			WRS	X^2=2.14 P=0.14
0	73 (44%)	92 (51%)		
1	88 (53%)	86 (48%)		
2	6 (4%)	3 (2%)		
3	0 (0%)	0 (0%)		
4	0 (0%)	0 (0%)		
Incidence of Prior Therapies				
Surgery Yes (%) No (%)	 132 (79%) 36 (21%)	 140 (77%) 41 (23%)	PCS	X^2=0.08 P=0.78
Radiotherapy Yes (%) No (%)	 44 (26%) 124 (74%)	 45 (25%) 136 (75%)	PCS	X^2=0.08 P=0.78
Chemotherapy Yes (%) No (%)	 72 (43%) 96 (57%)	 63 (35%) 118 (65%)	PCS	X^2=2.38 P=0.12

[a]FXT=Fisher's Exact test; WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square; LR=Logrank.
[b]Time between initial disease diagnosis and first relapse.

21 F

TABLE 4A *(cont'd)*

BASELINE DISEASE STATUS

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
WBC ($x10^3/mm^3$)			WRS	X^2=2.22 P=0.14
Mean	8.0	7.3		
Median	7.6	6.9		
St. Dev.	2.6	2.3		
(min., max.)	3.2, 16.4	2.9, 15.4		
n	67	54		
Absolute Granulocytes ($x10^3/mm^3$)			WRS	X^2=1.79 P=0.18
Mean	5.661	5.029		
Median	4.993	4.876		
St. Dev.	2.498	2.150		
(min., max.)	2.112, 14.612	1.914, 14.168		
n	66	54		
Platelets ($x10^3/mm^3$)			WRS	X^2=0.06 P=0.80
Mean	340.6	346.9		
Median	319.0	334.0		
St. Dev.	120.6	119.5		
(min., max.)	104, 763	148, 868		
n	67	54		
Hemoglobin (g/dl)			WRS	X^2=0.07 P=0.78
Mean	12.7	12.6		
Median	12.9	12.6		
St. Dev.	1.6	1.4		
(min., max.)	9, 16.7	8.6, 15.4		
n	67	54		

[a]FXT=Fisher's Exact test; WRS=Wilcoxon Rank Sum; PCS=Pearson Chi Square; LR=Logrank.
[b]Time between initial disease diagnosis and first relapse.

6.3.2 10:1 Patients

Table 6.3.2 indicates the number of patients summarized in the statistical analyses.

TABLE 6.3.2
10:1 Patients

Number of Patients Participating in Data Summaries

	DZR	PLA
No. Randomized	168	181
Cardiotoxicity Efficacy Time to Event (Drops in LVEF, CHF)	168	181
Changes in LVEF Baseline 150 mg/M^2 300 mg/M^2 400 mg/M^2 500 mg/M^2 550 mg/M^2 600 mg/M^2	 168 131 77 33 26 21 17	 181 147 99 65 34 19 8
Antitumor Efficacy Response Rates Intent-to-Treat[a] Evaluable[a,b]	 141 116	 152 137
Disease Progression	168	181
Disease-Free Survival	168	181
Survival	168	181
Safety Course 1 *(Clinical Toxicities, Hematologies)*[c] Course 3 *(Lab Toxicities)* All Courses	 165 127 165	 179 152 180
Quality of Life Changes in Performance Status Changes in Body Weight	 168 168	 181 181

[a]Randomized with bidimensional, measurable disease.
[b]≥3 courses of therapy with objective assessment of disease status and not in gross violation of the protocol.
[c]Maximum number of patients participating in at least one analysis.

TABLE 5B

TIME TO CARDIAC EVENT

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
No. Events (%)	19 (11%)	52 (29%)		
Median Event Time (mg/M^2 of DOX[b])	1156.57	503.18		
Hazard Ratio (P:D)	2.875		LR	X^2=15.52 P<0.001
95% C.I. of (P:D)	(1.654, 4.997)		GW	X^2=1.94 P=0.16

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin

Figure 1B Time to Cardiotoxic Event
10:1 Patients

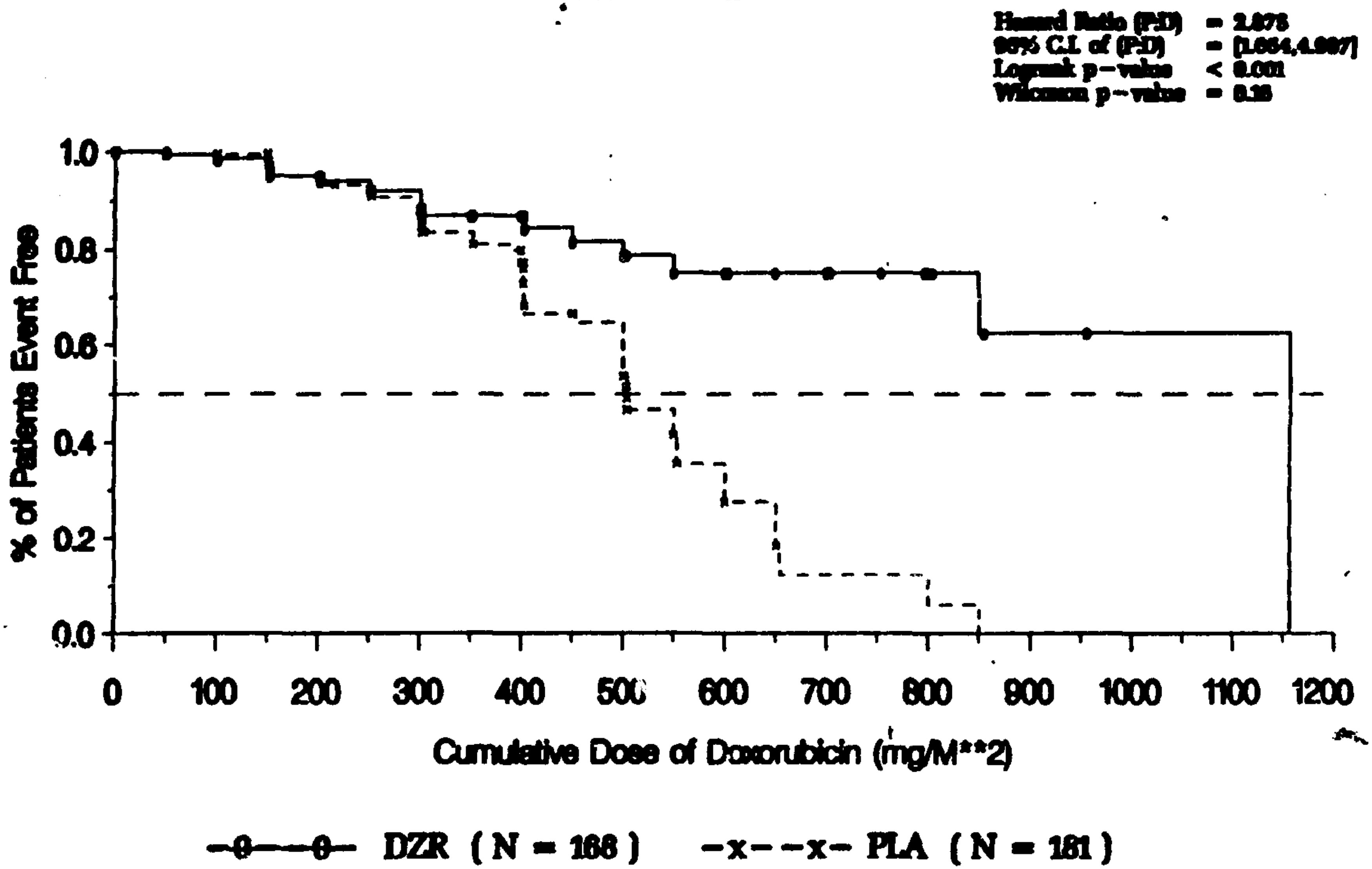

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TABLE 6B

TIME TO CONGESTIVE HEART FAILURE

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
No. Events (%)	2 (1%)	10 (6%)		
Median Event Time (mg/M^2 of DOX[b])	1150	_[c]		
Hazard Ratio (P:D)	10.776		LR	X^2=7.73 P=0.005
95% C.I. of (P:D)	(1.356, 85.648)		GW	X^2=4.03 P=0.045

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin
[c]The median is unestimable.

Figure 2B Time to Congestive Heart Failure
101 Patients

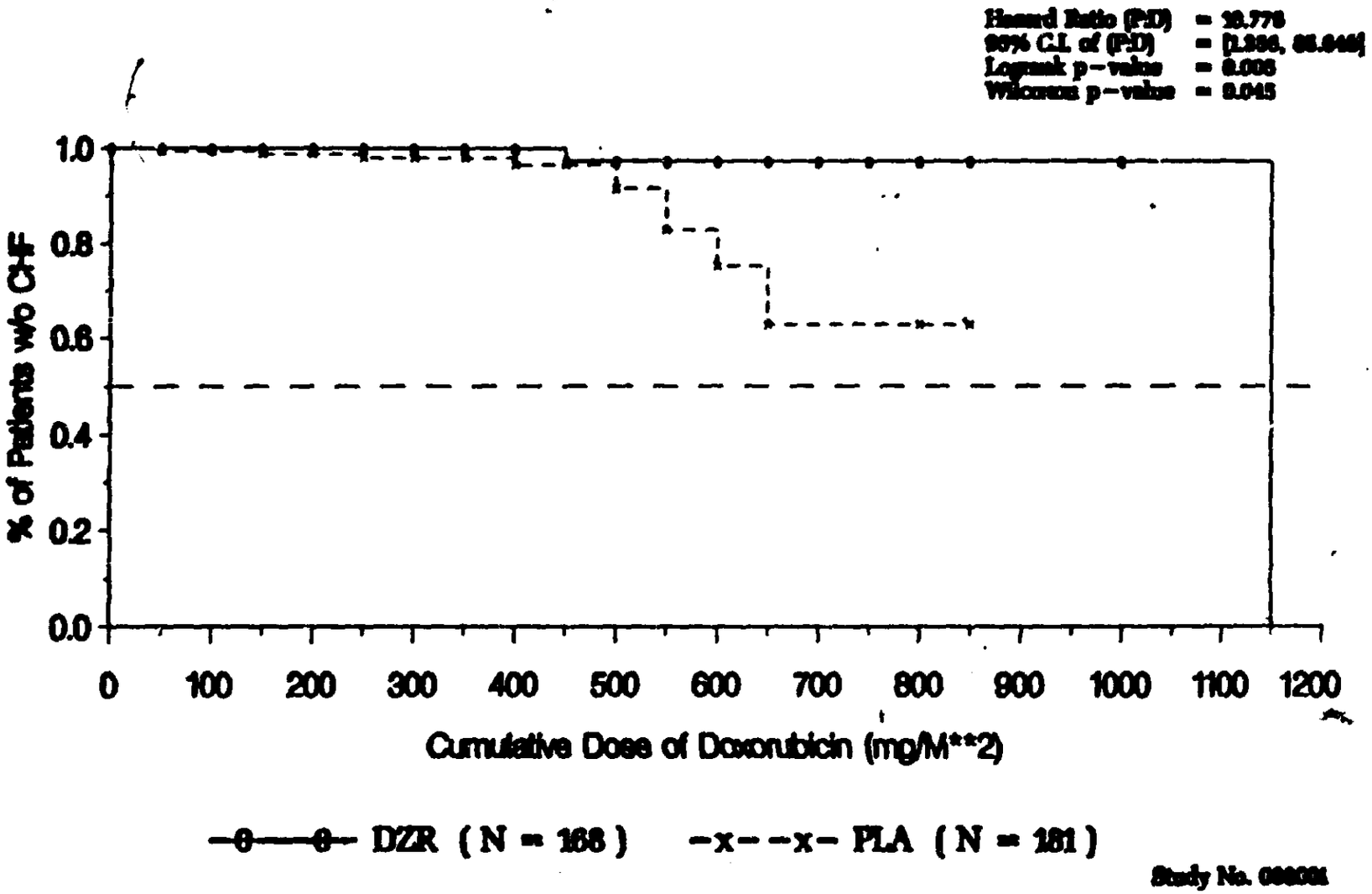

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TABLE 7B

CHANGES IN LVEF (%) OVER CUMULATIVE DOSE OF DOXORUBICIN
10:1 Patients

	Cumulative Dose of Doxorubicin (mg/M²)													
	Baseline		150		300		400		500		550		600	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Mean	63.7	63.5	62.3	61.0	62.6	59.0	64.7	56.4	62.7	53.7	61.7	52.1	62.8	52.3
Median	62.0	63.0	63.0	60.0	63.0	58.0	65.0	59.0	61.0	53.0	59.0	52.0	60.0	53.0
Std. Dev.	9.8	9.5	8.6	8.7	8.9	8.5	6.4	10.4	7.6	9.5	9.4	11.0	8.5	11.2
n	168	181	131	147	77	99	33	65	26	34	21	19	17	8
Estimate of Effect[a]	0.2		1.3		3.6		8.3		9.0		9.6		10.5	
p-value[b]	0.94		0.10		0.008		<0.001		<0.001		0.004		0.020	
Change From Baseline														
Mean			-1.4	-2.8	-2.5	-3.9	-0.3	-7.0	-1.3	-8.9	-1.7	-11.8	-0.2	-9.8
Median			-1.0	-2.0	-2.0	-4.0	1.0	-5.0	-1.0	-8.0	-4.0	-10.0	-2.0	-11.0
Std. Dev.			9.0	8.3	9.3	8.5	9.2	11.1	8.8	10.6	9.8	10.5	11.0	7.3
n			131	147	77	99	33	65	26	34	21	19	17	8
Estimate of Effect[a]			1.4		1.4		6.7		7.6		10.1		9.6	
p-value[b]			0.18		0.38		0.005		0.003		0.007		0.036	

[a]Mean Difference between treatment group means: D minus P.
[b]Wilcoxon rank sum test.

TABLE 6.5.1.2
10:1 Patients

Number of Patients Included in Response Rate Summaries

	DZR	PLA
No. of Randomized Patients	168	181
No. of Patients with Measurable Disease[a]	141	152
No. of Intent-to-Treat Patients	141	152
No. of Patients with Tumor Assessment	127	143
No. of Off-Study Patients without Tumor Assessment[b]	14	9
Reasons Off-Study[c]		
Progressive Disease	0	0
Cardiotoxicity	1	0
Adverse Reaction	2	0
Patient Refusal	1	3
Protocol Violation	4	2
Death	3	1
Lost to Follow-Up	1	1
Randomized, Not Treated	1	0
Other	1	2
No. of Evaluable Patients[d]	116	137

[a]Patients with nonmeasurable disease were excluded from the response rate analyses.
[b]These patients were categorized as treatment failures in the intent-to-treat analysis.
[c]Patients lacking objective determination of tumor status while on-study.
[d]Patients receiving ≥3 courses of treatment, had objective measurement of their disease, and were not in gross violation of the protocol.

In the 10:1 group, unexpectedly, 15% fewer of the intent-to-treat patients responded on the DZR arm compared to the control group (Table 8B). This difference was statistically significant at a test size of 1% (p=0.007). The rates of response in the intent-to-treat cohort were 48% (67/141) and 63% (96/152) on the DZR and control arms, respectively.

TABLE 8B

RESPONSE RATES
10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Randomized Patients				
No. Patients	141	152		
Best Response				
CR (%)	13 (9%)	20 (13%)		
PR (%)	54 (38%)	76 (50%)		
SD (%)	31 (22%)	30 (20%)		
PD (%)	29 (21%)	17 (11%)		
NA (%)[b]	14 (10%)	9 (6%)		
Response Rate[c]	67/141 (48%)	96/152 (63%)		
Estimate of Effect[c,d]	-15%		PCS	X^2=7.25 P=0.007
95% C.I. of Effect[c,d]	(-27%, -4%)			
Evaluable Patients				
No. Patients	116	137		
Best Response				
CR (%)	12 (10%)	20 (15%)		
PR (%)	50 (43%)	75 (55%)		
SD (%)	29 (25%)	27 (20%)		
PD (%)	25 (22%)	15 (11%)		
Response Rate[c]	62/116 (53%)	95/137 (69%)		
Estimate of Effect[c,d]	-16%		PCS	X^2=6.74 P=0.009
95% C.I. of Effect[c,d]	(-28%, -4%)			

[a]PCS=Pearson chi square (comparing proportion of responders to nonresponders).
[b]NA=Not assessed while on-study; categorized as treatment failures.
[c]Response=CR+PR.
[d]Difference between treatment groups in response rates: D minus P.

TABLE 9B

TIME TO DISEASE PROGRESSION

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
No. Events (%)	89 (53%)	96 (53%)		
Median Failure Time (days)	232	243		
Hazard Ratio (P:D)	0.908		LR	X^2=0.43 P=0.51
95% C.I. of (P:D)	(0.680, 1.212)		GW	X^2=2.91 P=0.09

[a]LR=Logrank; GW=Generalized Wilcoxon.

Figure 3B Time to Disease Progression
10:1 Patients

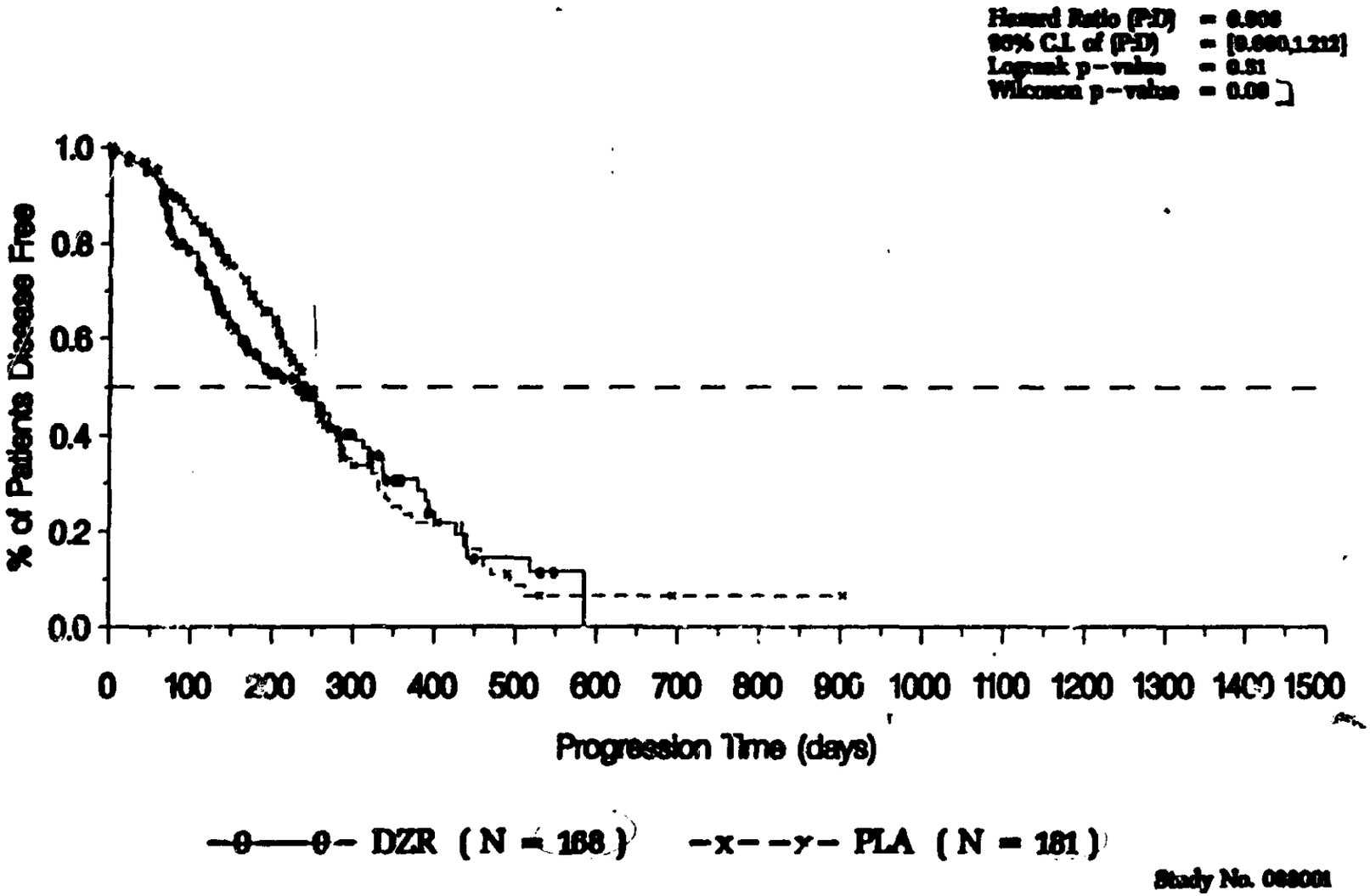

TABLE 10B

DISEASE FREE SURVIVAL

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181	[redacted]	[redacted]
No. Events (%)	99 (59%)	112 (62%)	[redacted]	[redacted]
Median Failure Time (days)	271	258	[redacted]	[redacted]
Hazard Ratio (P:D)	0.918		LR	X^2=0.38 P=0.54
95% C.I. of (P:D)	(0.700, 1.204)		GW	X^2=1.80 P=0.18

[a]LR=Logrank; GW=Generalized Wilcoxon.

Figure 4B Disease Free Survival
101 Patients
Hazard Ratio (PLA) = 0.608
95% C.I. of (PLA) = [0.700,1.204]
Logrank p-value = 0.54
Wilcoxon p-value = 0.38
% of Patients Alive & Disease Free
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
Failure Time (days)
DZR (N = 188)
PLA (N = 181)
Study No. 060501

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TABLE 11B

SURVIVAL

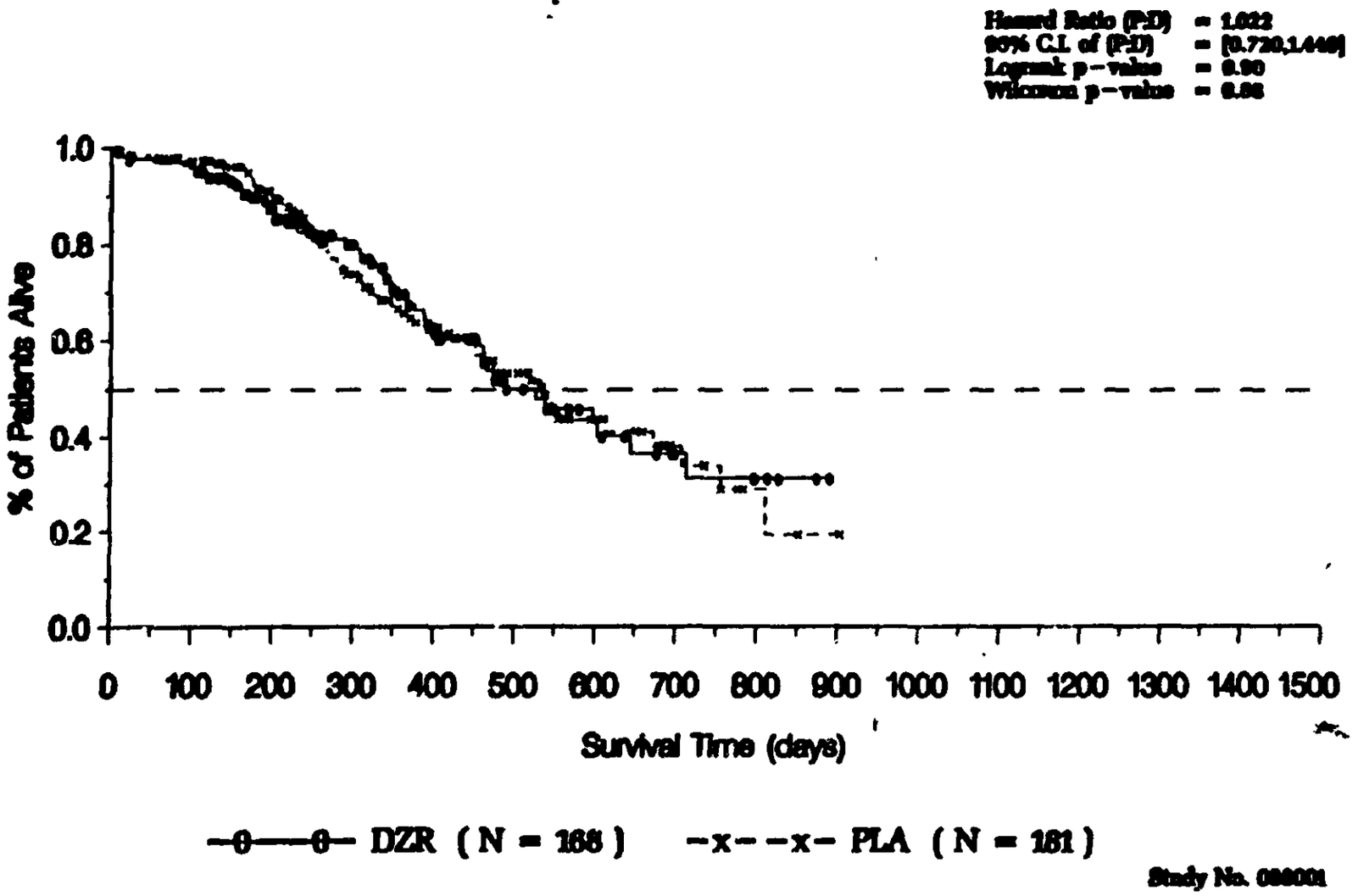
10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
No. Dead (%)	58 (35%)	69 (38%)		
Median Survival (days)	526	537		
Hazard Ratio (P:D)	1.022		LR	X^2=0.01 P=0.90
95% C.I. of (P:D)	(0.720, 1.449)		GW	X^2=0.02 P=0.88

[a]LR=Logrank; GW=Generalized Wilcoxon.

26 A

Figure 5B Survival
10:1 Patients



26 B

TABLE 32B

QUALITY OF LIFE MEASURES

TIME TO DECREASED STATUS

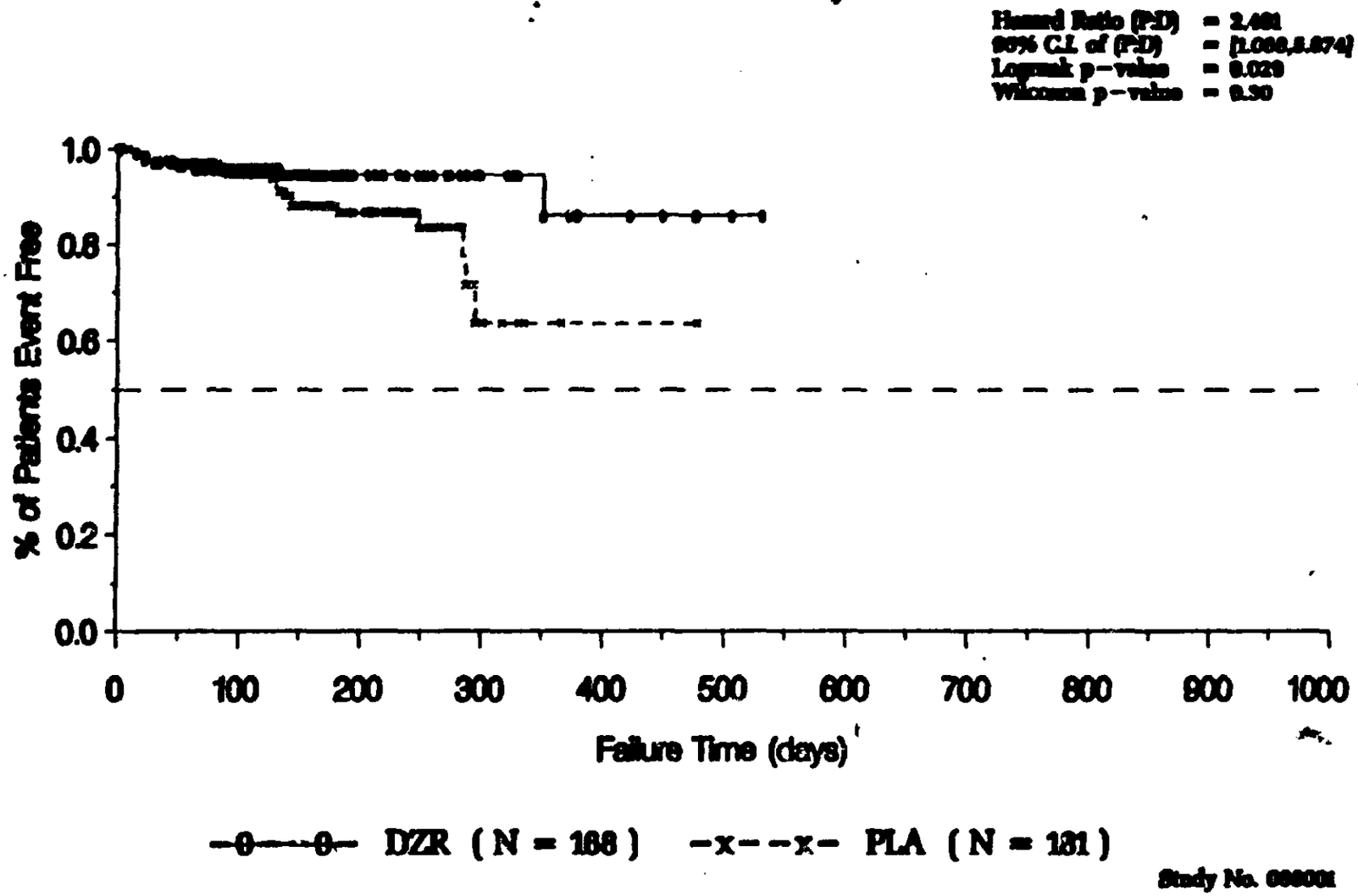
10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
Performance Status ≥3 (On-Study)				
No. Events (%)	8 (5%)	20 (10%)		
Median Time (days)	--[c]	--[c]		
Hazard Ratio (P:D)	2.461		LR	X^2=4.76 P=0.029
95% C.I. of (P:D)	(1.068, 5.674)		GW	X^2=1.09 P=0.30
Performance Status ≥3 (Any Time)				
No. Events (%)	47 (28%)	52 (29%)		
Median Time (days)	482	563		
Hazard Ratio (P:D)	0.944		LR	X^2=0.08 P=0.78
95% C.I. of (P:D)	(0.636, 1.402)		GW	X^2=0.12 P=0.73
≥15% Decrease in Body Weight[b]				
No. Events (%)	15 (9%)	13 (7%)		
Median Time (days)	--[c]	--[c]		
Hazard Ratio (P:D)	0.681		LR	X^2=1.03 P=0.31
95% C.I. of (P:D)	(0.323, 1.435)		GW	X^2=4.50 P=0.034

[a]LR=Logrank; GW=Generalized Wilcoxon.
[b]Relative to baseline.
[c]The median is unestimable.

26 A

Figure 6B Time to Performance Status ≥ 3
101 Patients – On–Study



2 6 C

TABLE 33B

PATIENT DISPOSITION

10:1 Patients

	DZR	PLA
No. Randomized	168	181
No. On-Study (%)	31 (18%)	17 (9%)
No. Off-Study (%)	137 (82%)	164 (91%)
Primary Reason Off-Study (%)		
Progressive Disease	70 (51%)	59 (36%)
Cardiac Toxicity[a]	13 (9%)	46 (28%)
Adverse Reaction[b]	5 (4%)	4 (2%)
Patient Refusal	23 (17%)	21 (13%)
Protocol Violation	10 (7%)	13 (8%)
Death	4 (3%)	6 (4%)
Lost to Follow-Up	1 (1%)	2 (1%)
Other	9 (7%)	13 (8%)
Randomized, Not Treated	2 (2%)	0 (0%)

[a]Includes drop in LVEF, CHF, and other cardiac related toxicities.
[b]Non-cardiac related reasons.

26 D

TABLE 35B

TIME TO OFF-STUDY

ANY REASON

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic*	Result
No. Randomized	168	181		
No. Off-Study (%)	137 (82%)	164 (91%)		
Median Time (days)	139	156		
Hazard Ratio (P:D)	1.108		LR	X^2=0.77 P=0.38
95% C.I. of (P:D)	(0.880, 1.394)		GW	X^2=1.28 P=0.26

*LR=Logrank; GW=Generalized Wilcoxon.

Figure 9B Time to Off-Study
10:1 Patients

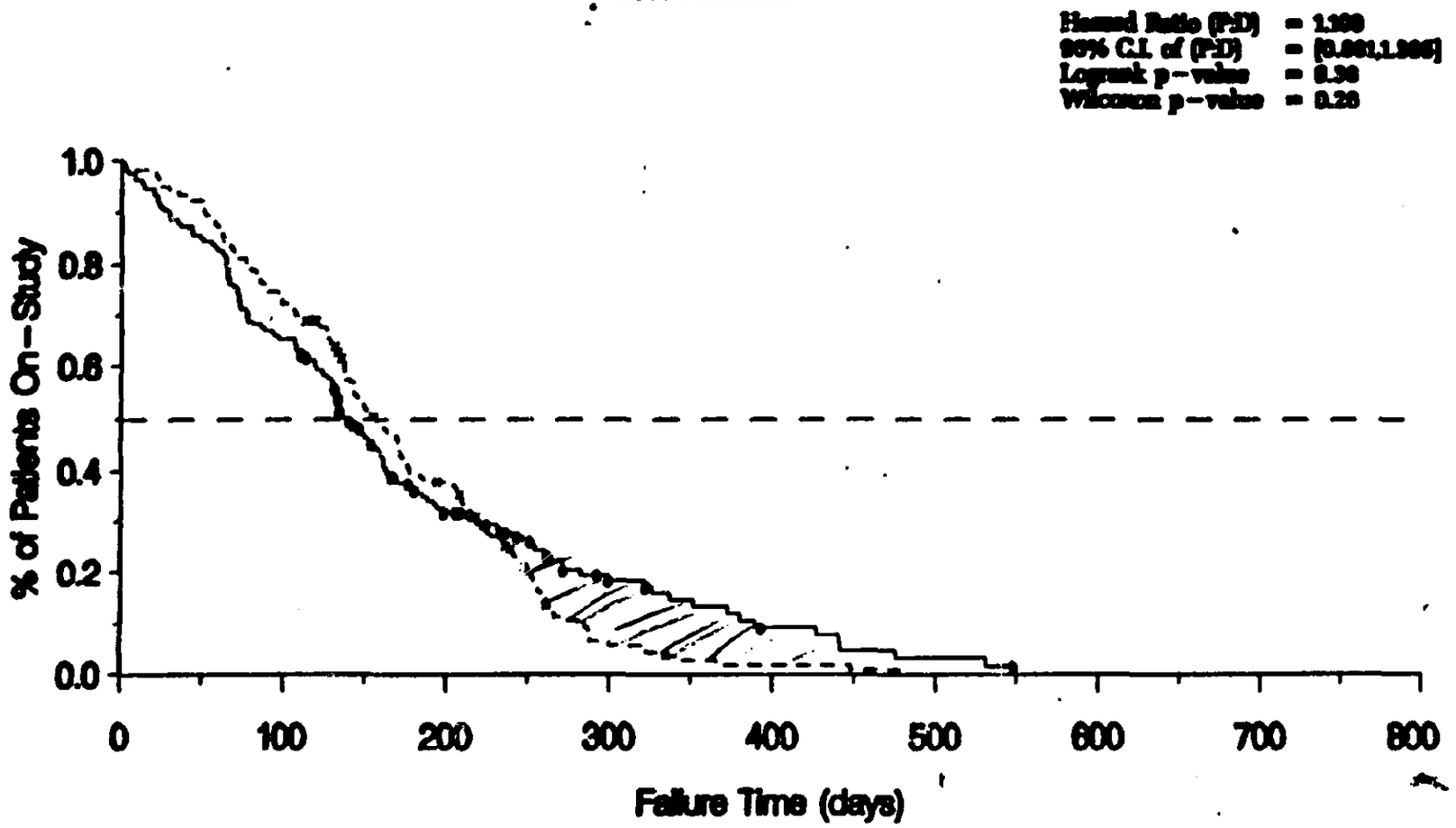


TABLE 34B

PATIENT DISPOSITION BY COURSE

10:1 Patients

	Course																					
	1		2		3		4		5		6		7		8		9		10		>10	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P
No. Patients On-Study	165[a]	181	149	170	142	162	115	141	106	123	91	117	68	80	57	72	53	55	44	45	35	25
No. Patients Off-Study	16	11	7	8	27	21	9	18	15	6	23	37	11	8	4	17	9	10	9	20	35	25
Reason Off-Study:																						
Progressive Disease	2	0	3	2	21	11	4	10	11	3	8	12	3	4	2	2	0	5	4	5	12	5
Cardiac Toxicity[b]	2	0	0	0	3	4	0	2	0	0	4	10	0	1	1	10	1	0	0	10	2	9
Adverse Reaction[c]	1	0	1	0	1	2	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0
Patient Refusal	1	3	2	2	1	0	2	2	2	3	6	2	5	1	0	2	0	0	2	3	2	3
Protocol Violation	4	4	1	3	0	1	2	1	0	0	2	1	0	1	0	1	0	1	0	0	0	0
Death	4	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
Lost to Follow-Up	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	1	1	0	0	1	2	1	2	1	0	2	7	0	0	0	0	0	0	1	1	2	0
Censored	0	0	0	0	0	0	0	0	0	0	1	4	3	1	1	1	8	4	2	1	16	6

[a]Excludes two patients randomized, not treated; excludes one patient with protocol violation prior to dosing.
[b]Includes drop in LVEF, CHF, and other cardiac related toxicities.
[c]Non-cardiac related reasons.

TABLE 12B

CLINICAL TOXICITIES - INCIDENCE AND MEAN SEVERITY
MOST SEVERE GRADE AT COURSE 1
10:1 Patients

	Incidence (%)		Mean Severity Given Toxicity	
	DZR	PLA	DZR	PLA
No. Patients	165	179		
Esophagitis	6 (4%)	5 (3%)	1.8	1.2
Stomatitis	28 (17%)	33 (18%)	1.5	1.7
Alopecia	133 (81%)	133 (74%)	2.5	2.6
Streaking/Erythema	4 (2%)	2 (1%)		
Urticaria	0 (0%)	0 (0%)		
Recall Skin Reaction	0 (0%)	0 (0%)		
Pain on Injection†	7 (4%)	1 (1%)	1.7	1.0
Extravasation	0 (0%)	0 (0%)		
Phlebitis	3 (2%)	1 (1%)	1.3	1.0
Hemorrhage	1 (1%)	0 (0%)		
CHF	0 (0%)	1 (1%)		
Dysphagia	7 (4%)	5 (3%)	2.1	1.6
Anorexia	43 (26%)	46 (26%)	1.7	1.6
Nausea†	98 (59%)	125 (70%)	1.7	1.7
Vomiting	74 (45%)	97 (54%)	1.8	1.6
Diarrhea	19 (12%)	19 (11%)	1.6	1.4
Fatigue/Malaise	58 (35%)	62 (35%)	1.7	1.6
Fever†	40 (24%)	26 (14%)	2.0	1.8
Sepsis	19 (12%)	12 (7%)	3.0	3.0
Infection	21 (13%)	12 (7%)		
Neurotoxicity††	13 (8%)	3 (2%)		

† p<.05, Pearson chi square; †† p<.01, Pearson chi square, based on incidence proportions.
* p<.05, Wilcoxon rank sum; ** p<.01, Wilcoxon rank sum based on severity scores, excluding scores of zero.

TABLE 14B

CLINICAL TOXICITIES - INCIDENCE AND MEAN SEVERITY MOST SEVERE GRADE OVER ALL COURSES

10:1 Patients

	Incidence (%)		Mean Severity Given Toxicity	
	DZR	PLA	DZR	PLA
No. Patients	165	180		
Esophagitis †	7 (4%)	17 (10%)	2.0	1.8
Stomatitis	60 (36%)	74 (41%)	1.8	1.8
Alopecia	156 (95%)	169 (94%)	3.6	3.5
Streaking/Erythema	10 (6%)	9 (5%)		
Urticaria	5 (3%)	1 (1%)		
Recall Skin Reaction	3 (2%)	3 (2%)	1.0	1.7
Pain on Injection††	17 (10%)	5 (3%)	1.6	1.6
Extravasation	4 (2%)	1 (1%)		
Phlebitis	7 (4%)	10 (6%)	1.7	1.5
Hemorrhage	5 (3%)	4 (2%)		
CHF†	2 (1%)	10 (6%)		
Dysphagia	13 (8%)	18 (10%)	2.1	1.7
Anorexia	72 (44%)	87 (49%)	1.8	1.9
Nausea††	131 (79%)	160 (89%)	1.9	1.9
Vomiting††	103 (62%)	137 (77%)	1.9	1.8
Diarrhea	33 (20%)	40 (22%)	1.8	1.5
Fatigue/Malaise	93 (56%)	108 (60%)	1.7	1.8
Fever*	63 (38%)	59 (33%)	2.1	1.9
Sepsis	30 (18%)	28 (16%)	3.1	3.2
Infection	40 (24%)	35 (19%)		
Neurotoxicity	24 (15%)	18 (10%)		

† p<.05, Pearson chi square; †† p<.01, Pearson chi square based on incidence proportions.
* p<.05, Wilcoxon rank sum, based on severity scores, excluding scores of zero.

TABLE 17B *(cont'd)*

REPORTED ADVERSE EXPERIENCES - ALL COURSES

10:1 Patients

Adverse Experience	DZR (%)	PL[illegible] (%)
NER/CNS/B		
Abnormal Dreams	1 (1%)	0 (0%)
Acute Brain Syndrome	0 (0%)	1 (1%)
Agitation	0 (0%)	0 (0%)
Akathisia	0 (0%)	0 (0%)
Amnesia	0 (0%)	2 (1%)
Anxiety	18 (11%)	19 (10%)
Aphasia	0 (0%)	1 (1%)
Ataxia	0 (0%)	1 (1%)
Confusion	0 (0%)	5 (3%)
Convulsion	0 (0%)	3 (2%)
Delirium	1 (1%)	1 (1%)
Depersonalization	0 (0%)	1 (1%)
Depression	13 (8%)	16 (9%)
Dizziness	8 (5%)	14 (8%)
Dyskinesia	0 (0%)	0 (0%)
Emotional Lability	0 (0%)	2 (1%)
Extrapyramidal Syndrome	0 (0%)	0 (0%)
Hallucinations	0 (0%)	1 (1%)
Hemiplegia	0 (0%)	0 (0%)
Hostility	1 (1%)	0 (0%)
Hyperkinesia	1 (1%)	0 (0%)
Hypertonia	3 (2%)	3 (2%)
Hypokinesia	1 (1%)	0 (0%)
Insomnia	10 (6%)	10 (6%)
Nervousness	4 (2%)	7 (4%)
Psychotic Depression	0 (0%)	0 (0%)
Somnolence	5 (3%)	9 (5%)
Speech Disorder	0 (0%)	0 (0%)
Stupor	1 (1%)	1 (1%)
Thinking Abnormal	0 (0%)	0 (0%)
Tremor	1 (1%)	1 (1%)
Vertigo	0 (0%)	2 (1%)
NER/CNS/SC		
Paraplegia	0 (0%)	0 (0%)

TABLE 23B

MYELOSUPPRESSION - ABSOLUTE GRANULOCYTE COUNT ($x10^3/mm^3$)

NADIR COUNTS OVER FIRST TWO WEEKS

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic*	Result
Course 1				
Mean	0.631	0.943	WRS	X^2=11.36 P<0.001
Median	0.362	0.598		
St. Dev.	0.775	1.098		
n	147	161		
(min.-max.)	0, 5.9	0, 8.1		
All Courses				
Mean	0.402	0.522	WRS	X^2=4.38 P=0.036
Median	0.256	0.350		
St. Dev.	0.419	0.587		
n	157	173		
(min.-max.)	0, 1.76	0, 4.08		

*WRS=Wilcoxon rank sum.

TABLE 24B

MYELOSUPPRESSION - PLATELET COUNT ($x10^3/mm^3$)

NADIR COUNTS OVER FIRST TWO WEEKS

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Course 1				
Mean	188.4	216.3	WRS	X^2=12.00 P<0.001
Median	168.0	213.0		
St. Dev.	91.8	79.1		
n	151	162		
(min.-max.)	19, 523	25, 541		
All Courses				
Mean	137.6	154.3	WRS	X^2=5.47 P=0.019
Median	133.0	155.0		
St. Dev.	67.4	73.8		
n	160	174		
(min.-max.)	0.5, 316	15, 541		

[a]WRS=Wilcoxon rank sum.

TABLE 29B

EXTENT OF ON-STUDY DOSING

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
No. Courses Given			WRS	X^2=2.37 P=0.12
Median	5.5	6		
Range (min.-max.)	0, 23	1, 17		
CTX/Course (mg/M²)			WRS	X^2=0.25 P=0.62
Median	500	500		
Range (min.-max.)	0, 505.3	190.6, 500		
5-FU/Course (mg/M²)			WRS	X^2=0.25 P=0.62
Median	500	500		
(min.-max.)	0, 505.3	190.6, 500		
Cumulative CTX (mg/M²)			WRS	X^2=1.78 P=0.18
Median	2500	2931.5		
Range (min.-max.)	0, 11500	497, 8500		
Cumulative 5-FU (mg/M²)			WRS	X^2=1.78 P=0.18
Median	2500	2931.5		
Range (min.-max.)	0, 11500	497, 8500		

[a]WRS=Wilcoxon rank sum.
CTX=Cyclophosphamide; 5-FU=5-Fluorouracil.

TABLE 31B

DEVIATIONS FROM INTENDED DOSING SCHEDULE

10:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	168	181		
Median No. of Courses	5.5	6	WRS	X^2=2.37 P=0.12
No. of Patients with Dose Reductions			WRS	X^2=0.10 P=0.75
0	95 (57%)	100 (55%)		
1	11 (7%)	11 (6%)		
2	17 (10%)	16 (9%)		
3	10 (6%)	10 (6%)		
4	5 (3%)	13 (7%)		
≥5	30 (18%)	31 (17%)		
No. of Patients with Dose Delays			WRS	X^2=1.23 P=0.27
0	90 (54%)	74 (41%)		
1	28 (17%)	42 (23%)		
2	21 (13%)	30 (17%)		
3	11 (7%)	19 (11%)		
4	8 (5%)	8 (4%)		
≥5	10 (6%)	8 (4%)		
Dosing Intensity				
DOX	0.93	0.92	WRS	X^2=0.17 P=0.68
CTX/5-FU	0.90	0.86	WRS	X^2<0.01 P=0.99

[a]WRS=Wilcoxon rank sum.
CTX, 5-FU (CTX=Cyclophosphamide, 5-FU=5-Fluorouracil).
DOX (DOX=Doxorubicin).

TABLE 6.3

Number of Patients Participating in Data Summaries

	DZR	PLA
No. Randomized	81	104
Cardiotoxicity : Efficacy Time to Event (Drops in LVEF, CHF)	81	104
Changes in LVEF Baseline 150 mg/M^2 300 mg/M^2 400 mg/M^2 500 mg/M^2 550 mg/M^2 600 mg/M^2	 81 58 33 21 12 10 7	 104 80 46 35 23 17 9
Antitumor Efficacy Response Rates Intent-to-Treat[a] Evaluable[a,b]	 54 44	 69 59
Disease Progression	81	104
Disease-Free Survival	81	104
Survival	81	104
Safety Course 1 *(Clinical Toxicities, Hematologies)*[c] Course 3 *(Lab Toxicities)* All Courses	 79 58 79	 100 77 100
Quality of Life Changes in Performance Status Changes in Body Weight	 81 81	 104 104

[a]Randomized with bidimensional, measurable disease.
[b]≥3 courses of therapy with objective assessment of disease status and not in gross violation of the protocol.
[c]Maximum number of patients participating in at least one analysis.

TABLE 3

BASELINE CARDIAC RISK FACTORS

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
Age >65 years			PCS	X^2=0.62 P=0.43
Yes (%)	17 (21%)	27 (26%)		
No (%)	64 (79%)	77 (74%)		
Prior Radiation to Mediastinum			PCS	X^2=1.84 P=0.18
Yes (%)	3 (4%)	9 (9%)		
No (%)	78 (96%)	95 (91%)		
History of Heart Disease[b]			PCS	X^2=0.71 P=0.40
Yes (%)	9 (11%)	16 (15%)		
No (%)	72 (89%)	88 (85%)		
Hypertension[c]			PCS	X^2=1.97 P=0.16
Yes (%)	21 (26%)	37 (36%)		
No (%)	60 (74%)	67 (64%)		
Diabetes Mellitus[c]			PCS	X^2=0.16 P=0.69
Yes (%)	5 (6%)	8 (8%)		
No (%)	76 (94%)	96 (92%)		
LVEF ≤10% above lower limit of normal			PCS	X^2=1.12 P=0.29
Yes (%)	35 (43%)	37 (36%)		
No (%)	46 (57%)	67 (64%)		

[a]PCS=Pearson Chi Square.
[b]Previous myocardial infarction, significant arrhythmia, angina.
[c]Requiring medical therapy.

TABLE 5

TIME TO CARDIAC EVENT

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Events (%)	7 (9%)	22 (21%)		
Median Event Time (mg/M^2 of DOX[b])	–[c]	600		
Hazard Ratio (P:D)	2.297		LR	X^2=3.90 P=0.048
95% C.I. of (P:D)	(0.978, 5.396)		GW	X^2=0.56 P=0.46

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin
[c]The median is unestimable.

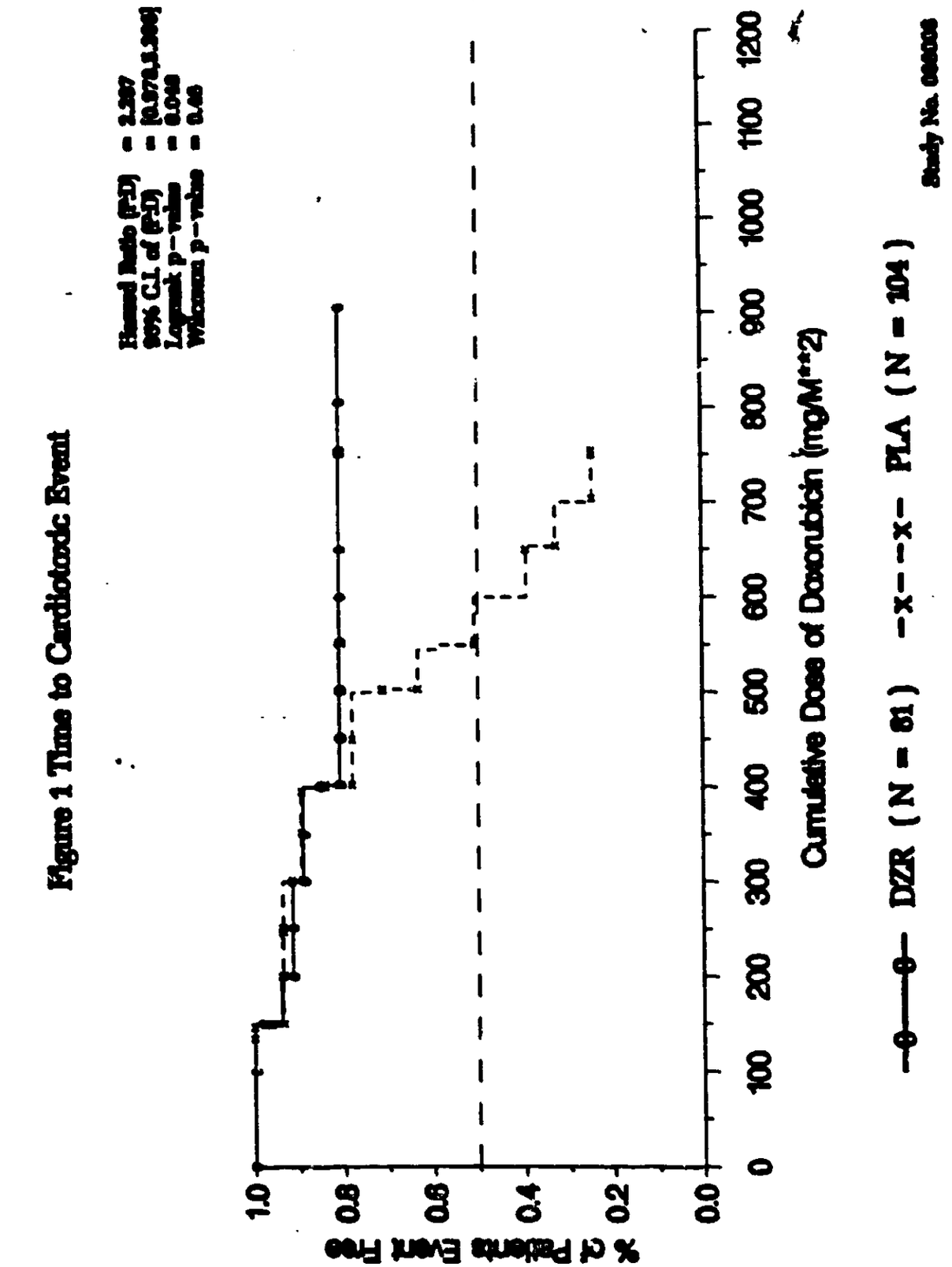


Figure 1 Time to Cardiotoxic Event
Hazard Ratio (DZR) = 2.357
95% C.I. of (DZR) = (1.377,4.036)
Logrank p-value = 0.002
Wilcoxon p-value = 0.00
% of Patients Event Free
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Cumulative Dose of Doxorubicin (mg/M**2)
—0— DZR (N = 61) -x--x- PLA (N = 104)
Study No. 000000

54B

TABLE 6

TIME TO CONGESTIVE HEART FAILURE

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Events (%)	0 (0%)	3 (3%)		
Median Event Time (mg/M^2 of DOX[b])	--[c]	--[c]		
Hazard Ratio (P:D)	5.161		LR	X^2=1.87 P=0.17
95% C.I. of (P:D)	(0.491, 54.350)		GW	X^2=1.85 P=0.17

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin
[c]The median is unestimable.

54C

Figure 2 Time to Congestive Heart Failure

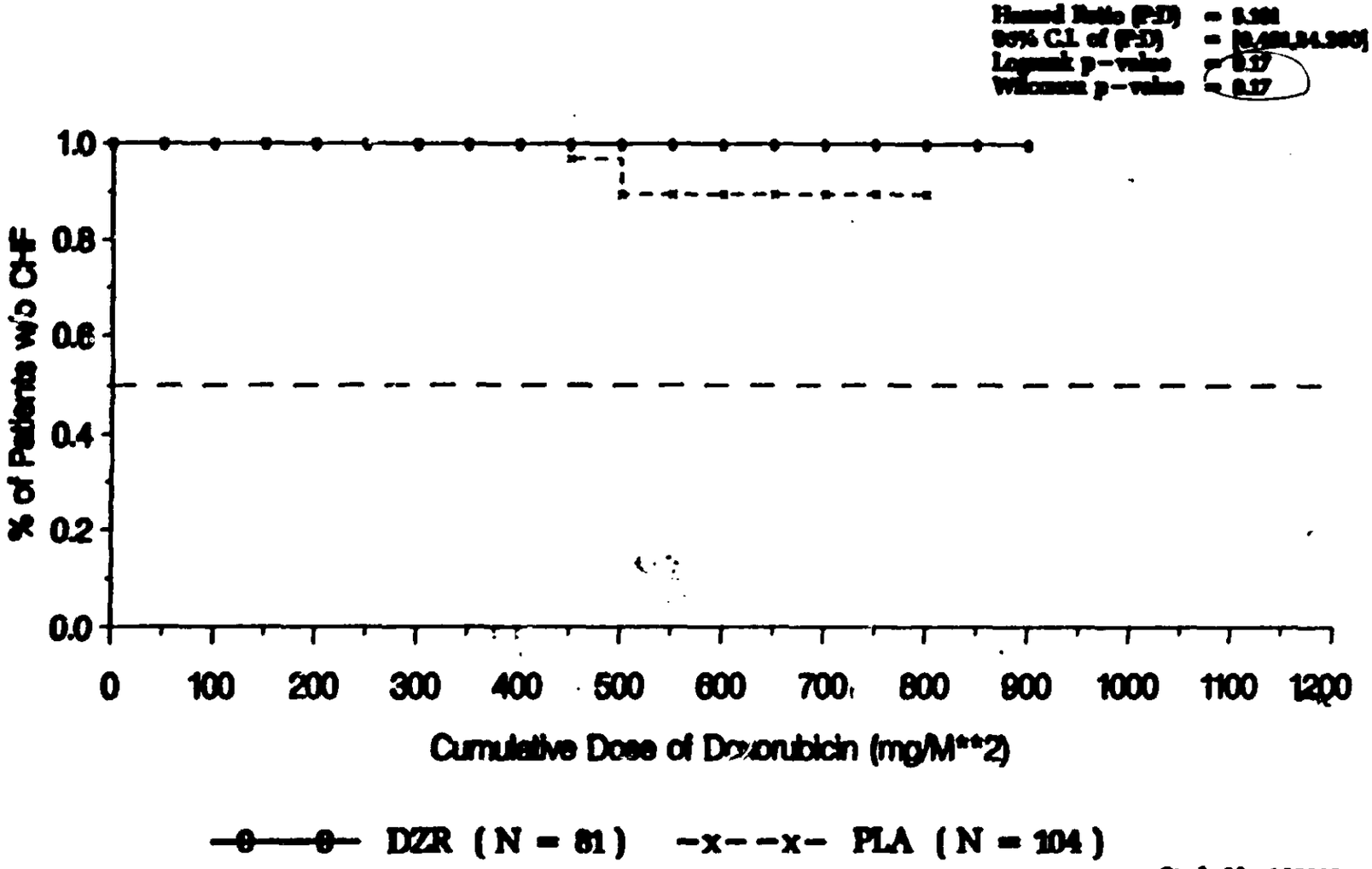


TABLE 7

CHANGES IN LVEF (%) OVER CUMULATIVE DOSE OF DOXORUBICIN

	Cumulative Dose of Doxorubicin (mg/M²)													
	Baseline		150		300		400		500		550		600	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Mean	63.5	65.1	60.8	62.3	60.4	60.7	60.3	58.4	59.5	56.1	61.3	54.9	60.4	53.2
Median	63.0	66.0	61.0	62.0	59.0	59.5	59.0	57.0	59.0	58.0	62.5	56.0	58.0	55.0
Std. Dev.	7.8	8.3	8.9	8.9	7.7	9.7	7.9	8.9	6.3	9.2	6.1	7.1	5.4	6.9
n	81	104	58	80	33	46	21	35	12	23	10	17	7	9
Estimate of Effect[a]	-1.6		-1.5		-0.3		1.9		3.4		6.4		7.2	
p-value[b]	0.08		0.29		0.99		0.43		0.46		0.031		0.09	
Change from Baseline														
Mean			-2.8	-3.7	-4.0	-5.0	-4.1	-7.9	-3.8	-10.0	-0.7	-12.4	-2.0	-13.3
Median			-3.0	-3.0	-5.0	-7.0	-5.0	-8.0	-4.0	-8.0	-2.0	-12.0	-2.0	-14.0
Std. Dev.			9.1	8.6	7.0	7.5	8.7	9.4	4.4	9.6	4.4	8.7	4.0	10.5
n			58	80	33	46	21	35	12	23	10	17	7	9
Estimate of Effect[a]			0.9		1.0		3.8		6.2		11.7		11.3	
p-value[b]			0.75		0.47		0.09		0.028		0.001		0.030	

[a]Mean difference between treatment group means: D minus P.
[b]Wilcoxon rank sum test.

TABLE 6.5.1

Number of Patients Included in Response Rate Summaries

	DZR	PLA
No. of Randomized Patients	81	104
No. of Patients with Measurable Disease[a]	54	69
No. of Intent-to-Treat Patients	54	69
No. of Patients with Tumor Assessment	51	64
No. of Off-Study Patients without Tumor Assessment[b]	3	5
Reasons Off-Study[c]		
Progressive Disease	0	0
Cardiotoxicity	0	0
Adverse Reaction	0	0
Patient Refusal	0	2
Protocol Violation	1	1
Death	1	1
Lost to Follow-Up	1	0
Randomized, Not Treated	0	0
Other	0	1
No. of Evaluable Patients[d]	44	59

[a]Patients with non-measurable disease were excluded from the response rate analyses.
[b]These patients were categorized as treatment failures in the intent-to-treat analysis.
[c]Patients lacking objective determination of tumor status while on-study.
[d]Patients receiving ≥3 courses of treatment, had objective measurement of their disease, and were not in gross violation of the protocol.

The point estimates in Table 8 show that 57% (31/54) of the DZR patients responded compared to 52% (36/69) on the control arm (p=0.56). Looking at a more stringent measure of successful activity, 11% (6/54) of the patients receiving DZR achieved a CR compared to 7% (5/69) for the control patients. The number of patients achieving progressive disease as their best response was 20% and 19% on the DZR and control arms,

TABLE 8

RESPONSE RATES

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Randomized Patients				
No. Patients	54	69		
Best Response				
CR (%)	6 (11%)	5 (7%)		
PR (%)	25 (46%)	31 (45%)		
SD (%)	9 (17%)	15 (22%)		
PD (%)	11 (20%)	13 (19%)		
NA (%)[b]	3 (6%)	5 (7%)		
Response Rate[c]	31/54 (57%)	36/69 (52%)		
Estimate of Effect[c,d]	5%		PCS	X^2=0.34 P=0.56
95% C.I. of Effect[c,d]	(-13%, 23%)			
Evaluable Patients				
No. Patients	44	59		
Best Response				
CR (%)	6 (14%)	5 (8%)		
PR (%)	23 (52%)	30 (51%)		
SD (%)	9 (20%)	15 (25%)		
PD (%)	6 (14%)	9 (15%)		
Response Rate[c]	29/44 (66%)	35/59 (59%)		
Estimate of Effect[c,d]	7%		PCS	X^2=0.47 P=0.50
95% C.I. of Effect[c,d]	(-12%, 26%)			

[a]PCS=Pearson chi square; Comparing responders to non-responders.
[b]NA=Not assessed while on-study; categorized as treatment failures.
[c]Response=CR+PR.
[d]Difference between treatment groups in response rates: D minus P.

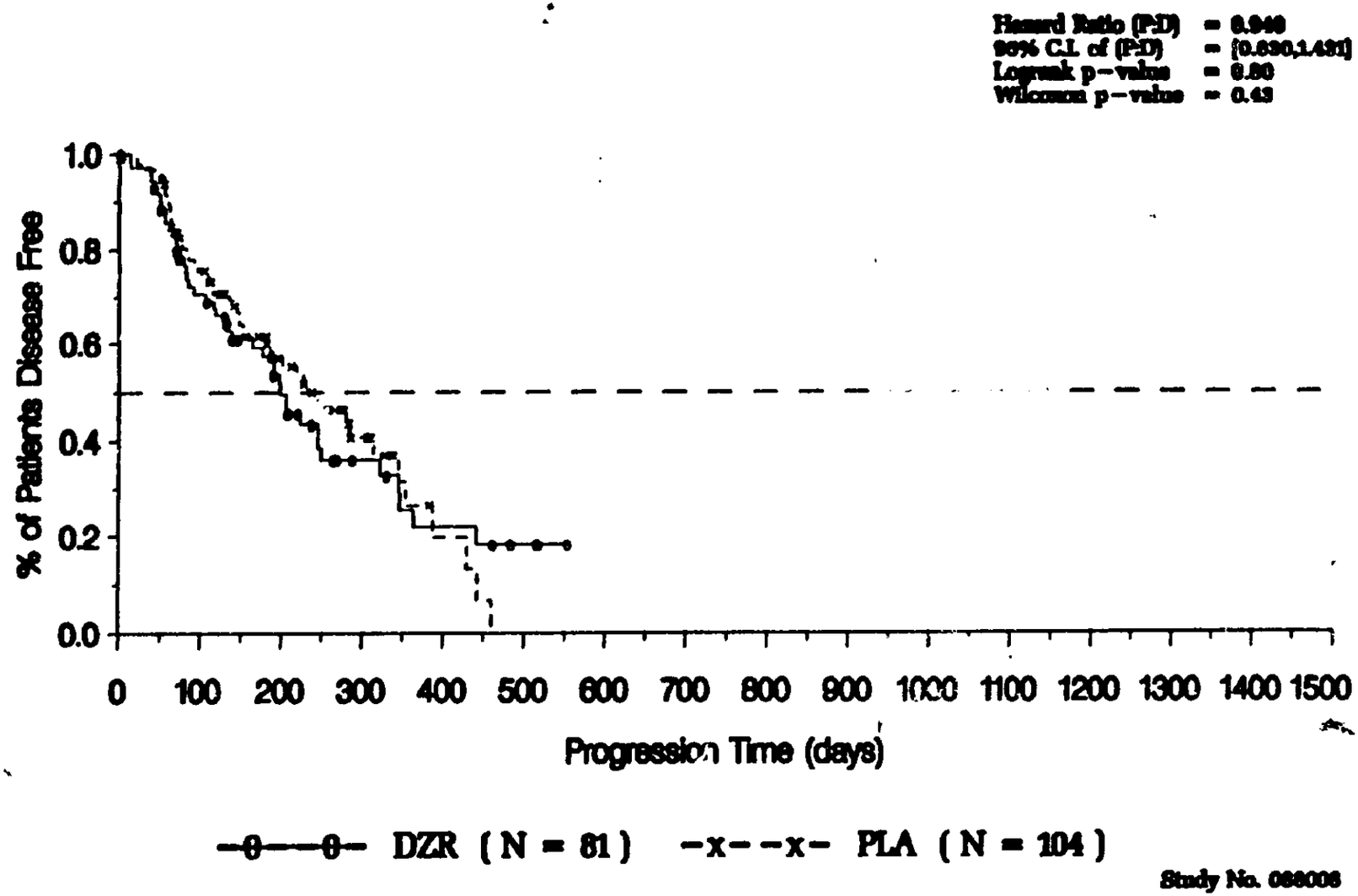
TABLE 9

TIME TO DISEASE PROGRESSION

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Events (%)	43 (53%)	51 (49%)		
Median Failure Time (days)	199	232		
Hazard Ratio (P:D)	0.949		LR	X^2=0.06 P=0.80
95% C.I. of (P:D)	(0.630, 1.431)		GW	X^2=0.62 P=0.43

[a]LR=Logrank; GW=Generalized Wilcoxon.

Figure 3 Time to Disease Progression



Study No. 000006

TABLE 10

DISEASE FREE SURVIVAL

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Events (%)	49 (60%)	58 (56%)		
Median Failure Time (days)	224	286		
Hazard Ratio (P:D)	0.832		LR	X^2=0.90 P=0.34
95% C.I. of (P:D)	(0.569, 1.218)		GW	X^2=0.88 P=0.35

[a]LR=Logrank; GW=Generalized Wilcoxon.

Figure 4 Disease Free Survival

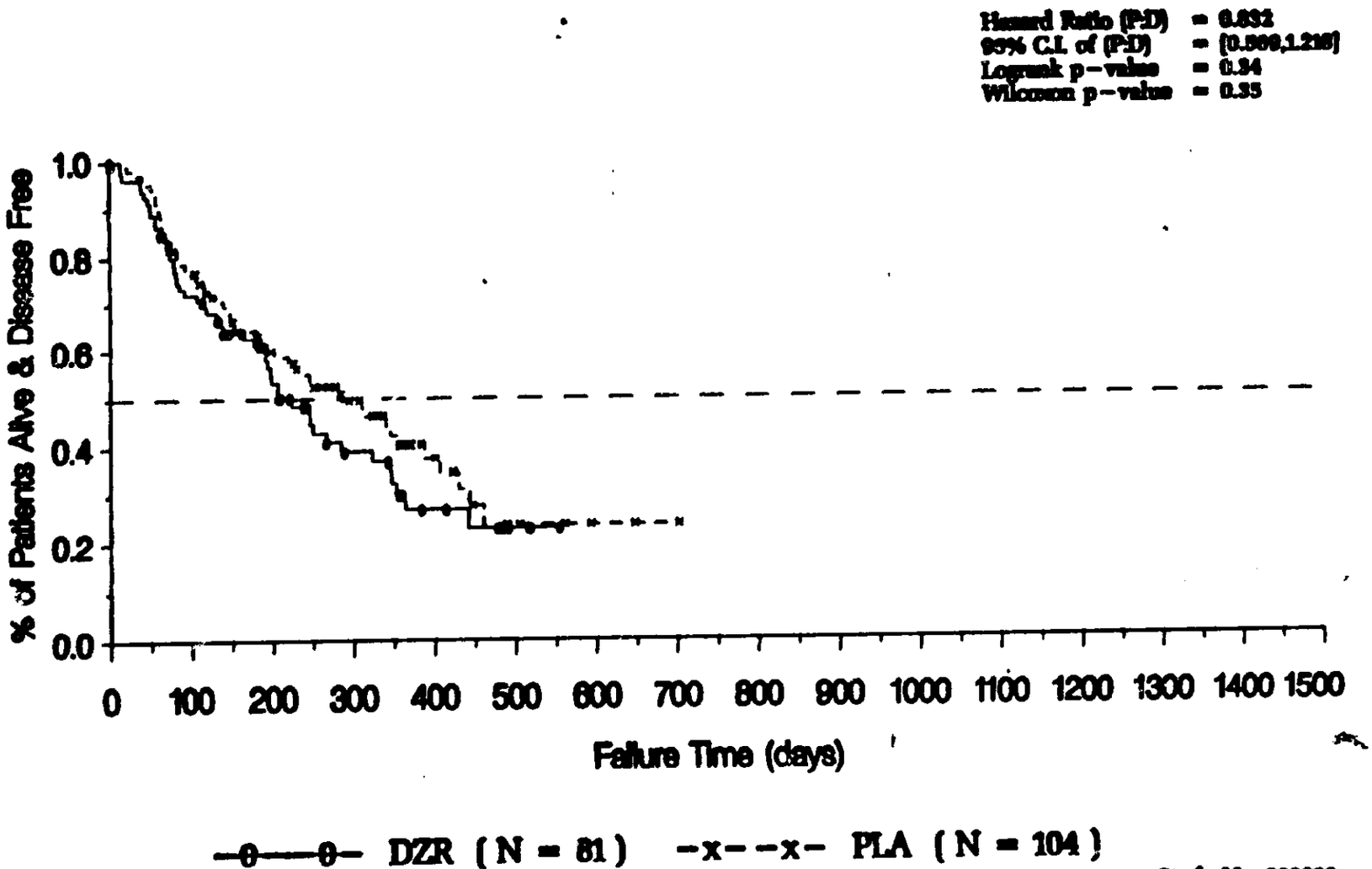


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TABLE 11

SURVIVAL

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Dead (%)	30 (37%)	25 (24%)		
Median Survival (days)	420	526		
Hazard Ratio (P:D)	0.604		LR	X^2=3.53 P=0.06
95% C.I. of (P:D)	(0.355, 1.028)		GW	X^2=2.41 P=0.12

[a]LR=Logrank; GW=Generalized Wilcoxon.

H55

Figure 5 Survival

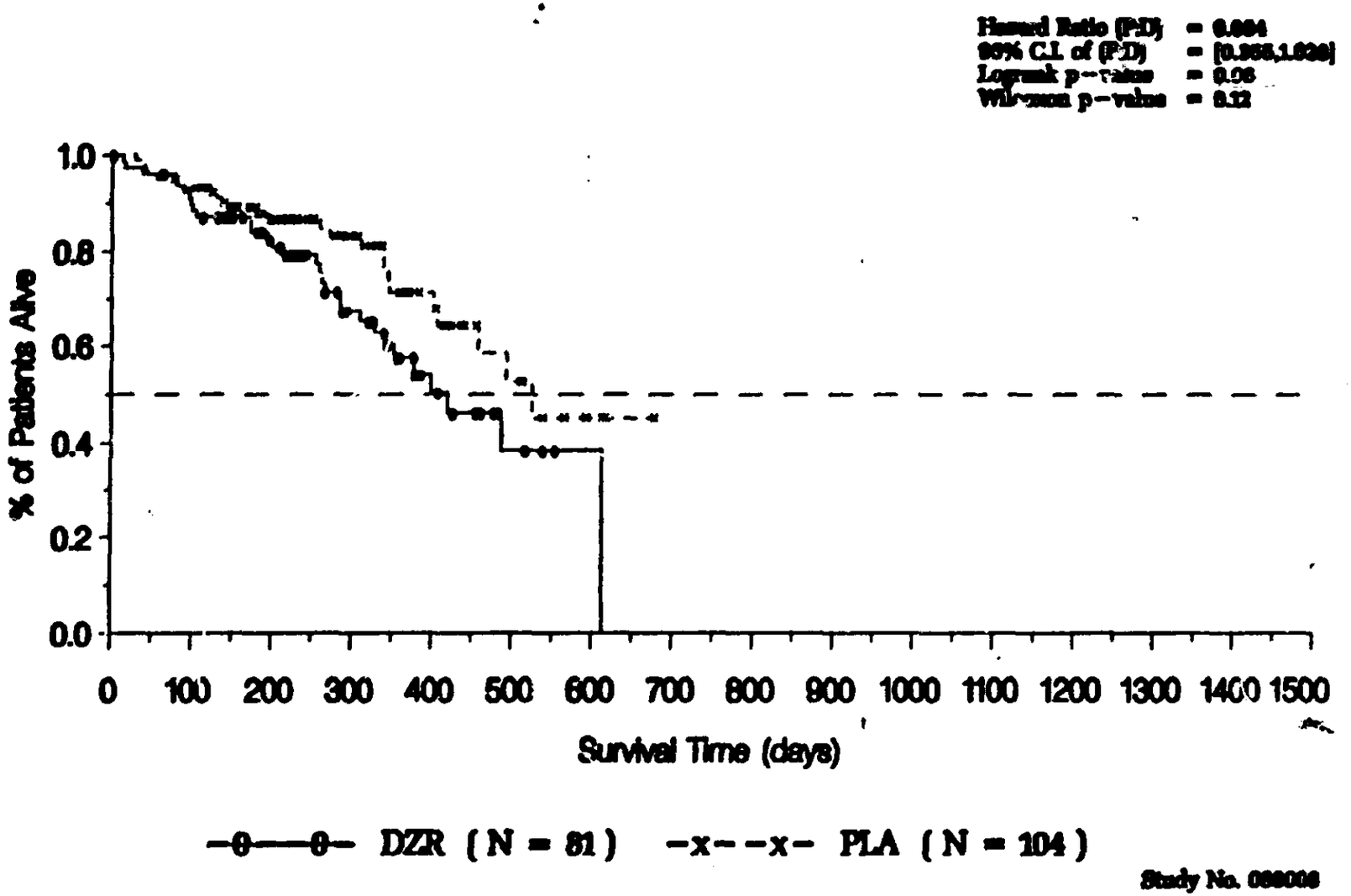


TABLE 14

CLINICAL TOXICITIES - INCIDENCE AND MEAN SEVERITY

MOST SEVERE GRADE OVER ALL COURSES

	Incidence (%)		Mean Severity	
	DZR	PLA	DZR	PLA
No. Patients	79	100		
Esophagitis	4 (5%)	7 (7%)	1.5	1.6
Stomatitis†	27 (35%)	52 (52%)	1.8	1.7
Alopecia	74 (94%)	99 (99%)	3.4	3.4
Streaking/Erythema	6 (8%)	5 (5%)		
Urticaria	5 (6%)	4 (4%)		
Recall Skin Reaction	0 (0%)	3 (3%)		
Pain on Injection	10 (13%)	5 (5%)	1.4	1.8
Extravasation	2 (3%)	2 (2%)		
Phlebitis	6 (8%)	4 (4%)	2.2	2.0
Hemorrhage	1 (1%)	2 (2%)		
CHF	0 (0%)	3 (3%)		
Dysphagia†	2 (3%)	10 (10%)	2.0	1.5
Anorexia	50 (63%)	59 (59%)	1.6	1.7
Nausea	68 (86%)	89 (89%)	1.8	2.0
Vomiting†	50 (63%)	78 (78%)	1.8	1.9
Diarrhea	20 (26%)	28 (28%)	1.5	1.8
Fatigue/Malaise*	59 (76%)	71 (71%)	1.7	2.0
Fever	23 (29%)	34 (34%)	2.3	2.1
Sepsis	13 (17%)	15 (15%)	3.2	3.1
Infection	19 (24%)	17 (17%)		
Neurotoxicity	15 (19%)	17 (17%)		

† p<.05, Pearson chi square; †† p<.01, Pearson chi square, based on incidence proportions.
* p<.05, Wilcoxon rank sum; ** p<.01, Wilcoxon rank sum, based on severity scores, excluding scores of zero.

TABLE 24

MYELOSUPPRESSION - PLATELET COUNT ($x10^3/mm^3$)

NADIR COUNTS OVER FIRST TWO WEEKS

Variable	DZR	PL[illegible]	Statistical Test	
			Statistic*	Result
Course 1				
Mean	185.6	215.1	WRS	X^2=7.33 P=0.007
Median	170.5	208.0		
St. Dev.	81.6	95.0		
n	74	96		
(min.-max.)	34, 514	15, 573		
All Courses				
Mean	138.3	150.2	WRS	X^2=1.59 P=0.21
Median	137.0	151.5		
St. Dev.	58.0	76.1		
n	76	98		
(min.-max.)	21, 296	4, 415		

*WRS=Wilcoxon rank sum.

TABLE 29

EXTENT OF ON-STUDY DOSING

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Courses Given			WRS	X^2=0.54 P=0.46
Median	5.0	6.0		
Range (min.-max.)	0, 18	0, 16		
CTX/Course (mg/M²)			WRS	X^2=2.02 P=0.16
Median	500	491		
Range (min.-max.)	0, 502	0, 500		
5-FU/Course (mg/M²)			WRS	X^2=1.60 P=0.21
Median	500	488		
(min.-max.)	0, 502	0, 501		
Cumulative CTX (mg/M²)			WRS	X^2=0.28 P=0.60
Median	2500	2600		
Range (min.-max.)	0, 8212	0, 7000		
Cumulative 5-FU (mg/M²)			WRS	X^2=0.28 P=0.60
Median	2500	2600		
Range (min.-max.)	0, 8212	0, 7000		

[a]WRS=Wilcoxon rank sum.
CTX=Cyclophosphamide; 5-FU=5-Flourouracil.

TABLE 31

DEVIATIONS FROM INTENDED DOSING SCHEDULE

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
Median No. of Courses	5	6	WRS	X^2=0.54 P=0.46
No. of Patients with Dose Reductions			WRS	X^2=0.29 P=0.59
0	48 (59%)	54 (52%)		
1	8 (10%)	12 (12%)		
2	2 (3%)	11 (11%)		
3	4 (5%)	7 (7%)		
4	4 (5%)	5 (5%)		
≥5	15 (19%)	15 (14%)		
No. of Patients with Dose Delays			WRS	X^2=0.12 P=0.73
0	43 (53%)	54 (52%)		
1	19 (23%)	21 (20%)		
2	5 (6%)	6 (6%)		
3	4 (5%)	11 (11%)		
4	2 (2%)	3 (3%)		
≥5	8 (10%)	9 (9%)		
Median Dosing Intensity				
DOX	0.96	0.94	WRS	X^2=0.42 P=0.52
CTX/5-FU	0.92	0.87	WRS	X^2=0.58 P=0.44

[a]WRS=Wilcoxon rank sum.
DOX=Doxorubicin; CTX=Cyclophosphamide; 5-FU=5-Flourouracil.

Figure 7 Time to Performance Status ≥ 3
Any Time

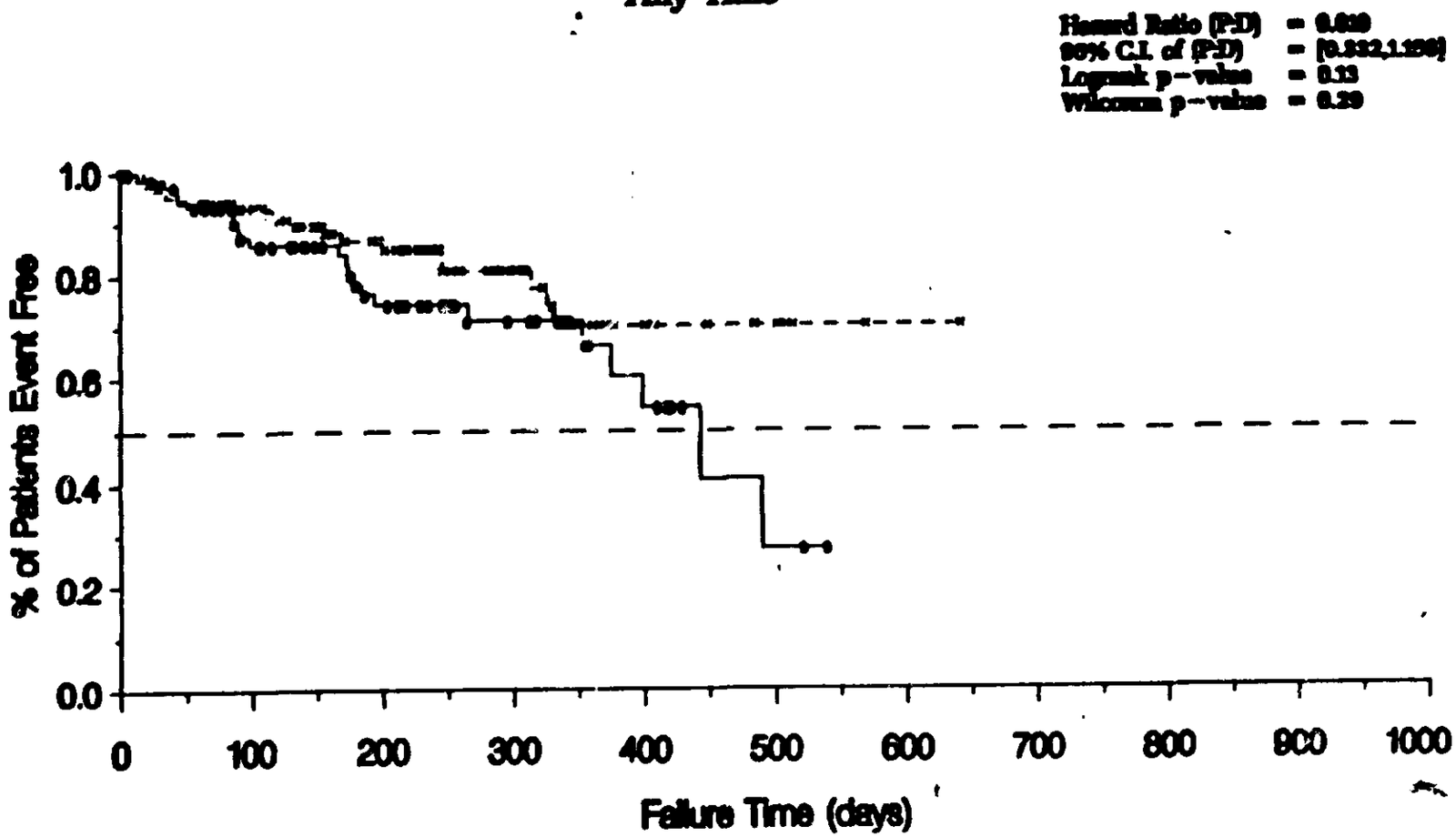


TABLE 33

PATIENT DISPOSITION

	DZR	PLA
No. Randomized	81	104
No. On-Study (%)	12 (15%)	20 (19%)
No. Off-Study (%)	69 (85%)	84 (81%)
Primary Reason Off-Study (%)		
Progressive Disease	32 (46%)	36 (43%)
Cardiac Toxicity[a]	8 (12%)	22 (26%)
Adverse Reaction[b]	3 (4%)	3 (4%)
Patient Refusal	10 (14%)	10 (12%)
Protocol Violation	5 (7%)	7 (8%)
Death	4 (6%)	3 (4%)
Lost to Follow-Up	1 (1%)	0 (0%)
Other	6 (9%)	3 (4%)

[a]Includes drop in LVEF, CHF, and other cardiac related toxicities.
[b]Non-cardiac related reasons.

TABLE 34

PATIENT DISPOSITION BY COURSE

	Course																					
	1		2		3		4		5		6		7		8		9		10		>10	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P
No. Patients On-Study	79[a]	101[b]	71	95	65	90	50	73	47	63	40	58	34	45	32	41	26	36	16	32	12	25
No. Patients Off-Study	8	6	6	5	15	17	3	10	7	5	6	13	2	4	6	5	10	4	4	7	12	25
Reason Off-Study:																						
Progressive Disease	1	0	3	3	11	11	2	3	3	2	2	4	1	2	0	2	5	2	1	2	3	5
Cardiac Toxicity[c]	0	0	0	0	2	4	0	2	0	0	2	3	1	0	2	1	0	1	0	4	1	7
Adverse Reaction[d]	0	0	1	0	0	0	0	1	0	0	0	2	0	0	1	0	0	0	0	0	1	0
Patient Refusal	1	2	0	1	1	0	0	3	3	2	1	0	0	2	1	0	1	0	0	0	0	0
Protocol Violation	3	3	1	0	0	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Death	2	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lost to Follow-Up	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Censored	0	0	0	0	0	0	0	0	1	0	0	3	0	0	2	2	4	1	1	1	4	12

[a]Excludes two patients refusing prior to dosing.
[b]Excludes one patient violating protocol prior to dosing; excludes two patients for other reasons.
[c]Includes drop in LVEF, CHF, and other cardiac related toxicities.
[d]Non-cardiac related reasons.

TABLE 35

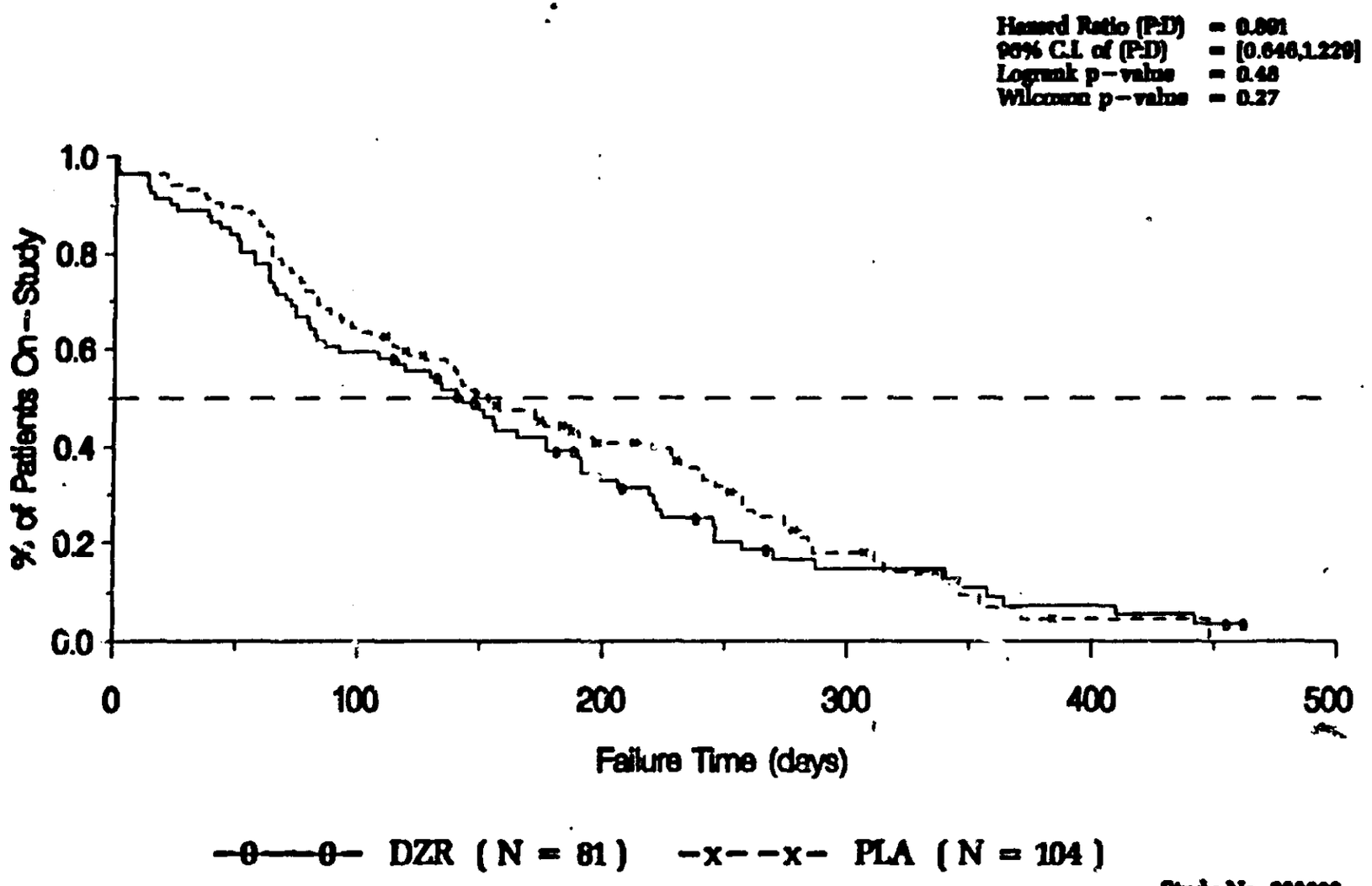
TIME TO OFF-STUDY

ANY REASON

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	81	104		
No. Off-Study (%)	69 (85%)	84 (81%)		
Median Time (days)	142	153		
Hazard Ratio (P:D)	0.891		LR	X^2=0.50 P=0.48
95% C.I. of (P:D)	(0.646, 1.229)		GW	X^2=1.23 P=0.27

[a]LR=Logrank; GW=Generalized Wilcoxon.

Figure 9 Time to Off-Study



3.7.2 Study Schema II - Amended Protocol (Effective date November 2, 1988)

RANDOMIZATION ASSIGNMENT	
TREATMENT A	**TREATMENT B**
C 750 mg/M^2 I.V. A 50 mg/M^2 I.V. V 2.0 mg I.V.	C 750 mg/M^2 I.V. A 50 mg/M^2 I.V. V 2.0 mg/M^2 I.V.
LM Control - 50 ml/M^2 I.V.	DZR-500 mg/M^2
↓	↓
Repeat every three weeks*	Repeat every three weeks*
↓	↓
Off-study if PD, cardiac event, unacceptable toxicity, patient refusal	Off-study if PD, cardiac event, unacceptable toxicity, patient refusal
↓	↓
Follow-up every three months until death	Follow-up every three months until death
*Provided recovery from toxicities had occurred.	

67A

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3.9 Schedule of Evaluations

The evaluations required by protocol are summarized in tabular form below:

	Baseline [j]	Wkly for 1st Four Courses [m]	After Every Course [n]	After Every 3rd Course	After DOX Cumulative Doses of: 150 mg/M², 300 mg/M², 400 mg/M², 500 mg/M², and Then After Every 50 mg/M²	End of Treatment	Follow-up q 3 Mos. until Death
General Medical History	X						
Cardiac History	X						
Complete Physical Examination	X					X	
Cardiac Signs and Symptoms	X				X[a]	X	X
BP, Pulse, Weight	X		X			X	
BSA	X						
Performance Status (ECOG)	X		X			X	
Signs/Symptoms[b]	X		X			X	
Toxicities[b]			X			X	
Disease Assessment[c]	X			X		X[l]	X[h] See Note
Bone Marrow Biopsy	X					X[f]	
CT Scan, Chest, Abdomen	X			X[f]		X[d]	X[d] See Note
CT Head	X						
Tc Bone Scan	X			X[f]		X[d]	X[d] See Note
Chest X-ray	X			X[f]	X[d]	X[l]	
EKG	X					X[l]	

70 A

	Baseline [1]	Wkly for 1st Four Courses [2]	After Every Course [3]	After Every 3rd Course	After DOX Cumulative Doses of: 150 mg/M², 300 mg/M², 400 mg/M², 500 mg/M², and Then After Every 50 mg/M² [4]	End of Treatment	Follow-up q 3 Mos. until Death
MUGA Scan (Resting LVEF)[5]	X				X[6]	X[7]	X[8]
Hemoglobin, Hematocrit, WBC, Differential Platelets	X	X	X			X	
Chemistries[9]	X			X	X		
Follow-up Form							X

[1]A course is defined as day of treatment +21 days, or in case of delays, until next treatment. Evaluations should take place prior to next treatment. All studies due at time/date indicated.
[2]Grade according to criteria in Appendix IE.
[3]Identify and record all sites of disease and measure all measurable disease.
[4]Obtain chest X-ray at each MUGA scan.
[5]Bilirubin (total), alkaline phosphatase, SGOT, LDH, BUN, creatinine, albumin, and total serum protein (TSP) Ca++, phosphorus.
[6]If abnormal at baseline, and required for response evaluation.
[7]The MUGA scan and recording of cardiac signs and symptoms were to be performed at the end of the course, prior to the next dose (within one week). NOTE: After a cumulative dose of Adriamycin® of 500 mg/M² has been reached, the MUGA was to be repeated after every 50 mg/M². Cardiac signs and symptoms were evaluated also. Tapes of MUGA scans were to be indexed for future retrieval and review. All MUGA scans were to be performed on the same equipment.
[8]Until relapse.
[9]At first three month follow-up only.
[1]Within one month of entry.
[2]If ≥three courses. Repeat MUGA scan required if drop in LVEF was the only reason for removal from study.
[3]If ≥three courses.
[4]Provision for weekly CBCs for first four courses added at initial protocol amendment.

NOTE: Disease assessment during follow-up was required only for patients who were still in response and who had not received antineoplastic treatment since discontinuing Adria study drugs.

To A

TABLE 5A

TIME TO CARDIAC EVENT

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	26	25	[redacted]	[redacted]
No. Events (%)	4 (15%)	9 (36%)	[redacted]	[redacted]
Median Event Time (mg/M^2 of DOX[b])	--[c]	450	[redacted]	[redacted]
Hazard Ratio (P:D)	2.263		LR	X^2=1.83 P=0.18
95% C.I. of (P:D)	(0.670, 7.641)		GW	X^2=0.24 P=0.62

[a]LR=Logrank; GW=Generalized Wilcoxon
[b]DOX=Doxorubicin
[c]The median is unestimable.

73C

performed compared to 12% of the control patients, p=0.15. The groups did not differ significantly for the other baseline measures of disease status.

6.3 Data Sets Analyzed

6.3.1 20:1 Patients

Following is a brief summary of the number of patients contributing data to the various statistical summaries. All patients, unless noted elsewhere, were included in the analyses.

TABLE 6.3.1
NUMBER OF PATIENTS INCLUDED IN DATA SUMMARIES

	DZR	PLA
Number Randomized	26	25
Cardioprotection Efficacy: Time to Cardiotoxic Event (Drops in LVEF, CHF)	26	25
Changes in LVEF:		
Baseline	26	25
150 mg/M²	17	20
300 mg/M²	10	14
400 mg/M²	7	9
500 mg/M²	7	3
550 mg/M²	3	1
600 mg/M²	3	1
Antitumor Efficacy:		
Response Rates		
Intent-to-Treat[a]	26	25
Evaluable[a,b]	19	20
Disease Progression	26	25
Disease-Free Survival	26	25
Survival	26	25
Safety:		
Course 1 *(Clinical Toxicities, Hematologics)*[c]	25	25
Course 3 *(Lab Toxicities)*	17	19
All Courses	25	25
Quality of Life:		
Changes in Performance Status	26	25
Changes in Body Weight	26	25

[a]Randomized with measurable disease.
[b]three courses of therapy with objective assessment of disease status and not in gross violation of the protocol.
[c]Maximum number of patients included in at least one analysis.

73 B

08-02105

TABLE 3A

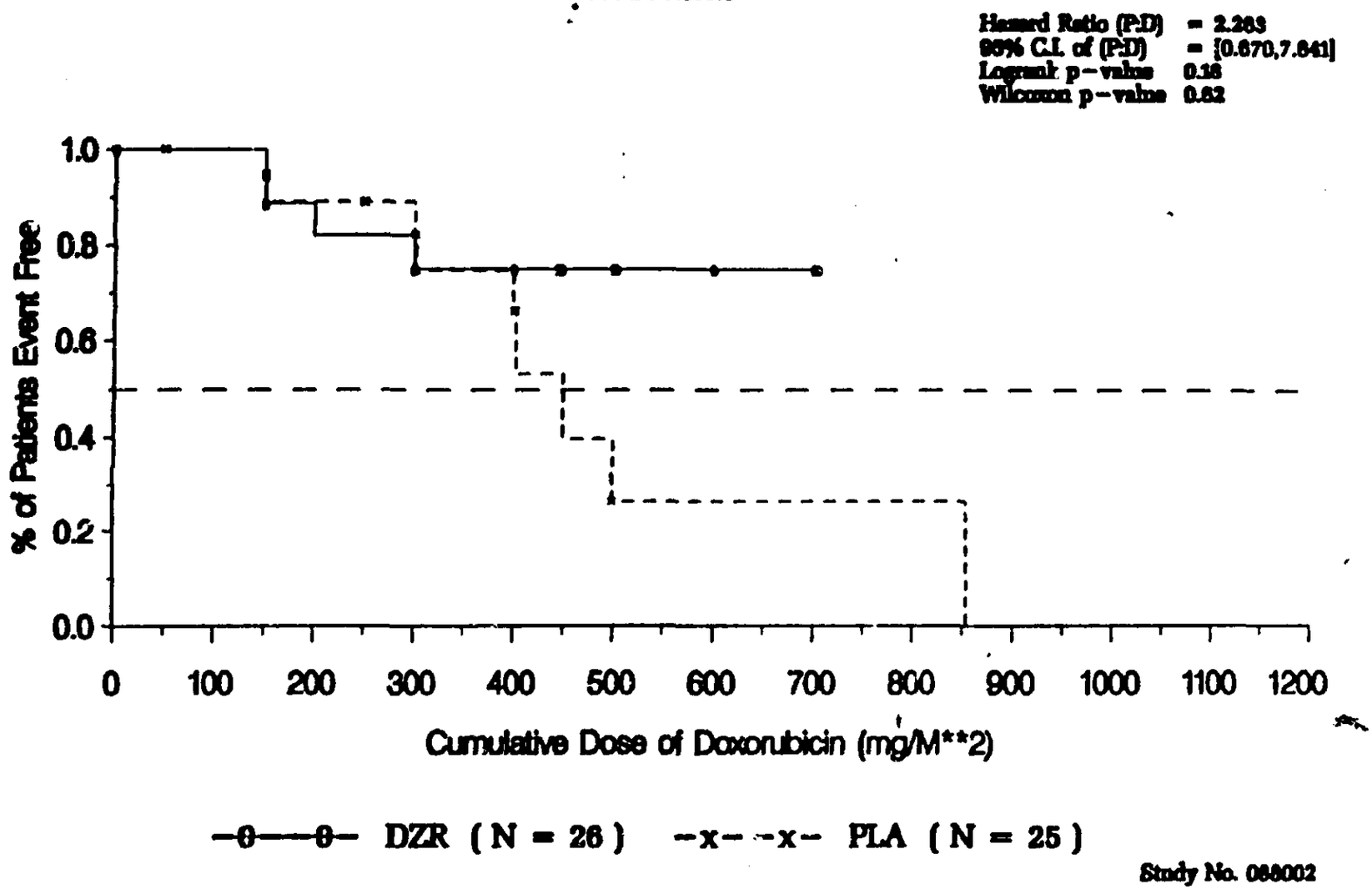
BASELINE CARDIAC RISK FACTORS

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	26	25		
Age >65 years			PCS	X^2=2.42 P=0.12
Yes (%)	7 (27%)	12 (48%)		
No (%)	19 (73%)	13 (52%)		
Prior Radiation to Mediastinum			PCS	
Yes (%)	0 (0%)	0 (0%)		
No (%)	26 (100%)	25 (0%)		
History of Heart Disease[b]			FXT	P=1.00
Yes (%)	5 (19%)	4 (16%)		
No (%)	21 (81%)	21 (84%)		
Hypertension[c]			PCS	X^2=0.05 P=0.83
Yes (%)	8 (31%)	7 (28%)		
No (%)	18 (69%)	18 (72%)		
Diabetes Mellitus[c]			FXT	P=0.051
Yes (%)	0 (0%)	4 (16%)		
No (%)	26 (100%)	21 (84%)		
LVEF ≤10% above lower limit of normal			PCS	X^2=0.06 P=0.81
Yes (%)	7 (27%)	6 (24%)		
No (%)	19 (73%)	19 (76%)		

[a]PCS=Pearson Chi Square; FXT=Fisher's Exact test.
[b]Previous myocardial infarction, significant arrhythmia, angina.
[c]Requiring medical therapy.

Figure 1A Time to Cardiotoxic Event
201 Patients



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88220-80

TABLE 6.4.2.1
20:1 PATIENTS WITH CHF

Patient No.	Treatment Arm	Time of CHF	Final LVEF	Cumulative Dose of Doxorubicin (mg/M^2)	Reason Off Study
4201					
5201					
38201					
52102					
54201					
64201					
65202					

[a]Definitive diagnosis of CHF not established. Not considered a cardiotoxic event in the analysis.
[b]Patient expired off-study with congestive heart failure and progressive disease.
[c]Patient received an additional three doses of doxorubicin.

Individual patient information for these patients is located in Appendix X.

TABLE 7A

CHANGES IN LVEF (%) OVER CUMULATIVE DOSE OF DOXORUBICIN
20:1 Patients

	Cumulative Dose of Doxorubicin (mg/M²)													
	Baseline		150		300		400		500		550		600	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Mean	63.3	66.1	56.6	59.1	59.2	54.4	69.4	52.4	63.9	53.3	59.3	57.0	60.7	56.0
Median	64.5	66.0	55.0	59.0	58.0	57.5	66.0	57.0	64.0	55.0	60.0	57.0	64.0	56.0
Std. Dev.	8.2	8.7	8.3	6.6	7.7	10.2	7.3	10.3	7.6	7.6	2.1	--	7.6	--
n	26	25	17	20	10	14	7	9	7	3	3	1	3	1
Estimate of Effect[a]	-2.8		-2.5		4.8		17.0		10.6		2.3		4.7	
p-value[b]	0.32		0.48		0.54		<0.001		0.11		--		--	
Change from Baseline														
Mean			-5.8	-7.0	-2.9	-9.4	3.4	-14.3	-1.6	-13.7	-5.7	-9.0	-4.3	-10.0
Median			-3.0	-6.5	-4.0	-9.0	1.0	-14.0	-2.0	-11.0	-7.0	-9.0	-1.0	-10.0
Std. Dev.			9.3	8.4	6.6	6.6	7.0	11.0	6.9	5.5	4.2	--	8.5	--
n			17	20	10	14	7	9	7	3	3	1	3	1
Estimate of Effect[a]			1.2		6.5		17.7		12.1		3.3		5.7	
p-value[b]			0.54		0.050		0.004		0.040		--		--	

[a]Mean difference between treatment group means: D minus P.
[b]Wilcoxon rank sum test.

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TABLE 8A

RESPONSE RATES

20:1 Patients

Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
Randomized Patients				
No. Patients	26	25		
Best Response				
CR (%)	7 (27%)	4 (16%)		
PR (%)	8 (31%)	13 (52%)		
SD (%)	4 (15%)	2 (8%)		
PD (%)	0 (0%)	1 (4%)		
NA (%)[b]	7 (27%)	5 (20%)		
Response Rate[c]	15/26 (58%)	17/25 (68%)		
Estimate of Effect[c,d]	-10%		PCS	X^2=0.58 P=0.45
95% C.I. of Effect[c,d]	(-36%, 16%)			
Evaluable Patients				
No. Patients	19	20		
Best Response				
CR (%)	7 (37%)	4 (20%)		
PR (%)	8 (42%)	13 (65%)		
SD (%)	4 (21%)	2 (10%)		
PD (%)	0 (0%)	1 (5%)		
Response Rate[c]	15/19 (79%)	17/20 (85%)		
Estimate of Effect[c,d]	-6%		FXT	P=0.70
95% C.I. of Effect[c,d]	(-30%, 18%)			

[a]PCS=Pearson chi square; FXT=Fisher's exact test.
[b]NA=Not assessed while on-study; categorized as treatment failures.
[c]Response=CR+PR.
[d]Difference between treatment groups in response rates: D minus P.

TABLE 6.5.1.1
NUMBER OF PATIENTS INCLUDED IN RESPONSE RATE SUMMARIES

20:1 Patients	DZR	PLA
No. of Randomized Patients	26	25
No. of Patients with Measurable Disease[a]	26	25
No. of Intent-to-Treat Patients:	26	25
No. of Patients with Tumor Assessment	19	20
No. of Off-Study Patients without Tumor Assessment	7	5
Reasons Off-Study[c]:		
Progressive Disease	0	0
Cardiotoxicity	0	2
Adverse Experience	2	1
Patient Refusal	0	0
Protocol Violation	0	1
Death	4	1
Lost to Follow-up	0	0
Randomized, not Treated	0	0
Other	1	0
No. of Evaluable Patients[d]	19	20

[a]Patients with non-measurable disease were excluded from the response rate analyses.
[b]These patients were categorized as treatment failures in the intent-to-treat analysis.
[c]Patients lacking objective determination of tumor status while on-study.
[d]Patients receiving ≥three courses of treatment, had objective measurement of their disease, and were not in gross violation of the protocol.

The point estimates in Table 8A (intent-to-treat) show that 10% more of the control patients responded relative to the DZR group (p=0.45). Fifty-eight percent (58%; 15/26) of the DZR patients responded compared to 68% of patients (17/25) on the control arm. Twenty-seven percent (27%) of patients receiving DZR achieved a CR (7/26) compared to 16% for the control group (4/25). Only one patient (on the control arm) experienced progressive disease as the best response. A similar picture was seen for the evaluable patient subgroup: 79% responded on DZR relative to 85% on the control arm. Again,

74 D

TABLE 9A

TIME TO DISEASE PROGRESSION

20:1 Patients

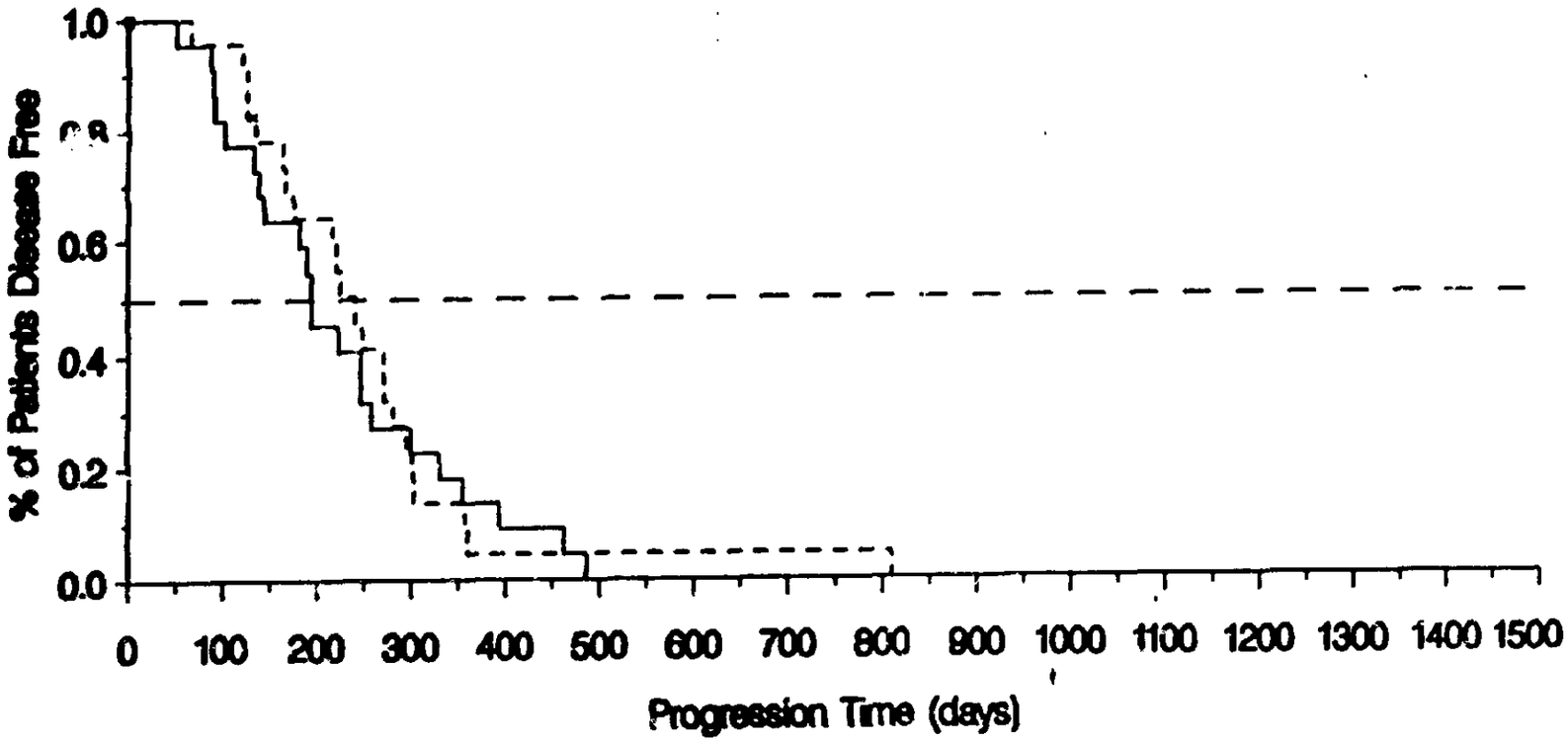
Variable	DZR	PLA	Statistical Test	
			Statistic[a]	Result
No. Randomized	26	25		
No. Events (%)	22 (85%)	22 (88%)		
Median Failure Time (days)	195	241		
Hazard Ratio (P:D)	0.908		LR	X^2=0.10 P=0.75
95% C.I. of (P:D)	(0.496, 1.659)		GW	X^2=0.47 P=0.49

[a]LR=Logrank; GW=Generalized Wilcoxon.

75 B

Figure 3A Time to Disease Progression
201 Patients

Hazard Ratio (P:D) = 0.906
95% C.I. of (P:D) = [0.496,1.656]
Logrank p-value = 0.75
Wilcoxon p-value = 0.49



—0—0— DZR (N = 26) -x--x- PLA (N = 25)

Study No. 088002

could not be evaluated by the Bertazzoli method, hence were evaluated somewhat differently (see above table).

This study, as in other studies of this nature, confirms the cardioprotective effect of ADR-529 (ICRF-187) against doxorubicin induced cardiomyopathies. There was also a protective effect against the cardiac toxicity produced by isoproterenol. Carcinogenicity testing would be required for ADR-529 if it were to be used with other non-cytotoxic drugs.

35-WEEK COMPARATIVE CARDIOTOXICITY STUDY OF DOXORUBICIN AND DOXORUBICIN + ADR-529 IN RATS.
VOL. 1.14, P. 05 01051
REPORT # 4521

Compound: ADR-529 (lyophilized formulation), Batch #TF/23409
Dox-HCl, Batch #3008B622, 99.5% HPLC assay
Formulation: ADR-529 in M/6 Na lactate
Dox-HCl in physiological NaCl
Route: IV, caudal veins, 2.5 mL/Kg at 2.5-3 mL/min
Dosage Levels:

	Treatment	
	Dox	ADR-529
Group	(mg/Kg)	(mg/Kg)
1	0	0
2	1	0
3	1	5
4	1	10
5	1	20

Strain: Male Crl:CD(SD)BR, 47-48 days of age, 230 g body weight
Number: 36/dose level
Control: (-) control Na lactate followed 30 min later by saline
(+) control Na lactate followed 30 min later by
1 mg/Kg doxorubicin
Study Site: /Oct-88 to
Sept-91
GLP/QAU Statements: Both present and signed

Groups were administered iv ADR-529, followed 30 minutes later by 1 mg/Kg doxorubicin iv (about 1/13 of LD50). The control groups received either Na lactate and 1 mg/Kg doxorubicin 30 minutes later or Na lactate followed 30 minutes later by normal saline. Animals were dosed 1 x/week for 7 weeks, with the dosage adjusted to body weight on the day of treatment. The study includes daily observation, body weight (prior to weekly dosing, then every other week), organ weights (kidneys, liver, heart), gross pathology, and histopathology on

hearts. The first 6 animals with the lowest identification number/group were sacrificed at Weeks 4, 8, 12, 22, 26, and 35. Hearts were scored by severity (1 and 2) and extent of altered myocytes (0.5 to 5). Mean total scores (MTS) were calculated for each group according to the following formula:

$$MTS = \sum (s \times e)/\text{number of animals}$$

Results

-deaths: 3 in G2 week 22 and later (all showed marked cardiac lesions
-tail ulcers on some animals in G2 and G5 (one)
-pallor, dyspnea, ruffled fur in animals dying
-body weight: significantly reduced G2 (23%)
G(3-5) also significant (9%-18%)
-testes: decreased size/flacid-slight to marked G(2-5) dose related-slightly reversible
-epididymides: decreased size-slight to marked G(2-5) only at Week-12
-thymus: decreased size-slight to moderate G(2-5) hemorrhage G(4 and 5) Week-12
-kidneys: increase in size G(2-4)
medullary hyperemia (G3-5)
pitted surface G(2-5)
-organ weights (absolute): significant increases vs G1

	Kidney	Liver	Heart
Week-8			G4
Week-12	G2	G2	
Week-22	G2,3,4	G2,3	
Week-26	G2,3	G2,3,4	

The following table (in part from p. 05-01117) shows heart scores:

Mean Total Score Of Cardiac Lesions
(left ventricle + interventricular septum)
Statistical Comparison vs Control

	Week					
Group	4	8	12	22	26	35
1	0	0	0	0	0	0
2	0.5	3.33**	5.00**	5.00**	4.00**	4.67**
3	0.5	3.20	3.67	3.17**	2.67**	2.50**
4	0.17	1.50	2.17	2.67*	1.50	1.50*
5	0	0.80	2.00	1.33	0.83	0.83

* $p<0.05$
** $p<0.01$

Observed Lesions
Number of Damaged Hearts/Number Examined

	Week					
Group	4	8	12	22	26	35
1	0/6	0/6	0/6	0/6	0/6	0/6
2	3/6	6/6	6/6	6/6	6/6	3/3
3	3/6	5/5	6/6	6/6	6/6	6/6
4	1/6	3/6	6/6	6/6	5/6	6/6
5	0/6	3/5	6/6	6/6	4/6	5/6

Heart lesions occurred in 50% of Group 2 by Week 4, became more severe (moderate to marked) at each scheduled sacrifice, and were maximum at Weeks 12 and 22 where scores were 5.00. Heart scores were less in those groups treated with ADR-529 and were described as slight to moderate. All animals sacrificed Weeks 12 and 22 in Groups 2, 3, 4, and 5 developed cardiac lesions. Only in Groups 4 and 5 were 1 or 2 animals free of cardiac lesions by Weeks 26 and 35.

In conclusion, cardioprotection was afforded rats treated with ADR-529 when administered 30 minutes prior to doxorubicin. Cardioprotection was dose-dependent, with maximum protection observed with a 20:1 ratio of ADR-529 to doxorubicin.

35 WEEK COMPARATIVE CARDIOTOXICITY STUDY OF EPIRUBICIN AND EPIRUBICIN + ADR-529 IN RATS.
VOL. 1.14, P. 05 01237
REPORT # 4511

Compound: ADR-529 (lyophilized formulation), Batch #TF/23409
Epirubicin HCl, Batch #7005B86, 96.4% HPLC assay
Formulation: ADR-529 in M/6 Na lactate
Epirubicin in normal saline, 0.9%
Route:IV, caudal veins
Dosage Levels:

	Treatment		
Group	Epi (mg/Kg)	ADR-529 (mg/Kg)	Ratio Epi:ADR-529
1	0	0	
2	1.13	0	
3	1.13	5.65	1:5
4	1.13	11.30	1:10
5	1.13	22.60	1:20

Strain: Male Crl:CD9SD)Br, 47-48 days of age, 220-230 g body wt
Number: 36/dose level

Control: (-) control Na lactate followed 30 min later by saline
(+) control Na lactate followed 30 min later by 1.13 mg/Kg epirubicin
Study Site: */6-88 to 9-91
GLP/QAU Statements: Both present and signed.

Animals were administered ADR-529, followed 30 minutes later by 1.13 mg/Kg epirubicin HCl (about 1/13 of the epirubicin LD50 in the rat). The positive control received Na lactate and 1.13 mg/Kg epirubicin 30 minutes later. The negative control received Na lactate, followed 30 minutes later by normal saline. Animals were dosed 1/week for 7 consecutive weeks, with dosage adjusted to body weight on the day of treatment. The study includes daily observation, body weight (prior to dosing, weekly, then every other week), organ weights (kidneys, liver, heart), gross pathology, and histopathology on hearts. The first 6 animals with the lowest identification number/group were sacrificed at Weeks 4, 8, 12, 22, 26, and 35. Hearts were scored by severity and extent of altered myocytes, as in the previous study.

Results

Cardiac scores are presented in the following tables (from Table 6, p. 05-01305):

Mean Total Score of Cardiac Lesions
(left ventricle + interventricular septum)
Statistical Comparison vs Control

	Week					
Group	4	8	12	22	26	35
1	0	0	0	0	0	0
2	0.7	3.2**	5.0**	3.2**	3.0**	4.0
3	0	2.5**	3.0**	1.8**	1.0	0.5
4	0	1.0	2.3*	1.2	0.4	0.8
5	0	0.2	1.3	0.8	0.2	0.2

* $p<0.05$
** $p<0.01$

NDA 20-212

Observed Lesions
Number of Damaged Hearts/Number Examined

	Week					
Group	4	8	12	22	22	35
1	0/6	0/6	0/6	0/6	0/6	0/6
2	3/6	6/6	6/6	6/6	6/6	2/2
3	0/6	6/6	6/6	6/6	5/6	3/6
4	0/6	5/6	6/6	5/6	4/6	4/5
4	0/6	1/6	5/6	4/6	1/6	1/6

-deaths: 4 in G2 beginning Week 25 (all showed marked cardiac lesions)

1 in G4 Week 31 (showed very slight cardiac lesions)

-tail ulcers in some animals in G3-G5
-pallor, ruffled coat and weight loss in dying animals
-body weight: reduced in G3 (9%), G4 (13%), and G5 (10%)
-testes and epididymides: decreased size G3, G4, and G5 (slight to marked)
-thymus: decreased size (slight/moderate) G3, G4, and G5
-seminal vesicles/prostate: decreased size G2, Weeks 26 and 35
-kidney: absolute (significant) and relative weight increase G2
-liver: absolute (significant) and relative weight increase G2 beginning Week 12
-heart: sarcoplasmic vacuolation of myocytes-more evident in left ventricle and interventricular septum G2-G4

As in the previous 35 week study, heart lesions increased in severity in G2 by Week 8, were maximum at Week 12, and occurred in all six animals. Cardiac scores were less severe in G3, G4, and G5, were maximum Week 12, and tended to become less severe over the rest of the study.

In conclusion, the results of this study were similar to those observed in the previous 35 week study with doxorubicin. Treatment with ADR-529 produced a significant and dose-related decrease in cardiac scores. The 1:20 ratio (Group 5) also significantly reduced cardiotoxicity when compared to Group 2.

DOXORUBICIN CARDIOTOXICITY IS REDUCED BY PRETREATMENT WITH THE IRON CHELATOR ADR-529.
DANESI, R., ET AL.,EUR. J. PHARMACOL. 183 (5): 1703, 1990.
VOL. 1.15, P. 05-01439
GB0116

Male SD rats (200-220 g) were administered iv doxorubicin 1 mg/Kg /week x5 iv. A second cycle of doxorubicin was administered after 2 weeks. ADR-529 was given 30 min prior to doxorubicin: 1) beginning Week 1 of doxorubicin administration, 2) beginning Week 3, and 3) beginning Week 8. Significant changes in body weight were not observed. By Week 10 significant changes occurred in the EKGs (widening of the S, T and QRS complex, and flatening of T waves. These changes were significant to termination of study. EKG data were significantly reduced with ADR-529 treatment, even when ADR-529 was started at Week 3 or Wek 8.

EFFECT OF ICRF-187 PRETREATMENT ON THE INCIDENCE AND SEVERITY OF DOXORUBICIN-INDUCED CHRONIC CARDIONYOPATHY IN DOGS.
Vol. 1.15, p. 05-1440
REPORT # 4011

This study examined the effects of iv ADR-529 (ICFR-187) on cumulative doses of doxorubicin administered to male and female dogs up to 30 weeks. Dosing was as indicated below.

Group	# Dogs	Treatment
1	5M, 3F	1.75 mg/Kg iv doxorubicin Q3 weeks
2	5M, 3F	1.75 mg/Kg iv doxorubicin Q3 weeks + 25 mg/Kg iv ICRF-187 given 15 min prior to doxorubicin
3	5?	25 mg/Kg iv ICRF-187 followed in 15 min by iv 5 mL physiological saline
4	6?	two iv injections of saline 15 min apart

Both drugs were administered in 0.9% saline. Cardiovascular functions were measured prior to dosing and prior to each dosing at 3 week intervals. Echo- and electrocardiographic alterations were presented in graphic form. Hematology and blood chemistry parameters were determined predose and at intervals of 3 weeks. All surviving dogs were necropsied. Cardiac lesions were evaluated and scored as in other studies.

Results

The cardiomyopathy scores are indicated in the following table (from Table 1, p. 05-01457).

Gp	Doxorubicin Cumulative Dose (mg/Kg)	# of Doses	# of Animals	Number Dying	Cardiomopathy Scores 0	1	2	3	4
1	Dox (12.25)	7	2	2	0	0	0	2	0
	(14.00)	8	6	3	0	0	0	6	0
	Totals		8	5	0	0	0	0	0
2	ICRF/Dox (35.00)	20	1	1	1	0	0	0	0
	(43.75)	25	4*	2	3	1	0	0	0
	(52.50)	30	3	1	0	1	2	0	0
	Totals		8	4	4	2	2	0	0
3	-	30	5**	0	5	0	0	0	0
4	-	30	6***	0	6	0	0	0	0

* Two dogs were also euthanized after 25 injections due to dificulty in dosing.
** Three dogs were euthanized after the 25th dose.
*** Two were euthanized after the 8th dose.

The remaining controls were euthanized three weeks after the 30th dose. Group 1 dogs developed alopecia after 3 doses. Food consumption was reduced the first 2 days after dosing with doxorubicin or doxorubicin + ICRF-187. Body weight was reduced during the first 4 treatments in all groups, then weight gain occurred. Myocardial lesions that were observed in these animals were said to be typical of those described in other species, including humans. Cytoplasmic vacuolization and myofibrillar loss, both of which increased in severity, were prominent. Other tissues which developed changes with doxorubicin only were liver and renal congestion, and loss of epithelial cells at the tips of the villi in the small intestin. Animals treated with both drugs developed a loss of lymphoid tissue in the spleen, and hepatic and renal congestion. Testicular atrophy was seen in two dogs receiving 30 doses of the combination. Serum creatine kinase became elevated in Group 2 during the end of the study. Decreases occurred in RBCs, Hb concentration, and Hct in Group 1. Electrocardiographic changes occurring in Group 2 were prolongation of PQ and QRS interval, AV block, and ventricular premature depolarization, which occurred singly or in short paroxysms of tachycardia. No significant change in heart rate or mean systemic arterial pressure occurred.

In conclusion, doxorubicin cardiotoxicity was reduced in dogs treated with ICRF-187 (ADR-529) at a ratio of 14.3:1 (ICRF-187:Doxorubicin) dosed every three weeks for up to 90 weeks.

ICRF-187 EXERTS CARDIOPROTECTION WHEN ADMINISTERED SIMULTANEOUSLY WITH OR 2 HOURS AFTER DOXORUBICIN IN BEAGLE DOGS. HJJERMAN, E.H., ET AL., PROC AMER ASSN CANCER RES 31:442, 1990. VOL. 1.15, P. 05-01468
GB0106

This short abstract describes the cardioprotective results of ICRF-187 when administered 30 minutes prior to doxorubicin, with doxorubicin, or 2 hours after doxorubicin. Cardiac lesions were more severe when ICRF-187 was administered 2 hours after doxorubicin than when administered simultaneously with doxorubicin. Cytoplasmic vacuolization and myofibrillar loss were the lesions mentioned.

EFFECT OF ICRF-187 ON DOXORUBICIN-INDUCED MYOCARDIAL EFFECTS IN THE MOUSE AND GUINEA PIG. PERKINS, W.E., SCHROEDER, R.L., CARRANO, R.A., AND IMONDI, A.R. BR. J. CANCER (1982) 46,662. VOL. 1.15, P. 05-01469.
GB0045

Studies were done to evaluate the cardioprotection afforded by ICRF-187 in mice and guinea pigs treated with doxorubicin. Mice were injected ip with ICRF-187 (50 mg/Kg) 30 min prior to doxorubicin HCl (4 mg/Kg). Injections were on Tuesday and Friday of Weeks 1, 2, 5, 6, and 7. Sacrifice was Week 11. Male Hartley guinea pig hearts (atria) were evaluated in vitro for contractile frequency and response to histamine after being treated with doxorubicin (single 1 mg/Kg ip) or doxorubicin (1 mg/Kg) plus ICRF-187 (12.5 mg/Kg 2x/day on Days 1, 8, and 15). The results in mice, presented below, are from Table I of the reprint.

Histopathological Effects of Doxorubicin on Hearts and Kidneys of Female Cox ICR Mice

Heart:	Saline ip Saline iv	Saline ip Dox iv	ICRF-187 ip Dox iv
No. examined	10	15	17
Incidence of damage	0%	53%	18%*
No. with myocardial vacuolation			
Grade 1	0	6	3
Grade 2	0	2	0
Grade 3	0	0	0
Grade 4	0	0	0
Focal mononuclear cellular infiltration	0	2	0

Kidney			
No. examined	10	15	17
Incidence of renal damage	10%	13%	6%
No. of kidneys with:			
Focal mineralization(papilla)	1	0	1
Tubular basophilia	0	1	0
Cystic tubule	0	1	

* Significantly less than Saline-Dox: p <0.05

Results in the guinea pig showed that three weekly ip administrations of doxorubicin had no significant effect on the rate of atria contraction. Significant reductions in the chronotropic response to histamine were observed only after 2 weekly injections. ICRF-187 did not block this effect. No evidence of vacuolar cardiomyopathy in the heart or degenerative or inflammatory changes in the cortex of the kidneys were reported in guinea pigs receiving the third 1 mg/Kg doxorubicin dose.

EFFECT OF ICRF-187 PRETREATMENT AGAINST DOXORUBICIN-INDUCED DELAYED CARDIOTOXICITY IN THE RAT. VILLANI, F., ET AL. TOXICOL. APPL. PHARMACOL. 102, 292-299(1990).
VOL. 1.15, P. 05-01475
GB0103

Female CD rats were evaluated for general and myocardial toxicity. Doxorubicin was administered iv at 3 mg/Kg/week (one dose/week for 5 weeks). ICRF-187 was administered ip in water at 125 mg/Kg, 60 min prior to doxorubicin.

Results

Blood chemistry was not altered by the treatment 5 weeks after the last doxorubicin administration. No significant changes appeared in myocardial Ca and Fe contents. No animals died during the study. Body weights of the rats treated only with ICRF-187 were significantly reduced compared to controls, while the body weight of those treated with ICRF-187 plus doxorubicin was somewhat higher. Progressive and significant prolongation of QT and ST intervals appeared in doxorubicin treated rats. These changes were prevented with ICRF-187 up to Week 6, then the intervals began to increase. No significant changes in serum electrolytes were found. The most prominent cardiac findings in left ventricle tissue were myocyte vacuolization and myofibriller loss. These lesions were significantly reduced (p<0.001) in the group administered

ICRF-187. The contractile properties (df/dt, g/sec) of atria isolated from treated animals were significantly reduced with a high load (4.25 g) in the doxorubicin only group. AUC values for myocardial tissue are indicated below:

Doxorubicin = 39.67 + 1.76 µg/g/hr
Doxorubicin + ICRF-187 = 47.33 + 1.86 µg/g/hr

PROTECTIVE EFFECT OF ICRF-187 ON DOXORUBICIN-INDUCED CARDIAC AND RENAL TOXICITY IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS. HERMAN, E.H., EL-HAGE, A., AND FERRANS, V.J., TOXICOL.APPL. PHARMACOL. 92(1): 42-53, 1988.
VOL. 1.15, P. 05-01485
GB0064

The results from these studies were little different from what has been reported in other research papers on this topic. This study, however, also evaluated the effect of ICRF-187 on renal toxicity. Cardiomyopathy scores and treatment of adult male SHR and WKT rats (5/group) are shown below. Cardiac lesions were graded on a scale of 0 to +4 on the basis of the number of muscle cells showing myofibriller loss and cytoplasmic vacuolization. Renal damage was also based on a scale of 0 to +4. Results for the WKY rats are indicated in the following table in parentheses.

	Cardiomyopathy Scores				
Treatment SHR (WKY)	0	1	2	3	4
Dox(1 mg/Kg/week iv x 12)	0(1)	0(3)	2(1)	3(0)	0(0)
Dox+ICRF (1 mg/Kg/week + 25 mg/Kg/week ip x 12)	0(5)	5(0)	0(0)	0(0)	0(0)
ICRF(25 mg/Kg/week ip x 12)	5(5)	0(0)	0(0)	0(0)	0(0)
Saline	5(5)	0(0)	0(0)	0(0)	0(0)

One SHR rat died 3 days after the 12th dose. Weight gain was significantly less in the doxorubicin treated group by Week 6 and significantly less by Week 12 in the combination group. Loss of body weight, renal lesions, and cardiomyopathy were the main toxicity observed in SHR and WKY rats. Pretreatment with ICRF-187 reduced the severity of weight loss and cardiac and renal lesions in SHR and WKY rats. Renal toxicity (rare in humans) consisted of atrophy and dilation of tubules, protein casts in the tubular lumina, and glomerular vacuolization. Serum triglycerides and cholesterol were elevated in SHR and WYK rats administered doxorubicin. In general, toxicity was more severe in SHR rats.

REDUCTION BY ICRF-187 OF ACUTE DAUNORUBICIN TOXICITY IN SYRIAN GOLDEN HAMSTERS. HERMAN, E.H., ET AL., RES. COMM. CHEM. PATHOL. PHARMACOL. 40 (2):217-231, 1983.
VOL. 1.15, P. 05-01505
GB0055

Variations in ip ICRF-187 doses and treatment times were evaluated in hamsters administered daunorubicin (25 mg/Kg iv). Body weight, food consumption, and lethality were reduced by ICRF-187 administration. Doses greater than 12.5 mg/Kg afforded protection, and survival was maximum when ICRF-187 (100 mg/Kg) was administered 3 hours before to 3 hours after daunorubicin. The most prominent histopathologic changes in hamsters receiving daunorubicin were found in the GI tract, and included ulcerative lesions on the tongue, stomach, duodenum, and colon. Kidney toxicity consisted of tubular dilation and deposition of large amounts of proteinaceous material. Only two of the hamsters developed cardiac lesions, characterized as minimal cellular vacuolization. These lesions were reduced with ICRF-187 administration.

REDUCTION OF DAUNORUBICIN LETHALITY AND MYOCARDIAL CELLULAR ALTERATIONS BY PRETREATMENT WITH ICRF-187 IN SYRIAN GOLDEN HAMSTERS.
HERMAN, E., ET AL., CANCER TREAT. REP. 63 (1): 89-92, 1978
VOL. 1.15, P. 05-01520
GB0001

These authors looked at the protective activity of ICRF-187 against daunorubicin induced cardiac and hepatic toxicity. Hamsters (70-110 g) were administered 100 mg/Kg ICRF-187 ip in physiologic saline, followed in one hour by 25 mg/Kg daunorubicin iv or saline. Twenty saline treated animals served as a control. WBCs were counted 72 hours after similar treatment in other groups of animals. Other animals similarly treated were used to evaluate the histopathology of the heart and liver at intervals of 1, 2, 4, 6, and 10 weeks after daunorubicin administration.

By Week 3 only 10% of the daunorubicin group were alive, compared to 85% in the ICRF-187 pretreated group and 100% in the control group. By Week 5 all in the daunorubicin group were dead, compared to 20% in the ICRF-187 pretreated group. After 4 months, 36% of the pretreated group were still alive. A 36% body weight reduction occurred in the daunorubicin group by Week 3, contrasted to 10% weight loss in the ICRF-187 group.

WBCs were reduced 68% in the daunorubicin group and 86% in the pretreated ICRF-187 group. Diffuse vacuolation of myocardial cells were comparable in both ICRF-187 pretreated and saline control animals at Weeks 1 and 2. At Week 4, areas of focal and diffuse vacuolation of cardiac muscle cells were present in both groups. By Week 6, vacuolation was diminished in the ICRF-187 pretreated animals; by Week 10, essentially no morphologic difference in cardiac cells was seen in control and ICRF-187 pretreated animals, when compared to the daunorubicin group. Liver histopathology showed comparable slight vacuolation of the hepatocytes, with some recovery noted with time. Vacuolation of hepatocytes was still evident at Week 10 in ICRF-187 pretreated animals.

COMPARISON OF THE PROTECTIVE EFFECT OF ICRF-187 AND STRUCTURALLY RELATED ANALOGUES AGAINST ACUTE DAUNORUBICIN TOXICITY IN SYRIAN GOLDEN HAMSTERS.
HERMAN, E.H., ET Al., RES COMM CHEM PATHOL PHARMACOL 48(1): 39-55, 1985.
VOL. 1.15, P. 05-01524
GB0065

This paper compared structure activity of ICRF-187 analogues in preventing daunorubicin induced toxicity in hamsters. Both enantiomers of ADR-529, ie., ICRF-187 (d-enantiomer) and (l-enantiomer) showed similar protective activity against daunorubicin lethality. Bimolane was as effective as ICRF-187 in reducing the mortality of daunorubicin. Other analogues that were evaluated did not show protection.

EXAMINATION OF THE POTENTIAL LONG-LASTING PROTECTIVE EFFECT OF ICRF-187 AGAINST ANTHRACYCLINE-INDUCED CHRONIC CARDIOMYOPATHY.
HERMAN, E. H., AND FERRANS, V. J., CANCER TREAT REVIEWS 17, 155-160 (1990)
VOL. 1.15, P. 05-01541
GB0120

The long-lasting protective effect of ICRF-187 against anthracycline toxicity was studied in NZ rabbits treated with daunorubicin. In one study, a total of five dosings/animal were administered at 3 week intervals over a period of 15 weeks. Another study treated animals 6 times at 3 week intervals over an 18 week period. Blood chemistry and hematology (Chem-screen 25) were evaluated. Histopathology evaluation was conducted on the heart, liver, kidney, lung, small intestine, and skeletal muscle. Cardiac scores for the

left ventricle were graded on a scale of 0 to 4 on the basis of the number of muscle cells showing myofibriller loss and cytoplasmic vacuolization. Tissue sections were evaluated blinded 3 weeks and 3 months after the last treatment.

The observed cardiac alterations were comparable to what has been previously seen in rabits [Jaenke, R.S. Lab. Invest. 30:292-304 (1974)]. Cardiomyopathy scores were also comparable to what has been reported in other species. Rabbits given a cumulative dose of 19.2 mg/Kg over 18 weeks, evaluated 3 months after the last treatment, did not appear to show an increase in cardiac scores. Pretreatment with ICRF-187 significantly reduced cardiomyopathy scores when tissues were evaluated 3 weeks or 3 months after the last treatment. No morphologic changes related to daunorubicin or ICRF-187 were said to be found in the lung, small intestine, liver, or skeletal muscle. Also, no consistent changes were said to occur in blood chemistry or in WBCs, RBCs, or hemoglobin.

REDUCTION OF CHRONIC DOXORUBICIN CARDIOTOXICITY IN DOGS BY PRETREATMENT WITH (±)1,2-BIS(3,5-DIOXOPIPERAZINYL-1-YL (ICRF-187).
HERMAN, E. H. AND FERRANS, V.J., CANCER RES. 41(9): 3436-3440, 1991.
VOL. 1.15, P. 05-01547
GB0032

Adult beagle dogs treated with doxorubicin (1 mg/Kg/week x15 iv) alone or 30 minutes after ICRF-187 (12.5 mg/Kg/week x15 ip) administration developed cardiac alterations consisting of cytoplasmic vacuolization and myofibriller loss similar to what has been reported in humans and other animals. The doxorubicin group showed the most severe changes. General toxicity observed with doxorubicin treatment was alopecia, which was not prevented with ICRF-187. No histological lesions attributed to doxorubicin or ICRF-187 were reported in the kidney, lung, small intestine, diaphragm, or skeletal muscle. Serum iron levels were significantly reduced in groups given doxorubicin alone or with ICRF-187. RBCs, WBCs, and hemoglobin values were also significantly reduced in these groups. Hematocrit was significantly low in the doxorubicin only group.

COMPARISON OF THE EFFECTIVENESS OF (±) 1,2-BIS(3,5-DIOXO-PIPERAZINYL-1-YL)PROPANE (ICRF-187) AND N-ACETYLCYSTEINE IN PREVENTING CHRONIC DOXORUBICIN CARDIOTOXICITY IN BEAGLES. HERMAN, E. H., ET AL., CANCER RES. 45(1): 276-281, 1985
VOL. 1.15, P. 05-01552
GB0031

This report examined the use of N-acetylcysteine (NAC) and ICRF-187 alone and in combination with doxorubicin in preventing cardiotoxicity. The results indicated that pretreatment with NAC offered no protection in reducing the doxorubicin cardiotoxicity in dogs.

INFLUENCE OF VITAMIN E AND ICRF-187 ON CHRONIC DOXORUBICIN CARDIOTOXICITY IN MINIATURE SWINE. HERMAN, E. H., AND FERRANS, V. J. LAB. INVEST. 49(1): 69-77, 1983
VOL. 1.15, P. 05-01558
GB0046

This study evaluated the use of vitamin E in preventing doxorubicin induced cardiotoxicity in miniature swine. No reduction in the incidence of cardiac lesions occurred when vitamin E was administered with doxorubicin; however, severity of the lesions decreased. Cardiac changes were similar to those reported in dogs, rabbits, rats, pigs, and humans.

CELLULAR PHARMACOLOGY OF ADR-529 IN ADULT RAT HEART MYOCYTES.
VOL. 1.15, P. 05001567
PH-001

Intact, beating, rat heart myocytes were used to study the uptake, efflux, and metabolism of ICRF-187. ICRF-198, the major open ring metabolite, was also evaluated. The following results are verbatim from the summary section of the report (p. 5).

1. Both doxorubicin and daunorubicin produced dose- and time-dependent cytotoxicity in adult rat heart myocytes in vitro.

2. ADR-529 produced significant protection against doxorubicin related myocyte killing at a 2.5:1 (ADR-529:doxorubicin) molar ratio; ICRF-198 was protective only at a 10:1 molar ratio.

3. Clear cut morphological evidence of protection against

doxorubicin cardiac toxicity was obtained with ADR-529 using electron microscopic evidence of heart damage as the major end point.

4. In the same model that clearly predicts the features of doxorubicin cardiotoxicity, the uptake of ADR-529 into rat heart myocytes was found to be rapid, with peak levels of cell-associated drug measurable within 20-30 sec after drug addition. Cell accumulation increased with the concentration of the extracellular drug and varied as a function of the number of myocytes exposed.

5. Accumulation of ADR-529 was not affected by changes in temperature (from 14-37 degrees C), or the addition of sodium azide, rotenone, or deoxyglucose.

6. At equimolar extracellular concentrations, ICRF-198 accumulated to a significantly lesser degree in myuocytes than ADR-529.

7. Loss of ADR-529 and its hydrolysis products from myocytes is very rapid, occurring within 1-2 minutes after cells are placed in fresh buffer.

SELECTIVE ALTERATIONS IN RAT CARDIAC mRNA INDUCED BY DOXORUBICIN: POSSIBLE SUBCELLULAR MECHANISMS.
PAPOIAN, T., AND LEWIS, W., CIRCULATION 82 (4) SUPPL. 291, 1990 VOL. 1.15, P. 05-01593
GB0123

This short abstract described results observed on isolated myocardial mRNAs from SD rats treated with ICRF-187 and doxorubicin. The isolated mRNA was transferred to nitrocellulose and hybridized with 32P-cDNA probes (alpha c-actin, troponin C (TnC), BamHI fragment of mouse mitochondria, and glyceraldehyde-3-phosphate dehydrogenase (G3PD). Only myocardial alpha c-actin mRNA was depressed. Troponin C, mouse mitochondria or G3PD were not depressed. It was suggested that

a change in myocardial alpha c-actin mRNA, rather than free radical involvement, is involved in the mechanism of action.

ANTHRACYCLINE-INDUCED HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS: EFFECT OF ICRF-187.
DECORTI, G., ET AL., MED. SCI. RES. 15 (21/22): 1405-1406, 1987.
VOL. 1.15, P. 05-01594
GB0062

The results of incubating rat peritoneal mast cells in saline with calcium, with EDTA (2 mM), or ICRF-187 (2 and 10 mM) on mast cell histamine release induced by doxorubicin (100 µg/mL), daunorubicin (100 µg/mL), epirubicin (100 µg/mL), and a compound called 48/80 (1 µg/mL) were investigated. The results indicated ICRF-187 (10 mM) significantly blocked histamine release when incubated in saline solution with calcium, but no difference in histamine release was seen when the cells were incubated in calcium free saline alone. These authors suggest that ICRF-187 acts mainly by chelating intracelluler calcium.

MODULATION OF IN VITRO FUNCTIONAL AND BIOCHEMICAL EFFECTS OF DOXORUBICIN BY IRON-COMPLEXATING AGENTS IN MOUSE HEART.
DE JONG, J., ET AL., EUR .J. PHARMACOL. 183 (5): 1715-1716, 1990.
VOL. 1.15, P. 05-01596
GB0118

These authors reported that lipid peroxidation induced by doxorubicin is decreased in isolated mouse left atria when incubated with ICRF-187. They suggested the mechanism of action of ICRF-187 is related to inhibition of free radicals.

ROLE OF (±)-1,2-BIS(3,5-DIOXOPIPERAZINYL-1-YL)PROPANE (ICRF-187) IN MODULATING FREE RADICAL SCAVENGING ENZYMES IN DOXORUBICIN-INDUCED CARDIOMYOPATHY.
ALDERTON, P., ET AL., CANCER RES. 50 (16): 5136-5142, 1990
VOL. 1.15, P. 05-01597
GB0110

This study measured levels of superoxide dismutase (SOD), glutathione peroxidase (GP), catalase, and reduced glutathione in the heart, liver, kidney, and skeletal muscle of mice treated with doxorubicin, with and without added ICRF-187. No significant differences were observed with acute treatment or with chronic exposure to doxorubicin or doxorubicin + ICRF-187.

In chronic exposure, electron microscopy conclusively showed cardiotoxicity in doxorubicin treated mice, i.e., gross mitochondrial degeneration and disorganization of the precise alignment of myofilaments. Such changes were absent in hearts treated with the doxorubicin plus ICRF-187. These authors favor an iron chelating mechanism for ICRF-187 cardioprotection.

ADRIAMYCIN-INDUCED FREE RADICAL FORMATION IN THE PERFUSED RAT HEART: IMPLICATIONS FOR CARDIOTOXICITY.
RAJAGOPALAN, S., ET AL., CANCER RES. 48, 4766-4769, 9/1/88
VOL. 1.15, P. 05-01604
GB0078

These studies indicated ·OH radical formation is stimulated by Adriamycin in perfused rat hearts. The hydroxyl radical was analyzed by ESR spectroscopy using DMPO as a spin-trapping agent. When 600 units/mL SOD were added, hydroxyl radical formation was inhibited by about 3 times—superoxide is dismutated by SOD into hydrogen peroxide and oxygen. Catalase (550 units/mL) abolished formation of the hydroxyl radical. It was postulated that in cardiac cells, a one electron reduction of the anthracycline occurs in the presence of oxygen, leading to superoxide formation which is dismutated by SOD into oxygen and hydrogen peroxide. In the presence of Fe II, hydrogen peroxide is reduced back to the hydroxyl radical and produces cardiac injury. When ADR-529 is present, it is hydrolyzed to the open rings which can now chelate Fe II and prevent reduction of hydrogen peroxide to hydroxyl radicals. These studies also showed that Adriamycin stimulated free radical formation was suppressed in the presence of ADR-529, and that free radical formation was not suppressed in the absence of Adriamycin.

THE INTERACTION OF THE CARDIOPROTECTIVE AGENT ICRF-187 [(+)-1,2- BIS(3,5-DIOXOPIPERAZINYL-1-YL)PROPANE]; ITS HYDROLYSIS PRODUCT (ICRF-198); AND OTHER CHELATING AGENTS WITH THE Fe(III) AND Cu(II) COMPLEXES OF ADRIAMYCIN.
BRIAN B. HASINOFF, AGENTS AND ACTIONS, VOL. 26, 3/4(1989)
VOL. 1.15, P. 05-01608
GB0085

This paper examined the reactions of ICRF-187 and its hydrolyzed product, ICRF-198, with Fe(III)-Adriamycin and Cu(II)-Adriamycin complexes. ICRF-198 completely removed Fe(III) and Cu(II) from the Adriamycin complex. ICRF-187 was also capable of removing metal ions from the complexes, but was

slower than ICRF-198. Both ICRF compounds protected the Fe(III)-adriamycin induced inactivation of cytochrome c oxidase activity of submitochondrial particles.

THE IRON(III) AND COPPER(II) COMPLEXES OF ADRIAMYCIN PROMOTE THE HYDROLYSIS OF THE CARDIOPROTECTIVE AGENT ICRF-187 [(+)-1,2-BIS(3,5-DIOXOPIPERAZINYL-1-YL)PROPANE].
HASINOFF, B.B., AGENTS AND ACTIONS VOL. 29, 374(1990).
VOL. 1.15, P. 05-01616
GB0100

These studies indicated that both the Fe(III) and Cu(II) Adriamicin complexes are capable of promoting ring opening hydrolysis of ICRF-187 and complexing Fe(III) and Cu(II) ions with ICRF-198. ICRF-187 also aforded protection against Fe(III)-Adriamycin inactivation of cytochrome c oxidase and NADH cytochrome c reductase activity.

ROLE OF DAUNOSAMINE AND HYDROXYACETYL SIDE CHAIN IN REACTION WITH IRON AND LIPID PEROXIDATION BY ANTHRACYCLINES.
GIANNI, L., ET AL., JNCI VOL. 80, NO. 14, SEPTEMBER 21, 1988
VOL. 1.15, P. 05-01624
GB0239A

These authors looked at doxorubicin, daunorubicin, and analogs of doxorubicin modified in the amino sugar moiety of the molecule (daunosamine) for their ability to react with Fe (III) and to sustain lipid peroxidation of isolated human platelet membranes. The C14-OH and C3'-NH2 appear to act as a bidentate ligand for iron.

dl-N,N'-DICARBOXAMIDOMETHYL-N,N'-DICARBOXYMETHYL-1,2-DIAMINO-PROPANE (ICRF-198) AND d-1,2-BIS(3,5-DIOXOPOPERAZINE-1-YL)-PROPANE (ICRF-187) INHIBITION OF FE (III) REDUCTION, LIPID PEROXIDATION, AND CaATPase INACTIVATION IN HEART MICROSOMES EXPOSED TO ADRIAMYCIN.
VILE, G.F. AND WINTERBOURN, C. C., CANCER RESEARCH 50, 2307-2310, APRIL 15, 1990
VOL. 1.15, P. 05-01632
GB0096

This paper examined the effect of ICRF-198 or ICRF-187 on iron dependent lipid peroxidation in an incubation system with rabbit heart microsomes. Microsomal reduction of Fe (III) chelates and CaATPase inactivation was also evaluated. The results showed a 10% inhibition of Fe (III), Fe (III)ADP, or

Fe(III)-ferritin by rabbit heart microsomes in nitrogen in the presence of Adriamycin, NADPH, and ICRF-187. This system was inhibited by 77% with ICRF-198. Only partial inhibition of CaATPase and lipid peroxidation was observed with ICRF-187 but were greatly reduced with ICRF-198.

HUMAN FERRITIN (HLF) AS IRON SOURCE FOR LIPID PEROXIDATION BY ADRIAMYCIN (ADR).
GIANNI, L., ET AL., PROC. AM. ASS. CANCER RES. 29: A1108, 1988
VOL. 1.15, P. 05-01636
GB0071

This short abstract reported Adriamycin supported lipid peroxidation in human platelet membranes in vitro and could be completely inhibited by 50 μM ICRF-198 in the presence of iron-loaded human ferritin.

EFFECTS OF DOXORUBICIN AND ADR-529 ON LIPIP PEROXIDATION AND DISTRIBUTION OF IRON IN RAT HEART AND LIVER.
REPORT # 088915-000, Vol. 1.15, p. 05-01637

This study was conducted by Steven D. Aust, Ph.D. at Utah State University. The effects of doxorubicin on hepatic and cardiac iron distribution and how these parameters were influenced by ADR-529 in vivo studies were examined.

Lipid peroxidation was not observed with ADR-529. With hydrolyzed product (ICRF-198), oxidation was inhibited when it was in excess of the iron concentration. No inhibition occurred when the system was preincubated with microsomes. Ferritin levels in the heart and liver of rats determined 24 hours after administering 20 mg/Kg doxorubicin, 200 mg/Kg Dexrazoxane, or both together at the above concentrations are indicated in the following table (from Tables 2 through 5).

Effect of Doxorubicin And ADR-529 On Ferritin Levels

Gp	Treatment	Hepatic Ferritin (μg/g tissue)	Cardiac Ferritin (μg/g tissue)	Hepatic Ferritin Fe Content (μMol/mg)	Cardiac Ferritin Fe Content (μMol/mg)
1	Control	158±37	39±5	1.9±0.4	91±15
2	ADR-529	183±64	47±4	1.4±0.5	12±27
3	ADR-529+Dox	209±58	43±5	0.8±0.4	95±35
4	Dox	244±51	46±4	0.8±0.3	72±15

It was suggested that doxorubicin toxicity is related to the iron that may be released from ferritin and the generation of free radicals.

PHARMACOLOGIC STUDIES TO DETERMINE THE POTENTIAL OF ADR-529 TO LIMIT THE EXTENT OF MYOCARDIAL REPERFUSION INJURY IN EXPERIMENTALLY INDUCED MYOCARDIAL INFARCTION IN THE CANINE HEART.
VOL. 1.16, P. 05-01690
REPORT # 2111, 4171

ADR-529 was evaluated for its ability to protect against myocardial infarct size associated with reperfusion in canine hearts. After isolating and occluding the left circumflex coronary artery for 15 to 90 minutes, 200 mg/Kg ADR-529 infusion or 0.9% saline infusion was administered over 2 hours, begining 15 minutes before the start of reperfusion and ending 105 minutes after the initiation of reperfusion. Reperfusion was maintained for 6 hours, then the hearts were removed and the infarct size determined. Based on these studies, there was no reduction in the size of the infarcts when compared to saline control.

THE EFFICACY OF ADR-529/DOXORUBICIN COMBINED FORMULATION IN MOUSE MODELS OF MURINE CANCER.
VOL. 1.16, P. 05-01779.
REPORT # PLS 88-2

L1210 murine leukemia ip implants and B16 murine melenoma sc implants were treated with a combined formulation of ADR-529/doxorubicin and compared to seperate administration of Dox and ADR-529. The doxorubicin doses were chosen to give an antitumor response appropriate for each type of tumor. CDF1 mice were used for evaluation of L1210 leukemia and BDF1 mice were used for the B16 studies. Drugs were administered by the iv route in 0.9% saline solution on days 1, 5, and 9. Median survival time (MST) was used to compare antitumor activity. The results are indicated in the following tables (from Tables 1 and 2 of the report). The study was conducted by Adria Labs.

Dox & ADR = seperate solutions
Dox/ADR = combination solution

Results

Effect of ADR-529 and Doxorubicin on Implanted L1210 Leukemia

Treatment (mg/Kg)	MST (days)	T/C %	LTS	Toxic Deaths
Saline	8.33	-	-	-
Dox (4)	9.5	114	0/9	0/9
(6)	11.1	133	0/9	0/9
(8)	14.0	168	0/9	0/9
ADR (80)	9.07	108	0/9	0/9
(120)	9.22	111	0/9	0/9
(160)	9.22	111	0/9	0/9
(200)	9.17	110	0/9	0/9
Dox & ADR (4+80)	12.5	150	0/9	0/9
(6+120)	14.75	177	0/9	0/9
(8+160)	17.0	204	0/9	0/9
Dox/ADR (4+80)	12.5	150	0/9	0/9
(6+120)	14.7	176	0/9	0/9
(8+160)	16.0	192	0/9	0/9

Effect of ADR-529 and Doxorubicin on Implanted B16 Melanoma

Treatment (mg/Kg)	MST (days)	T/C %	LTS	Toxic Deaths
Saline	18.21	-	-	-
Dox (2)	20.5	113	0/9	0/7
(4)	25.75	141	0/8	0/8
(6)	29.67	163	0/8	0/8
(8)	31.5	173	0/9	0/9
ADR (40)	19.5	107	0/7	0/7
(80)	17.5	96	0/7	0/7
(120)	20.25	111	0/7	0/7
(160)	19.0	104	0/7	0/7
Dox & ADR (2+40)	21.5	118	0/9	0/9
(4+80)	27.5	151	0/9	0/9
(6+120)	31.5	173	0/9	0/9
(8+160)	32.5	178	0/9	0/9

DOX/ADR-529	(2+40)	22.5	124	0/9	0/9
	(4+80)	28.0	154	0/9	0/9
	(6+120)	32.5	178	0/9	0/9
	(8+160)	33.0	181	0/9	0/9

There were no toxic deaths (deaths before saline controls with >4 g weight loss) or long term survivors (LTS-surviving up to or beyond 60 days) in the L1210 or the B16 melanoma animals. MST was calculated according to the procedure. No antitumor effect (%T/C <125) was observed with ADR-529 in either tumor model. Dose dependent increases in antitumor activity (MST and T/C %) occurred in both tumor models with doxorubicin, with the combination formulation, and with the addition of separate solutions. ADR-529 alone appeared to slightly increase MST above the control, but these changes were not significant. No difference was seen with either tumor model in administering the combination formulation or separate administration of the two drugs.

COMPARISON OF THE EFFICACY OF ADRIAMYCIN ALONE AND IN COMBINATION WITH ADR-529 AGAINST THE P388 TUMOR MODEL WHEN ADMINISTERED SEPARATELY OR TOGETHER AS ONE SOLUTION.
VOL. 1.16, P. 05-01788.
REPORT # 227I, PLS 90-01

ADR-529 and Adriamycin RDF were administered separately (less than one minute apart) or in combination in the same solution to CDF1 mice to determine if an antitumor difference exists against the P388 murine leukemia model. Adriamycin RDF is a rapid dissolution formula that has not been used in animal studies before (this study was conducted June 1990). Both drugs were administered iv in normal saline on days 1, 5, and 9 after implanting 10^6 P388 cells. Neither compound required sonication to aid dissolution. Drugs were administered at 0, 1, or 4 hours after preparation. Median survival time (MST) was used to compare antitumor activity. The results are shown in the following table (from Table 1, p. 05-01798).

Compound	Dose (mg/Kg/inj)	MST (Days)	%T/C	LTS	Toxic Death
Saline		11.17		0/7	0/7
Adriamycin RDF	4.0	15.50	139	0/7	0/7
	6.0	18.50	166	0/7	0/7
	8.0	18.50	166	0/7	0/7

ADR-529	80	11.17	100	0/7	0/7
	120	14.50	130	0/7	0/7
	160	14.10	126	0/7	0/7
Time 0					
Adriamycin + ADR-529	4+80	18.17	163	0/7	0/7
(seperate solutions)	6+120	19.50	175	0/7	0/7
	8+160	17.50	157	0/7	0/7
Time 0					
Adriamycin + ADR-529	4+80	18.50	166	0/7	0/7
	6+120	22.00	197	0/7	0/7
	8+160	15.50	139	0/7	0/7
Time = 1 hour					
Adriamycin + ADR-529	4+80	18.00	161	0/7	0/7
(seperate solutions)	6+120	17.25	154	0/7	0/7
	8+160	17.50	157	1/7	0/7
Time = 1 hour					
Adriamycin + ADR-529	4+80	18.17	163	0/7	0/7
(same solution)	6+120	22.00	197	0/7	0/7
	8+160	15.50	139	0/7	0/7
Time = 4 hours					
Adriamycin + ADR-529	4+80	17.50	157	0/7	0/7
	6+120	18.50	166	0/7	0/7
	8+160	18.50	166	0/7	0/7
Time = 4 hours					
Adriamycin + ADR-529	4+80	19.00	170	0/7	0/7
(same solution)	6+120	17.50	157	0/7	0/7
	8+160	17.00	152	1/7	0/7

There were no toxic deaths (animals dying before saline control with >4 g weight loss) in the study. Long term survivors (LTS) were seen in the maximum combination dose at 1 and 4 hours. ADR-529 increased the MST by about 29% above the saline control. No differences were seen in MST between administration of the drugs immediately after preparation or up to 4 hours after preparation.

EFFECT OF ADR-529 ON THE ANTITUMOR ACTIVITY OF EPIRUBICIN AGAINST L1210 LEUKEMIA AND B16 MELANOMA WHEN ADR-529 IS ADMINISTERED IN THE SAME SOLUTION AS THE ANTHRACYCLINE.
VOL. 1.16, P. 1799.
REPORT # 2191, PLS 89-9

Antitumor activity was evaluated in CDF1 mice after ip inoculation with 10^5 L1210 cells, followed by iv administration of drugs on days 1, 5, and 9 after tumor implantation. The B16 melanoma was evaluated in BDF1 mice inoculated ip with tumor brei (0.5 mL of a 1:10 weight:volume), then dosed with drugs on days 1, 5, and 9 after tumor implantation. Both drugs were

reconstituted in 0.9% saline aided with sonication. Antitumor activity was compared using MST values determined according to the method. The results are shown in the following table (from Tables 1 and 2, p. 05-01809 and 05-01810).

Effect of ADR-529 and Epirubicin Against L1210 Leukemia

Compound	Dose (mg/Kg/inj)	MST (Days)	%T/C	LTS	Toxic Deaths
Saline		8.3			
Epirubicin	6	11.3	135	0/9	0/9
	8	11.3	135	0/9	0/9
	10	13.0	156	0/9	0/9
ADR-529	120	9.2	111	0/9	0/9
	160	9.3	112	0/9	0/9
	200	9.2	111	0/9	0/9
Epirubicin + ADR-529 (separate solutions)	6 + 120	13.8	166	0/9	0/9
	8 + 160	14.5**	174	0/9	0/9
	10 + 200	15.5*	186	0/9	0/9
Epirubicin + ADR-529 (same solution)	6 + 120	13.5	162	0/9	0/9
	8 + 160	14.3**	174	0/9	0/9
	10 + 200	15.5*	186	0/9	0/9

Effect of ADR-529 and Epirubicin Against B16 Melanoma

Saline		19.0			
Epirubicin	6	26.5	139	0/9	0/9
	8	31.0	163	0/9	0/9
	10	29.0	153	0/9	0/9
ADR-529	120	23.0	121	0/9	0/8
	160	20.5	108	0/9	0/9
	200	23.0	121	0/9	0/9
Epirubicin + ADR-529 (separate solutions)	6 + 120	30.8*	162	0/9	0/9
	8 + 160	31.0	163	0/9	0/9
	10 + 200	32.5*	171	0/9	0/9
Epirubicin + ADR-529 (same solution)	6 + 120	30.9*	163	0/9	0/9
	8 + 160	31.5	166	0/9	0/9
	10 + 200	32.9*	173	0/9	0/9

1 is significantly greater (p<0.01) than the group
the same dose of epirubicin alone.
val is significantly greater (p<0.001) than the group
the same dose of epirubicin alone.

There were no toxic deaths or long term survivors in either tumor model. A 21% increase in MST occurred in the group receiving ADR-529 alone. The above data showed no significant differences between administration of both drugs in the same solution or administration in separate solutions; however, a significant increase in MST occurred with some of the combination doses compared to the same dose of epirubicin.

EFFECT OF ADR-529 LOW pH FORMULATION (ADR-529 LpH) ON THE ANTITUMOR ACTIVITY OF METHOTREXATE, CYTARABIN (ARA-C) AND CISPLATIN IN MURINE TUMOR MODELS.
Vol. 1.16, P. 05-01887.
REPORT # 2201, PLS 89-7

This study was done to determine if ADR-529 LpH interferes with the antitumor activity of methotrexate, Ara-C, or cisplatin in murine P388 and L1210 leukemia models or in the B16 melanoma model. ADR-529 LpH was reconstituted in 1.87% M/6 sodium lactate; the other drugs were reconstituted in 0.9% saline. CDF1 or BDF1 mice were used in the evaluation studies. All drugs were administered iv on days 1, 5, and 9 after ip implanting tumors. In the combination drug groups, the drugs were administered within one minute of each other. Median survival times (MST), based on the NCI protocol, were used to compare and evaluate antitumor activity.

The results did not show any interference in the antineoplastic activity of methotrexate, Ara-C, or cisplatin in P388, L1210, or B16 murine tumor models.

SYNERGISTIC ACTIVITY OF DOXORUBICIN AND THE BISDIOXOPIPERAZINE (+)-1,2-BIS(3,5-DIOXOPIPERAZIN-1-YL)PROPANE (ICRF-187) AGAINST THE MURINE SARCOMA S180 CELL LINE.
WADLER, S., GREEN, M.D., AND MUGGIA, F.M., CANCER RES. 46 (3): 1176-1181, 1986.
VOL. 1.16, P. 05-01917.
GB 0034

Murine sarcoma S180 cells were used to evaluate the effect of ICRF-187 and doxorubicin in in vitro clonogenic and nonclonogenic assays. Cell suspensions with doxorubicin, with or without added ICRF-187, were evaluated in 1 and 24 hour

incubations. For these studies, doxorubicin was reconstituted in sterile 0.15 M NaCl and ICRF-187 was reconstituted from lyophilized powder in sterile, preservative-free saline.

The effect of varying doses of doxorubicin or ICRF-187 on colonly forming efficiency produced IC50 = 45 µg/mL for ICRF-187 and 0.175 µg/mL for doxorubicin. When colony forming efficiency was determined in a 36 hour incubation using 10 µg/mL ICRF-187 or 0.075 µg/mL, a linear decrease resulted over 16 hours with ICRF-187, followed by no further increase in cell kill. Doxorubicin alone resulted in cell kill decreasing up to 24 hours, followed by no further kill. Incubation of cells for 1 hour with 0.1 µg/mL doxorubicin or 10 µg/mL ICRF-187 decreased colony formation by 20% with doxorubicin, 44% with ICRF-187, and 59% with the drug combination. A 24 hour exposure of the two drugs at concentrations which resulted in no activity to cells (0.01 µg/mL doxorubicin and 0.1 µg/mL ICRF-187) resulted in a 64% decrease in colony formation. A synergistic cytotoxic and dose dependent effect was also produced.

LETHAL AND SUBLETHAL EFFECTS OF THE COMBINATION OF DOXORUBICIN AND THE BISDIOXOPIPERAZINE, (+)-1,2-BIS(3,5-DIOXOPIPERAZIN-1-YL PROPANE (ICRF-187), ON MURINE SARCOMA S180.
WADLER, S., GREEN, M.D., BASCH, R., AND MUGGIA, F.M., BIOCHEM. PHARMACOL. 36 (9): 1495-1501, 1987.
VOL. 1.16, P. 05 01923
GB 0015

These authors used cell cycle analysis to help discriminate cytokinetic from lethal effects of a combination of doxorubicin and ICRF-187. A 24 hour flow cytometric study showed a progressive accumulation of S180 cells in the G2M phase and a decrease of cells in the S-phase when incubated with increasing concentrations of doxorubicin. The ability of doxorubicin to arrest cells in the G2M phase was also increased in the presence of ICRF-187. Reduced colony forming activity, as a result of lethality to cells, was increased with the combination. Intracellular levels of doxorubicin were measured in cells that had been incubated previously with ICRF-187, during continuous exposure to doxorubicin (influx), and following loading (efflux) with doxorubicin. Preincubation of the cells with ICRF-187 decreased the uptake of doxorubicin, but no statistically significant difference in rate of uptake or total uptake was detected.

POTENTIATION OF DOXORUBICIN CYTOTOXICITY BY (+)-1,2-BIS-(3,5-DIOXOPIPERAZIN-1-YL) PROPANE (ICRF-187) IN HUMAN LEUKEMIC HL-60 CELLS.
MONTI, E., AND SINHA, B.K., CANCER COMMUN. 2 (4): 145-149 1990.
VOL. 1.16, P. 05 01930
GB 0109

These studies showed synergistic cell death in human HL-60 cells with concentrations of 5-10 nM doxorubicin and 2-10 μM ICRF-187, following a 96 hour exposure. This increase in cytotoxicity was not related to increases in cellular accumulation of the drug. The data suggested ICRF-187 significantly enhanced doxorubicin dependent free radical-OH formation.

POIKILOCYTOSIS IN DOGS WITH CHRONIC DOXORUBICIN TOXICOSIS.
BADYLAK, S.F, ET AL., AN. J. VET. RES. 46(2): 505-508, 1985.
VOL. 1.16, P. 05 01935
GB 0058

The administration of ICRF-187 did not prevent the occurrence of poikilocytosis in doxorubicin treated dogs. N-Acetylcystine administered with doxorubicin produced a mild decrease in poikilocytosis.

ICRF-187 REDUCES BLEOMYCIN-INDUCED PULMONARY TOXICITY IN MICE.
HERMAN, E.H., ET AL. (no information on where published).
VOL. 1.16, P. 05 01939
GB 0303A

This abstract reported ICRF-187 can protect against pulmonary injury in male adult CD-1 mice treated ac with 15 mg/Kg bleomycin 2x/week.

ATTENUATION OF BLEOMYCIN-INDUCED PULMONARY AND RENAL TOXICITY IN MICE BY ICRF-187.
HERMAN, E.H., ET AL., FASEB. J. 4(3): A615, 1990.
VOL. 1.16, P. 05 01940
GB 0114

Pretreatment of male or female adult C57/BL6 mice with ICRF-187 attenuated all aspects of pulmonary toxicity produced by bleomycin. The attenuation was more pronounced in males. Renal toxicity (hypertrophy of tubular epithelium and interstitial fibrosis), which occurred primarily in females, was also diminished by pretreatment with ICRF-187.

ICRF-187 AND POLYHYDROXYPHENYL DERIVATIVES FAIL TO PROTECT AGAINST BLEOMYCIN INDUCED LUNG INJURY. TRYKA, A. FRANCINE, TOXICONOGY 59(2): 127-138,1989.
VOL. 1.16, P. 05 01941
GB 0094

Treatment of hamsters with ICRF-187 failed to protect against bleomycin-induced lung injury. ICRF-187 enhanced the toxicity by increasing mortality (severe pneumonitis) when hamsters were exposed to low doses of bleomycin and 70% oxygen. No deaths occurred when the animals were treated with drugs alone. However, ICRF-187 has been shown to reduce doxorubicin cardiac toxicity in hamsters (Herman, E., et al., GB 001).

EXAMINATION OF THE PROTECTIVE EFFECT OF ICRF-187 AND DIMETHYL SULFOXIDE AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN SYRIAN GOLDEN HAMSTERS. EL-HAGE, A.N. ET AL., TOXICOLOGY 28(4): 295-303 1983.
VOL. 1.16, P. 05 01953
GB 0035

Both DMSO and ICRF-187 were examined as to their protective effect against acetaminophen-inducerd hepatotoxicity in Syrian golden hamsters. ICRF-187 (300 mg/Kg) administered 1 hour prior to the administration of hepatotoxic doses of acetaminophen attenuated the increases in alkaline phosphatase, SGPT, albumin, and globulin. Hepatotoxicity scores were also reduced by ip administration of DMSO or ICRF-187 in combination with acetaminophen.

EVALUATION OF THE ACUTE HEMODYNAMIC EFFECT OF ADR-529 IN ANESTHETIZED BEAGLE DOGS.
VOL. 1.16, P. 05 01979
REPORT # 2011, P-87-5, P-529-86-004.

Hemodynamic variables, such as mean arterial pressure (MAP), left ventricular pressure, cardiac contractility (dP/dt), and heart rate were evaluated in anesthetized beagle dogs treated with 81 or 200 mg/Kg ADR-529. No significant changes were reported for the above variables with 81 mg/Kg ADR-529. Values at the higher dose were also not significant when compared to the saline control. The average change in dP/dt was higher than the saline control (717 + 155 vs 294 + 58) at the high dose, but was not considered significant by the sponsor. Responses to norepinephrine (1 µg/kg), acetylcholine (2 µg/Kg), isoproterenol (0.25 µg/Kg), or a 30 second bilateral

carotid occlusion were not significantly altered when compared to the saline control. These studies did not show short term effects on hemodynamic function in dogs administered 81 or 200 mg/Kg ADR-529.

EFFECT OF ADR-529 ON GASTRIC EMPTYING OF A SEMI-SOLID TEST MEAL IN THE RAT.
VOL. 1.16, P. 05 01997
REPORT # 2041

Gastric emptying of gavaged Amberlite ion exchange resin pellets was not changed significantly when iv doses of ADR-529 (20 to 80 mg/Kg) were administered to rats. Atropine (1 mg/Kg iv), known to reduce gastric emptying in the human, reduced the emptying from 20% (control) to %4 in the rat. On the other hand, 12 mg/Kg metoclopramide, a drug used to aid GI emptying, increased the value to 60% in the rat.

EFFECT OF ADR-529 ON BASAL GASTRIC ACID SECRETION IN THE RAT.
VOL. 1.16, P. 05 02003
REPORT # 2021,P-87-4, P-329-87-005

Basal gastric acid secretion in the rat was studied with iv doses of 20, 40, 80, and 160 mg/Kg ADR-529. With 20 mg/Kg, a significant decrease occurred in the H ion concentration (5%). At the three higher doses of ADR-529, significant decreases occurred in volume, H ion concentration, and total acid output.

EFFECT OF ADR-529 AND ADR-529/HCl FORMULATIONS ON THE ANTITUMOR ACTIVITY OF VARIOUS ANTHRACYCLINES IN MURINE TUMOR MODELS.
Vol. 1.16, p. 05 01842
REPORT # 2131, PLS 89-1

This study was conducted to determine if the finished dosage formulations of ADR-529 or the hydrochloride salt of ADR-529 (ADR-529/HCl), which was developed as a more stable form of ADR-529, interfere with the antitumor activity of Adriamycin, idarubicin, or epirubicin in murine leukemias P388 and L1210, or Lewis lung carcinoma, and B16 melanoma. The studies compared antitumor activity of the anthracycline against the activity of the anthraclycline given in combination with either of the two formulations of drug. All drugs were administered on days 1, 5, and 9 after tumor implantation. Drug:anthracycline ratios were administered at various ratios,

but did not exceed 20:1. The study was done in the Pioneering Life Sciences Department of Adria Labs.

ADR-529/HCl was reconstituted in 1.87% M/6 Sodium Lactate; other compounds were reconstituted in 0.9% saline. Dissolution of anthracyclines was facilitated by sonication for 20-30 minutes. All drugs were diluted and administered iv to deliver the appropriate mg/Kg body dose in 10 mL/Kg. P388 (10^6 cells) and L1210 (10^5 cells) were inoculated ip into CDF1 mice; solid tumors were implanted ip into BDF1 mice.

Results

%T/C

Drug (mg/Kg)	P388	L1210	Lewis Lung	B16 Melanome
Adr(4,8)	151,202	114,154		
529(80,160)	106,121	110,114		
529/HCl(80,160)	107,111	108,113		
Adr+529(4,8/80,160)	172,251	171,199		
Adr+529/HCl(4,8/80,160)	181,237	158,189		
Adr(6,8)			142,167	129,150
529(160)			118	126
529/HCl(160)			118	100
Adr+529(6,8/120,160)			>400,88	164,167
Adr+529/HCl(6,8/120,160)			306,88	165,182
Ida(1.5,2)	205,258	176,171		
529(30,40)	107,104	103,113		
529/HCl(30,40)	103,105	111,109		
Ida+529(1.5,2/30,40)	216,254	200,215		
Ida+529/HCl(1.5,2/30,40)	216,263	219,221		
Epi(8,10)	231,231	112,142		149,177
529(160,200)	112,117	111,114		126,129
529/HCl(160,200)	117,112	110,115		100,110
Epi+529(8,10/160,200)	261,287	148,178		164,167
Epi+529/HCl(8,10/160,200)	306,320	151,198		151,177

In the Lewis lung study with Adriamicin, administeration of 8 mg/Kg Adriamicin with ADR-529/HCl or ADR-529 resulted in decreased values of %T/C, and toxic deaths (death before saline controls with >4 g weight loss) ran as high as 71%. Most of the other evaluations with the combinations resulted in %T/C values that were equal to or greater than values for the anthracyclines alone. Both ADR-529 and ADR-529/HCl produced

equivalent results, and it appeared that neither interfered with antitumor activity of Adriamycin, iderubicin, or epirubicin. Median survival time for many of the combination groups were significantly greater ($p<0.05$ to 0.001) than those groups given the same dose of anthracycline without ADR-529 or ADR-529/HCl. It is possible that 30 minutes of sonication could lead to decomposition of the anthracyclines. It must also be remembered that ADR-529/HCl is 11% HCl, i.e., 200 mg/Kg is equal to about 177 mg ADR-529.

EFFECT OF FINISHED DOSAGE FORMULATIONS OF ADR-529 AND ADR-529/HCl ON THE ANTITUMOR ACTIVITY OF 5-FLUOROURACIL OR CYCLOPHOSPHAMIDE IN MURINE TUMOR MODELS.
VOL. 1.16, P. 05 01872
REPORT # 2151, PLS 89-04

The objective of this study was to determine if finished dosage formulations of ADR-529 and ADR-529/HCl would interfere with the antitumor activity of 5-FU and cyclophosphamide in murine P388 and L1210 leukemia models and in the B16 melanoma model. P388 and L1210 cells were maintained by weekly ip passages in DBA/2 mice. For the study, 10^6 P388 and 10^5 L1210 cells were ip inoculated into CDF1 mice. The B16 tumor was maintained by passage in C57BL/6 mice. Tumor brei was implanted into BDF1 mice for testing. ADR-529/HCl was reconstituted with 1.87% M/6 Na lactate; the other drugs were reconstituted in 0.9% saline. Drugs were administered 1, 5, and 9 days after tumor implantation. Numbers in parentheses indicate %T/C values from a second study.

Results

Compound	mg/Kg/inj	%T/C L1210	%T/C P388	%T/C B16
5-FU	30	137	154(133)	
	60	199	135(128)	
ADR-529	200	114	117(116)	
ADR-529/HCl	200	115	112(133)	
5-Fu + ADR-529	30+200	155	125(133)	
	60+200	222	122(155)	
5-FU + ADR-529/HCl	30+200	163	117(123)	
	60+200	209	124(143)	

Cyclophos-	100	270	>544	151
phamide	200	135	>544	
	75			136
ADR-529	200	114	117	129
ADR-529/HCl	200	115	112	110
Cyclophos-	100+200	>360	>544	177
phamide +	200+200	141	>544	
ADR-529	75+200			156
Cyclophos-	100+200	>360	>544	164
phamide +	200+200	127	>544	
ADR-529/HCl	75+200			156

There were 1/7 toxic deaths (animals dying before saline control, with >4 g weight loss) each in the high dose cyclophosphamide and high dose cyclophosphamide + ADR-529/HCl groups. In the P388 study with 5-FU plus ADR-529/HCl or ADR-529, %T/C values were lower than with 5-FU alone, and the medium survival time with the 30 mg/Kg 5-FU + 200 ADR-529/HCl combination was significantly ($p<0.01$) lower than the mice given 30 mg/Kg 5-FU alone. Median survival time was significantly increased in the following groups: 5-FU + ADR-529 (30+200), 5-FU + ADR-529/HCl (30+200), and 5-FU + ADR-529/HCl (15+200 and 30+200), when compared to 5-FU alone.

THE INTERACTION OF THE CARDIOPROTECTIVE AGENT ICRF-187; ITS HYDROLYSIS PRODUCT ICRF-198; AND OTHER CHELATION AGENTS WITH THE Fe(III) AND Cu(II) COMPLEXES OF ADRIAMYCIN.
HASINOFF, B.B, AGENTS AND ACTIONS 26,3/4 (1989)
VOL. 1.15, P. 05 01608. GB 0085

This study reported on the efficient ICRF-198 (ring-opened product) removal of Fe(III) and Cu(II) from adriamycin complexes. The direct removal of Fe(III) from the Fe(III)-adriamycin complex by ICRF-187 resulted in hydrolysis to ICRF-198. Both ICRF-187 and the hydrolzed product could prevent Fe(III)-adriamycin induced inactivation of cytochrome c oxidase activity of submitochondrial particles.

EFFECTS OF ICRF-187 ON THE CARDIAC AND RENAL TOXICITY OF EPIRUBICIN IN SPONTANEOUSLY HYPERTENSIVE RATS. DARDIR, M., ET AL., CANCER CHEMOTHER AND PHARMACOL (1989)23:269-274.
VOL. 1.15, P. 05 01497
GB 0087

This study provided evidence that ICRF-187 could protect against the cardio- and nephrotoxicity produced by epirubicin in spontaneously hypertensive rats (SHR). Epirubicin treated rats (1.5 mg/Kg/week iv x 12 weeks, 18 mg/Kg total cumulative dose) developed severe myocardial changes, such as myofibril loss, cytoplasmic vacuolization caused by dilation of the sarcoplasmic reticulum, and cytoplasmic edema. Also severe renal changes occurred, consisting of atrophy and dilation of the tubules, protein casts in the lumina of tubules, and glomerular vacuolization. These cardiac lesions could be substantially reduced and the nephropathy could be moderately reduced by pretreatment with 50 mg/Kg ip ICRF-187 30 minutes before administration of epirubicin.

EFFECTS OF ADR-529 ON THE CENTRAL NERVOUS SYSTEM.
VOL. 1.16, P. 05 01962
REPORT # 2141

Compound: ADR-529, Batch No. 8001
Formulation: solution in 1.87% Na lactate (pH 5-6)
Route: iv
Dosage Levels: 0, 6.25 to 400 mg/Kg for general behavior study
0, 20, 60, 180 mg/Kg for other studies
Strain: male Crl:CD(SD)BR rats, 140-260 g body wt
male Crl:CD.1(ICR)BR mice, 20-25 g body wt
Number: 4 to 10 per group, depending on the test
Study Site/ Date: Farmitalia Carlo Erba Research and Development, Italy/June 13, 1989
GLP/QAU: no statements present

This study was designed to evaluate the effects ADR-529 may have on the CNS of mice and rats. General behavior (Irwin's test), body and skin temperature, neuromuscular coordination, anticonvulsant activity, and interaction with pentobarbital were investigated.

Results

In the Irwin test in mice (6/group) no animals died during 7 days after receiving 400 mg/Kg, the highest dose administered. Only slight hyperirritability, hyperreactivity, miosis and piloerection were observed at 400 mg/Kg. All doses (6.25 to 400 mg/Kg) produced slight fearfulness and flushing. In addition, at the high dose, snout and limb edema (marked) increased, and startle, exophthalmus, palpebral opening, and dyspnea were observed. The effect of ADR-529 (20, 60, 180 mg/Kg) on neuromuscular coordination (rotarod) in the rat, when

compared with diazepam, showed no change from the control. Oral diazepam (20 mg/Kg) greatly reduced neuromuscular coordination. Pentylenetetrazole induced tonic-extensor convulsions. Deaths in mice were not antagonized or inhibited by ADR-529 (20, 60 180 mg/Kg). Diazepam (1 mg/Kg) prevented convulsions and death by 90%, and pentobarbital-induced sleeping time of rats was not changed by 20, 60, or 180 mg/Kg ADR-529, while diazepam (20 mg/Kg) increased sleeping time by 66%.

The results from these studies indicate ADR-529 had no effects on the CNS in mice or rats at doses up to 180 mg/Kg, as judged by the above tests.

EFFECT OF ADR-529/HCl ON THE ANTILEUKEMIC ACTIVITY OF DAUNORUBICIN AND MITOXANTRONE AGAINST P388 AND L1210 LEUKEMIA.
VOL, 1.16, P. 05 01860
REPORT # 2181, PLS 89-6

This study was undertaken to determine if the HCl salt of ADR-529 would interfere with the antileukemic activity of daunorubicin or mitoxantrone when administered in combination. All drugs were administered by the iv route to female CDF1 and DBA/2 mice. For testing, mice were inoculated ip with 10^5 L1210 cells or 10 P388 cells. ADR-529 was reconstituted in 1.87% M/6 Na lactate.

Results

Drug	(mg/Kg/inj)	%T/C P388	%T/C L1210	%T/C P388	%T/C L1210
Daunorubicin	8	139	120		
	10	162	126		
	12	179	138		
ADR-529/HCl	160	133	144		
	200	120	144	120	144
	240	121	158		
Daunorubicin	8+160	156	160		
+	10+200	187	151		
ADR-529/HCl	12+240	197	153		
Mitoxantrone	0.75			152	147
	1.5			211	153
	3.0			349	135

Mitoxantrone	0.75+200	211	156
+	1.5+200	247	150
ADR-529/HCl	3.0+200	290	138

There were no toxic deaths (deaths before saline control with a weight loss >4 g). Long term survivors (>30 days) were seen in the 3 mg/Kg mitoxantrone (3/7) and 3 mg/Kg mitoxantrone + 200 mg/Kg ADR-529/HCl (2/7) P388 leukemia groups. Median survival time for ADR-529/HCl treated mice was longer (not significant) than saline controls, suggesting slight antitumor activity for ADR-529/HCl. These data also indicate the antileukemic activity of mitoxantrone or daunorubicin in mice is not diminished by combination with ADR-529/HCl. Median survival time was significantly greater in the daunorubicin + ADR-529/HCl (10+200, P388 study), daunorubicin + ADR-529/HCl (8,10,12/160,200,240, L1210 study), and mitoxantrone + ADR-529/HCl (0.75+200 and 1.5+200, P388 study) groups, when compared to groups given Daunorubicin or mitoxantrone.

FINAL REPORT ON MYELOSUPPRESSIVE EFFECT IN COMBINED APPLICATION OF THE CANCER CHEMOTHERAPY AGENTS, DOXORUBICIN AND ADR-529.
VOL. 1.17, P. 05 02082
Report # 2221, 088916-000

This study evaluated the in vivo effect of iv ADR-529 (0, 5, 10, and 20 x the iv doxorubicin dose) on the survival of CFU-S cells when administered in conjunction with doxorubicin (0, 3, 6, 9, 12, 18 mg/Kg). The response of CFU-GM (granulocyte and megakaryocyte) was also measured following doses of 0 to 240 mg/Kg ADR-529 administered 0.5 hr prior to 12 mg/Kg doxorubicin. The study was conducted by Allegheny-Singer Research Institute, Pittsburgh, PA.

Earlier studies indicated ADR-529 had no adverse effects on the survival of CFU-S cells when administered to mice alone by the iv route in doses less than 360 mg/Kg. The results from the present study indicated that low doses of ADR-529 (about 5 x greater than the doxorubicin dose) resulted in slight reduction of CFU-S survival below that of the value for doxorubicin give to mice alone. Higher doses of ADR-529 significantly reduced survival. On the other hand, there was no reduction in the survival of the mean number of CFU-GM colonies formed with increasing doses (0, 60, 120, 240 mg/Kg) of ADR-529 with 12 mg/Kg doxorubicin.

DELAYED TYPE HYPERSENSITIVITY TO SHEEP RED BLOOD CELLS IN MICE TREATED WITH THE TEST ARTICLE ADR-529.
VOL. 1.17, P. 05 02046
REPORT # 216i

Female BALB/c mice were evaluated to determine if ADR-529 had any potential in modulating a delayed type inflammatory reaction to SRBC, expressed as footpad swelling. Mice were immunized ip with $5x10^5$ SRBC on Day 0. An ip booster of SRBC ($5x10^5$) was injected Day 6, and Day 11 the mice were challenged by 50 µL of a 30% SRBC suspension in the right hind footpad. The left pad received 50 µL saline. Twenty-four hours later footpad swelling was measured. ADR-529 was tested at 100 and 200 mg/Kg iv in M/6 Na lactate, administered daily from Day -2 to Day + 11. The study was done by Instituto di Ricerche Biomediche "Anntoine Marxer", Italy.

The results of the foot pad swelling are indicated below:

Mean Values (S.D.) in Millimeters

0.9% NaCl		M/6 Na Lactate		ADR-529 100 mg/Kg		ADR-529 200 mg/Kg	
6.44	1.62	5.76	0.94	5.15	1.15	3.8	0.63*

*p<0.01

One death occurred in G3 after the 4th injection and two deaths occurred in G4 after the 12th and 13th injection. The results showed a reduction of footpad swelling and indicated ADR-529 had antinflammatory activity at 200 mg/Kg under the above conditions.

ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN MICE TREATED WITH THE TEST ARTICLE ADR-529.
VOL. 1.17, P. 05 02023
REPORT # 217i

The antibody response to SRBCs was evaluated in female mice by the hemagglutination test. Mice were immunized with SRBC and dosed with 100 or 200 mg/Kg/day iv x 14. Sera was tested for antibody titers. ADR-529 had a significant reduction ($p<0.001$) on the antibody titers with both low and high doses (0.9% NaCl control = 1280, M/6 Na lactate = 960, 100 mg/Kg ADR-529 = 40, 200 mg/Kg ADR-529 = 10). The study was conducted by Instituto di Ricerche Biomediche "Antoine Marxer", Italy.

EFFECT OF I.V. ADR-529, ALONE OR IN COMBINATION WITH DOXORUBICIN OR EPIRUBICIN, ON DIURESIS IN THE RAT.
VOL. 1.16, P. 05 02008
REPORT # 2211

This study, conducted by evaluated the potential of ADR-529 to increase diuresis and electrolyte excretion in male SD rats (275-300 g).

ADR-529 (100 and 120 mg/Kg) alone resulted in decreased urine volume at 120 mg/Kg and decreased K and Ca excretion with both doses. Doxorubicin (5 mg/Kg) alone produced proteinuria; epirubicin (6 mg/Kg) alone showed no effect on electrolytes or urine volume. ADR-529 in combination with epirubicin or doxorubicin showed the same effects as either anthracycline alone.

EFFECT OF ADR-529 ON THE ANTITUMOR ACTIVITY OF 5-FU, CYCLOPHOSPHAMIDE AND EPIRUBICIN AGAINST MURINE LEUKEMIAS AND SOLID TUMORS.
VOL. 1.16, P. 05 01811
Report # PLS 89-8, 2261

This study was undertaken to evaluate the cardioprotective effect of ADR-529 on antitumor efficacy of 5-FU, cyclophosphamide, and epirubicin against murine leukemias (P388, L1210, and Gross), B16 melanoma, and Lewis lung carcinoma. All drugs were administered iv on Days 1, 5, and 9 after tumor implantation. The studies were conducted by Adria Labs.

The results were similar to those obtained in studies conducted earlier. Based on %T/C values, it can be concluded that ADR-529 did not diminish the antitumor activity of 5-FU, cyclophosphamide, or epirubicin when administered in combination against the above murine tumors.

EVALUATION OF THE EFFECTS OF THE CARDIOPROTECTANT ADR-529 ON THE ANTITUMOR ACTIVITY OF BLEOMYCIN AND VINCRISTINE IN MURINE TUMOR MODELS.
VOL. 1.16, P. 05 01904
REPORT # PLS 90-4, 2231

ADR-529 was evaluated in combination with bleomycin and vincristine in the mouse P388 leukemia and B16 melanoma models. Mice were dosed Days 1, 5, and 9 after P388 tumor implantation and on Days 1 through 9 after B16 implantation. ADR-529 LpH

(the low pH formulation of ADR-529) was administered iv at 200 mg/Kg/inj within one minute of vincristine dosing and at 5:1, 10:1, 15:1, and 20:1 ADR-529:bleomycin. Bleomycin doses were 1, 3, 5, 6, and 8 mg/Kg/inj.

With vincristine plus ADR-529 LpH, %T/C values were not changed much; however, medium survival times were significantly increased with 0.5 and 1.0 vincristine + 200 mg/Kg ADR-529 LpH. No long term survivors or toxic deaths occurred. Bleomycin in combination with ADR-529 resulted in no significant changes in medium survival times. Long term survivals occurred with 6 and 8 mg/Kg bleomycin alone and somewhat more appeared with 6 + 60 and 90 and 8 + 40-160 bleomycin + ADR-529 combinations. No toxic deaths occurred. The results indicated ADR-529 did not interfere with the antileukemic activity of vincristine in the mouse P388 leukemia model or in the B16 melanoma model.

EFFECT OF ADR-529 (ICRF-187) ON CARDIOTOXICITY INDUCED BY A HIGH DOSE OF DOXORUBICIN IN THE BERTAZZOLI MOUSE MODEL.
VOL. 1.12, P. 05 00384
REPORT # 4041, PLS 87-06, P-529-86-001

This study was conducted by Adria Labs. to determine if administration of ADR-529 (40 or 80 mg/Kg/inj) in combination with 8 mg/Kg doxorubicin would reduce doxorubicin induced cardiotoxicity. Female ICR Swiss mice (30/group) received iv doses of saline, doxorubicin plus saline, and doxorubicin plus ADR-529. Mice were dosed 1x/week, two injections each day, during Weeks 1, 2, 5, 6, and 7 (total of 10 injections). All surviving mice were sacrificed Week 11. Histopathology was done on the heart, liver, kidneys, and spleen. Hematology and blood chemistry was evaluated. Light microscopic examination of the heart was done in random order and blinded, with exception of the negative control (saline) and positive control (dox 8 mg/Kg/inj). Cardiotoxicity scores are represented in the following table (from Vol. 1.12, p.05-00394):

Gp	Treatment (mg/Kg/inj)	Unached. Deaths	Number Examined	Scores 0	1	2	3	4	Mean±SD
1	Saline	0	30	30	0	0	0	0	0.0±0.0
2	Dox(8)	11	19	0	1	8	9	1	2.5±0.7
3	Adr(80)	3	27	25	2	0	0	0	0.1±0.3
4	Dox(8)+ADR(80)	12	18	0	6	11	1	1	1.7±0.6
5	Dox(8)+ADR(40)	13	17	2	3	8	3	1	1.9±1.1

Weight gain in the combination groups was higher than in the dox group, and organ weights were not affected by the

combination of drugs. Unscheduled deaths, characterized by acute weight loss, occurred in G2 (37%), G4 (40%), and G5 (43%). No significant changes occurred in any group with any of the blood parameters evaluated. Dox related lesions occurred in the liver (cytoplasmic rarefaction, increased karyomegaly, single-cell necrosis), kidney (multifocal cortical tubular degeneration, diffuse cortical degeneration, focal cortical tubular dilatation), and spleen (atrophy). No ADR-529 related lesions were present.

EVALUATION OF ADR-529 (ICRF-187) AS AN ANTIDOTE FOR PREVENTING ANTHRACYCLINE INDUCED CARDIOTOXICITY IN THE BERTAZZOLI MOUSE MODEL.
VOL. 1.12, P. 05 00427
REPORT # 4071, P-529-85-002, PLS 87-09

Female ICR Swiss mice (16/group) were dosed with iv saline, doxorubicin, iderubicin, epirubicin, or ADR-529 twice a week during Weeks 1, 2, 5, 6, 7 (total of 10 doses). This study was similar to the preceding study. ADR-529 doses ranged from 12 to 100 mg/Kg/inj. Cardiotoxic scores are indicated in the following table (Vol. 1.12, p. 05-00437):

Gp	Treatment (mg/Kg/inj)	Unsched. Deaths	Number Examined	Scores 0	1	2	3	4	Mean±SD
1	Saline	0	16	16	0	0	0	0	0.0±0.0
2	Dox(4)	0	16	0	2	2	10	2	2.8±0.9
3	Epi(5)	2	14	0	3	7	4	0	2.1±0.7
4	Ida(1)	2	14	3	3	7	1	0	1.4±0.9
5	Adr(100)	0	16	0	0	0	0	0	0.0±0.0
13	Dox(4)+ADR(48)	3	13	3	8	1	1	0	1.0±0.8
14	Dox(4)+ADR(80)	4	12	4	8	0	0	0	0.7±0.5
15	Ida(1)+ADR(12)	3	13	7	6	0	0	0	0.5±0.5
16	Ida(1)+ADR(20)	2	14	4	10	0	0	0	0.7±0.5
17	Epi(5)+ADR(60)	5	11	4	7	0	0	0	0.6±0.5
18	Epi(5)+ADR(100)	6	10	4	6	0	0	0	0.6±0.5

Combinations with ADR-529 did not significantly change body weight, weight gain, blood chemistry/hematology parameters, or histopathology of the liver, kidneys, and spleen; however, unscheduled deaths occurred with Dox plus ADR which did not occur in the groups treated individually with Dox or ADR. Increased numbers of unscheduled deaths appeared in the Epirubicin-ADR combination. Early deaths were characterized by acute weight loss. In conclusion, ADR-529 reduced the severity of heart lesions produced by doxorubicin, epirubicin, and iderubicin, when administered in combination and under the conditions of the study, but increased unscheduled deaths.

EFFECT OF ADR-529 (ICRF-187) ON THE DEVELOPMENT OF DELAYED DOXORUBICIN CARDIOTOXICITY IN THE BERTAZZOLI MOUSE MODEL.
VOL. 1.12, P. 05 00484
REPORT # P-529-86-003, PLS 90-5

Female ICR Swiss mice (30/group) were administered iv saline, dox (4 mg/Kg) or ADR-529 (20, 40, 60, or 80 mg/Kg) two times/week during Weeks 1, 2, 5, 6, and 7 (total of 10 doses). Drugs were made up in normal saline and solubilized by 20-30 min sonication. Combinations of drugs were administered within one minute of each other. All surviving animals were necropsied Week 11 (7 groups) or Week 22 (7 groups). Treatment in groups sacrificed Week 11 was identical to that in groups sacrificed Week 22. The study was done by Adria Labs. Light microscopic examination of heart, liver, kidney, and spleen were done in random order and blinded. Blood chemistry and hematology were also evaluated. Heart scores are indicated in the following table (Vol. 1.12, pp. 05-00508 and 05-00509):

Gp	Treatment (mg/Kg/inj)	Unsc Deaths	# Hearts Examined Week 11	Scores 0	1	2	3	4	Mean±SD
1A	Saline	0	30	29	1	0	0	0	0.033±0.18
2A	Dox(4)	6	24	0	0	5	15	4	2.96±0.62
3A	ADR(80)	3	27	25	2	0	0	0	0.07±0.27
4A	Dox+ADR(20)	10	20	0	5	14	1	0	1.8±0.52
5A	Dox+ADR(40)	11	19	1	12	6	0	0	1.26±0.56
6A	Dox+ADR(60)	15	15	1	7	5	2	0	1.53±0.83
7A	Dox+ADR(80)	15	15	0	12	3	0	0	1.2±0.41

Gp	Treatment (mg/Kg/inj)	Unscheduled Deaths Before Wk 11	After Wk 11	# Hearts Examined Wk 22	Scores 0	1	2	3	4	Mean±SD
1B	Saline	0	0	30	30	0	0	0	0	0
2B	Dox(4)	5	18	7	0	0	1	6	0	2.86±0.35
3B	ADR(80)	3	0	27	27	0	0	0	0	0
4B	Dox+ADR(20)	7	10	13	0	5	4	4	0	1.92±0.88
5B	Dox+ADR(40)	11	9	10	3	6	1	0	0	0.80±0.60
6B	Dox+ADR(60)	6	10	14	3	7	2	2	0	1.21±0.94
7B	Dox+ADR(80)	13	9	8	0	5	3	0	0	1.38±0.48

Body weight, weight gain, and organ weights did not change much in the ADR-529 + doxorubicin groups when compared with the dox only group, but again there were an increased number of unscheduled deaths that appeared with the combination. Cardiomyopathy was reduced significantly at Weeks 11 and 22 in groups administered ADR-529 in combination with doxorubicin. Liver lesions were notably reduced in the combination groups.

THE CARDIOPROTECTIVE EFFECT OF ADR-529 ADMINISTERED AS A SEPARATE BOLUS OR IN THE SAME INJECTION WITH DOXORUBICIN IN THE MOUSE.
VOL. 1.12, P. 05 00588
REPORT # P529-87-008, PLS 90-6

This study compared the effect of iv administration of ADR-529 followed immediately by iv doxorubicin, versus iv administration of a lyophilized combination of the two drugs. Female ICR Swiss mice (30/group) were administered saline, doxorubicin HCl, adriamycin (dox + lactose), or ADR-529 twice a week on Weeks 1, 2, 5, 6, and 7 (total of 10 injections). Doxorubicin and adriamycin were administered at 4 mg/Kg and ADR-529 was administered at 80 mg/Kg. All surviving mice were sacrificed Week 11. Heart, liver, kidneys, and spleen were examined blinded and at random with a light microscope. The results are indicated in the following table (Vol.,1.12, p. 05-00606).

Gp	Treatment (mg/Kg/inj)	Unached. Deaths	# Hearts Examined Week 11	Scores 0	1	2	3	4	Mean±SD
1	Saline	0	30	30	0	0	0	0	0
2	ADR	0	30	30	0	0	0	0	0
3	Adriamycin	4	26	0	1	9	12	4	2.7±0.76
4	Dox HCl	1	29	0	2	10	13	4	2.6±0.80
5	Adr+ADR sep.	4	26	5	19	2	0	0	0.88±0.50
6	Adr/ADR lyoph.	1	29	6	21	2	0	0	0.86±0.51
7	Dox+ADR sep.	2	28	7	18	3	0	0	0.86±0.58
8	Dox/ADR lyoph.	1	29	13	14	2	0	0	0.86±0.58
9	Dox/ADR lyoph.	3	27	10	17	0	0	0	0.63±0.48

The results indicate the incidence of cardiac and liver lesions were markedly reduced by the administration of ADR-529. It was not critical if the drugs were administered as seperate solutions or administered in the same solution.

PROTECTIVE EFFECT OF ICRF-187 AGAINST NORMAL TISSUE INJURY INDUCED BY COMBINED ADRIAMYCIN AND WHOLE BODY HYPERTHERMIA.
BABA, H., ET AL., PROC AMER ASSOC CANCER RES 31:391, 1990.
VOL. 1.15, P. 05 01504
GB0055

This abstract describes the results ICRF-187 had on adriamycin and whole body hyperthermia (WBH) in female F344 rats. The addition of ICRF-187 (70 mg/Kg ip) prior to WBH

(41.5 dregrees C for 2 hours) and 3.5 mg/Kg adriamycin given 30 minutes later during WBH significantly reduced the mean nephropathy and cardiomyopathy scores from 2.6 to 0.3 without changing the antitumor effect of adriamycin and WBH.

EFFECT OF ADR-529 ON BASAL GASTRIC ACID SECRETION IN THE RAT.
VOL. 1.16, P. 05 02003
REPORT # 2021, P-87-4, P-529-87-005

Male SD rats were administered iv saline vehicle, 20, 40, 80, or 160 mg/Kg ADR-529. Secreted gastric juice volume, hydrogen ion concentration, and total acid output were significantly inhibited at doses of 40 mg/Kg ADR-529 and higher.

MYELOSUPPRESSIVE EFFECTS IN COMBINED APPLICATION OF THE CANCER CHEMOTHERAPY AGENTS ADRIAMYCIN AND ICRF-187. I-1. HEMATOPOIETIC STEM CELL TOXICITY OF ICRF-187.
VOL. 1.17, P. 05 02069
REPORT # 088916-000-A

The administration of ICRF-187 (80 to 440 mg/Kg iv) to female SJL/J mice 12 weeks of age did not significantly change spleen weights. Likewise, no toxic effects were reported on the hematopoietic stem cell population (CFU-S per10 spleen cells) or number of CFU-S per whole spleen with doses of ICRF-187 up to 360 mg/Kg. At the two highest doses of 400 and 440 mg/Kg, a significant reduction (33 to 50%, respectively) occurred in the colony forming stem cells.

The 400 mg/Kg dose in the mouse would be equivalent to 1200 mg/sq M (32 mg/Kg) in the human. The recommended clinical dose of ZINECARD:DOX is 10:1, or 500 mg/sq M ZINECARD to 50 mg/sq M of doxorubicin. It must be remembered that ADR-529 can produce myelotoxicity when administered alone.

THE CYTOKINETIC AND CYTOTOXIC EFFECTS OF ICRF-159 AND ICRF-187 IN VITRO AND ICRF-187 IN HUMAN BONE MARROW IN VIVO.
WHEELER, R.H., ET AL., INVESTIGATIONAL NEW DRUGS 1, 283 (1983).
VOL. 1.17, P. 05 02103
GB0030

In this paper are described the cytokinetic and cytotoxic effects of ICRF-159 (the racemic mixture), and ICRF-187 (the S

or d isomer). Maximum cytotoxic effects were realized when cells were exposed to the drugs for twice the cell cycle time. Rapidly growing cells were more sensitive to drug exposure. Activity seemed to be present during the G2 phase of the cell cycle. Cytokinetic effects were determined in bone marrow of patients receiving ICRF-187. The results were similar to what was observed in vitro, i.e., the proliferating cells were blocked in the G2/M phase, with depletion of S phase cells.

KINETIC ANALYSIS OF DRUG-INDUCED G2 BLOCK IN VITRO.
KIMMEL, M., AND TRAGANOS, F., CELL TISSUE KINETICS 18(1): 91-110 (1985).
VOL. 1.17, P. 05 02116
GB0047

ICRF-187 was used to determine whether a modified stathmokinetic experiment could predict the effects of a continuous (0-48 hour) drug exposure in an in vitro L1210 murine leukemia cell system. The results failed to predict "after effects" of drug treatment which extend into the following cell cycle(s).

A SUMMARY OF THE ANTITUMOR ACTIVITY OF NSC-169780.
VOL. 1.17, P. 05 02136
NSC-169780

The data in this report are apparently from . The data show that both isomers of ICRF-159 produce similar responses by the ip or iv routes. Treatment was initiated 24 hours after ip implantation of L1210 cells. Both isomers were administered as solutions in water. There were no long term (30 day) survivors. All three forms of the drug (dl, d, or l) caused delayed toxicity in the non-tumor bearing animals, as reflected in the death pattern. NSC-169780 was also active against ip implanted P388 lymphocytic leukemia (ILS = 66-129%) and other tumor models. It was ineffective against the ip implanted B16 melanocarcinoma and the sc implanted Lewis lung tumor model.

MECHANISMS RESPONSIBLE FOR THE CARDIOTOXIC AND ANTINEOPLASTIC PROPERTIES OF ANTHRACYCLINES.
VOL. 1.17, P. 06 02144.
REPORT # PH-002

This report gives a good review of the role of free radical formation-lipid peroxidation in the mediation of anthracycline-induced cardiotoxicity and the interference with

topoisomerase II as a primary mechanism in the antitumor activity of anthracyclines. There are 205 references.

EFFECT OF DEXRAZOXANE ON THE EFFICACY
OF DOXORUBICIN IN MURINE TUMORS.
VOL. 1.17, P. 05 02205.
REPORT # PH-003

Detailed data are presented on the effect of dexrazoxane and doxorubicin in the experimental mouse tumor models, B16, melanoma, Madison lung, Gross leukemia, and Lewis lung assays. The results show that dexrazoxane did not affect the antitumor activity of doxorubicin when administered at optimal antitumor doses.

EFFECT OF ADR-529 ON THE ANTILEUKEMIC ACTIVITY OF IDARUBICIN AGAINST L1210 LEUKEMIA.
REPORT # PLS 90-3

Idarubicin was evaluated iv at 0.13 to 4.0 mg/Kg/inj on Days 1, 5, and 9 after L1210 leukemia tumor was implanted into mice. ADR-529:idarubicin ratios were 5:1, 10:1 15:1 and 20:1. %T/C was used to evaluate antitumor activity.

Idarubicin exhibited %T/C values ranging from 156 to 241 at doses of 1.5 to 4.0 mg/Kg. The highest values occurred at 2, 3, and 3.5 mg/Kg. Combinations with ADR-529 did not reduce %T/C values. Increases in median survival times were observed at combination doses of 2 + 10 to 2 + 40 Idarubicin + ADR-529. No long term survivors (animals living longer than 30 days) were observed with any single or combination dose. Significant increases ($p<0.05$-0.001) occurred with 0.5 and 1.5 Idarubicin and various doses of ADR-529.

CHARACTERIZATION OF THE CARDIOPROTECTIVE ACTIVITY OF ADR-529 (ICRF-187: A STUDY OF THE INFLUENCE OF DOSE AND THE TIME OF ADMINISTRATION.
REPORT # 403i, 87-04, P-529-001: VOL. 1.12, P. 05-00245

Female ICR Swiss mice were administered iv doses of doxorubicin (4 mg/Kg/inj) or ADR-529 (16, 32, 48, 64 mg/Kg/inj) twice/week on Weeks 1, 2, 5 ,6, and 7, given <1 minute before or 30 minutes before doxorubicin. The effect of 48 mg/Kg/inj ADR-529 administered from 120 minutes before to 60 minutes after 4 mg/Kg/inj doxorubicin was also determined. Both drugs were solubilized in normal saline using 20 to 30 minutes sonication to aid dissolution. The study determined the effect of dose and time of administration of ADR-529 on the cardiotoxicity of doxorubicin. Animals were sacrificed Week 11. Scoring of heart lesions was done randomized and blinded, except for saline and doxorubicin control groups. The study was conducted by Adria Labs., Ohio.

Heart pathology scores were graded from 1 to 4 as indicated:

Grade 0 = Normal histologic appearance
Grade 1 = Very slight; scattered, single myocardial fibers with vacuolation or degenerative changed.
Grade 2 = Slight; scattered small groups of altered myocardial fibers throughout the atrial and ventricular myocardium
Grade 3 = Moderate; disseminated myocardial fibers; vacuolation or degeneration with only occasional focal unaffected areas.
Grade 4 = Marked; confluent groups of affected myocardial fibers; most myocardial fibers affected.

Results

The mean cardiomyopathy scores were significantly reduced in all groups treated with ADR-529, and increasing the ADR-529 dose while keeping the doxorubicin dose the same reduced the cardiomyopathy scores. Doses ≥16 mg/Kg ADR-529 administered at an optimal time between 30 min before to 15 min after doxorubicin resulted in lower cardiomyopathy scores. When ADR-529 was maintained at 48 mg/Kg/inj and the administration time varied from 120 minutes before to 60 minutes after doxorubicin administration, mean cardiomyopathy scores were reduced in groups dosed 30 minutes before to 15 minutes after doxorubicin administration. Unscheduled deaths occurred as follows:

ADR-529 Dose (mg/Kg/inj)	Administration Time	Dox Dose (mg/Kg/inj)	Unscheduled Deaths
48	-30	4	2
48	-15	4	2
48	<1	4	1
4	+15	48	1
4	+30	48	3
4	+60	48	2

Combinations with doxorubicin did not alter body, spleen, or kidney weights, or change serum chemistry parameters.

EFFECTS OF GRADED DOSES OF ADR-529 (ICRF-187) ON DOXORUBICIN-INDUCED CARDIOTOCICITY IN THE BERTAZZOLI MOUSE MODEL.
REPORT # 405i, PLS 87-07, P-529-86-002: VOL. 1.12, P. 05-00327

In this study 20, 40, 60, and 80 mg ADR-529/Kg/inj were administered to 30 female ICR mice/group to determine the protective effect on the cardiotoxicity produced by a doxorubicin dose of 4 mg/Kg/inj. Mice were dosed 2 x /week during Weeks 1, 2, 5, 6, and 7. Drugs were administered iv within one minute of each other. Sonication in normal saline solution was used to aid dissolution of the drugs. Myocardial scores were graded as in the preceding study (0 to 4). Heart, liver, kidneys, and spleen were examined blinded and without knowledge of group assignment.

Results

The data in the following table is taken in part from Table 1, Vol. 1.12, p. 05-00337.

Gp	Treatment (mg/Kg/inj)	Unsch. Deaths	# Hearts Examined	Cardio Scores 0	1	2	3	4	Mean±SD
1	Saline	3	27	27	0	0	0	0	0.0
2	Dox(4)	8	22	0	5	4	10	3	2.55 ± 0.96
3	ADR(80)	2	28	28	0	0	0	0	0.0
4	Dox(4)+ADR(20)	8	22	0	8	12	2	0	1.73 ± 0.63
5	Dox(4)+ADR(40)	7	23	2	15	6	0	0	1.17 ± 0.58
6	Dox(4)+ADR(60)	8	22	10	6	5	1	0	0.86 ± 0.94
7	Dox(4)+ADR(80)	7	23	2	15	5	1	0	1.22 ± 0.67

Body weight and weight gain were reduced in all groups dosed with doxorubicin. Heart, liver, spleen, and liver weights showed variations in several of the groups, but increasing the dosage of ADR-529 did not appear to affect doxorubicin induced splenomegaly or hepatomegaly. A dose related increase was seen in WBCs in Groups 5, 6, and 7. The cytoplasmic rarefaction and increased karyomegaly which occurred in the liver of Group-2 were reduced in groups treated with ADR-529. Splenic atrophy and lymphoid hyperplasia in Group 2 was also reduced with concomitant

administration of ADR-529. Liver and spleen lesions were also reduced in those groups receiving ADR-529.

EVALUATION OF THE EFFECTIVENESS OF ADR-529 LpH, A FORMULATION OF ADR-529 LYOPHILIZED BY A NEW PROCESS, AND EDTA FOR PREVENTING ADRIAMYCIN INDUCED CARDIOTOXICITY IN THE MOUSE.
REPORT # P-529-88-002, PLS 90-8: VOL. 1.13, P. 05-00656

The cardioprotective effect of ADR-529 LpH, a new low Ph formulation of ADR-529 was evaluated in mice treated with 4 mg/Kg Adriamycin (doxorubicin HCl) and compared with 25.3 mg/Kg EDTA. ADR-529 LpH was reconstituted in 1.87% Na lactate. The other compounds were dissolved in normal saline. EDTA required 5-10 minutes of sonication to aid dissolution. Animals were dosed iv 2x/week during Weeks 1, 2, 5, 6, and 7. Combinations were administered within 1 minute of each other. The study includes daily observation, final body weight, blood chemistry, and hematology. Histopathology was done on liver, kidneys, spleen, and heart. Heart lesions were scored as in the above study. The cardiomyopathy scores are summarized in the following table (from p. 05-00678):

Results

Gp	Treatment (mg/Kg/inj)	Unsch. Deaths	# Hearts Examined	Cardiomyopathy Scores 0	1	2	3	4	Mean±SD
1	Saline	0	30	30	0	0	0	0	0
2	NaLactate	0	30	29	1	0	0	0	0.0 ± 0.2
3	EDTA(25.3)	12	18	15	2	1	0	0	0.2 ± 0.5
4	ADR(80)	1	29	28	1	0	0	0	0.0 ± 0.2
5	ADRLpH(80)	0	30	30	0	0	0	0	0
6	Dox(4)	1	29	1	0	7	19	2	2.7 ± 0.8
7	Dox+ADR-529(80)	4	26	4	7	15	0	0	1.4 ± 0.8
8	Dox+ADR-529(40)	0	30	1	5	20	4	0	1.9 ± 0.7
9	Dox+ADR-529(4)	2	28	0	0	5	18	5	3.0 ± 0.6
10	Dox+ADRLpH(80)	7	23	0	12	10	1	0	1.5 ± 0.6
11	Dox+ADRLpH(40)	3	27	0	6	15	6	0	2.0 ± 0.7
12	Dox+ADRLpH(4)	3	27	1	1	9	15	1	2.5 ± 0.8
13	Dox+EDTA(25.6)	8	22	1	0	5	11	5	2.9 ± 0.9

ADR = ADR-529, ADRLpH = ADR-529 LpH, Dox = Doxorubicin

The greatest number of unscheduled deaths were seen with EDTA and doxorubicin + EDTA. From the data, fewer unscheduled deaths were observed in those groups treated with ADR-529 in combination with doxorubicin than with combination with the new formulation (ADR-529 LpH). Body weight and weight gain were reduced more with doxorubicin by itself and in combination with ADR-529; however, combination with ADR-529 resulted in less reduction than

doxorubicin by itself. SGOT, SGPT, and LDH were elevated in Group 4 (1.4, 2.1, and 1.4 times, respectively). Absolute and relative heart weights were increased in Group 6. These weights were reduced somewhat and were similar to saline control values with the addition of ADR. The increase in liver karyomegaly, single cell necrosis, and hepatocyte hypertrophy produced by doxorubicin was reduced with ADR-529 but not with EDTA.

In summary, no significant differences were seen between the two ADR-529 formulations in protecting against doxorubicin induced cardiotoxicity. There appeared to be a slight advantage with ADR-529 than with ADR-529 LpH, and fewer unscheduled deaths were seen with ADR-529 in combination with doxorubicin than with ADR-529 LpH.

EFFECTIVENESS OF ADR-529 LpH FOR PREVENTING EPIRUBICIN-INDUCED CARDIOTOXICITY IN THE BERTAZZOLI MOUSE MODEL.
REPORT # PLS 90-11, P-529-89-001: VOL. 1.13, P. 05-00753

This study evaluated the cardioprotective effect of ADR-529 LpH in mice treated with epirubicin. Female ICR Swiss mice (21-26 g body weight) were administered iv 5 mg/Kg/epirubicin 2x/week during Weeks 1, 2, 5, 6, and 7, followed within one minute by 5, 25, 50, or 100 mg/Kg ADR-529 LpH. Other animals were dosed with Adriamycin (4 mg/Kg), epirubicin (5mg/Kg), and epirubicin + ADR-529 LpH (4+80 mg/Kg). Included in the study were daily observations, weekly body weight and weight gain, serum chemistry and hematology, organ weights (liver, kidneys, spleen, and heart), and histopathology (liver, kidneys, spleen,and heart). This study was conducted by Adria Labs., Ohio. Scoring of heart lesions was evaluated as in previous studies of this nature. Cardiomyopathy scores and unscheduled deaths are indicated in the following table (in part from Summary Table 5, p. 05-00771).

		Results						
				Cardiomyopathy Scores				
Gp	Treatment (mg/Kg/inj)	Unsch. Deaths	# Hearts Examined	0	1	2	3	4	Mean±SD
1	Saline	0	30	30	0	0	0	0	0
2	ADRHCl(80)	0	30	25	5	0	0	0	0.2±0.4
3	Adr(4)	2	28	0	1	4	19	4	2.9±0.7
4	Epi(5)	0	30	0	8	16	6	0	1.9±0.7
5	Adr(4)+ADRHCl(80)	5	25	6	15	4	0	0	0.9±0.6
6	Epi(5)+ADRHCl(100)	8	22	9	10	3	0	0	0.7±0.7
7	Epi(5)+ADRHCl(50)	4	26	10	16	0	0	0	0.6±0.5
8	Epi(5)+ADRHCl(25)	10	20	8	10	2	0	0	0.7±0.7
9	Epi(5)+ADRHCl(5)	3	27	8	15	4	0	0	0.9±0.7

ADRHCl = ADR-529 LpH, Epi = epirubicin, Adr = adriamycin (doxorubicin)

Results

Although cardiomyopathy scores were significantly reduced (Kruskal-Wallis test $p<0.001$) in Groups 5-9 treated with ADR-529 LpH (no dose relationship) compared to Group 5, the data indicated an increase in unscheduled deaths. Body weight by Week 7 was reduced by 7.1% in Group 9, compared to Group 4. Body weights of other groups were similar to Group 1. By Week 11, the final body weights of Groups 6 through 9 were reduced 2.6% to 4.8%, compared to Group 4. In terms of weight gain, all anthracycline treated groups were significantly reduced. SGOT and SGPT were elevated in all anthracycline treated groups, but significance was seen only for SGPT in Groups 3 and 4 compared to Group 1. Absolute and relative heart weights were increased in all anthracycline treated groups, and statistically significant increases in relative weights of heart, liver, and kidney were prevented with ADR-529 LpH. Liver toxicity (cytoplasmic rarefaction, mixed inflammatory cell infiltration, karyomegaly, focal necrosis) and kidney toxicity (cortical tubule degeneration, cortical/tubular cysts, cortical tubule hyperplasia, dilatation of the pelvis) seen in Group 4 was reduced with ADR-529 LpH.

In conclusion, ADR-529 LpH reduced heart, liver, and kidney toxicity in epirubicin treated mice. Adriamycin was more cardiotoxic than epirubicin in this study.

EFFECTIVENESS OF ADR-529 LpH IN PREVENTING DAUNORUBICIN-INDUCED CARDIOTOXICITY IN THE BERTAZZOLI MOUSE MODEL.
REPORT # PLS 90-10, P-529-88-005: VOL. 1.13, P. 05-00825

This study was designed to evaluate the effect of ADR-529 LpH in preventing daunorubicin induced cardiotoxicity in the mouse model (Bertazzoli mouse model). Female ICR Swiss mice, 30/group, were treated with daunorubicin (5 mg/Kg iv), administered 2x/week during Weeks 1, 2, 5, 6, and 7. ADR-529 LpH was administered within one minute of the anthracycline at doses ranging from 5 to 100 mg/Kg. ADR-529 LpH was reconstituted in Na lactate. The anthracyclines were reconstituted in normal saline. All surviving animals were necropsied Week 11. Treatment groups were dosed as indicated below. Cardiomyopathy scoring was done as previously indicated in an earlier study. The study was conducted by Adria Labs., Ohio.

Results

Gp	Treatment (mg/Kg/inj)	Unsch. Deaths	# Hearts Examined Week-11	Cardiomyopathy Scores 0	1	2	3	4	Mean±SD
1	Saline	0	30	28	1	1	0	0	0.1±0.4
2	ADR(80)	1	29	25	4	0	0	0	0.1±0.4
3	Dox(4)	4	26	2	0	8	12	4	2.6±1.0
4	Dau(5)	11	19	0	8	8	3	0	1.7±0.7
5	Dox(4)+ADR(80)	3	27	6	13	7	1	0	1.1±0.8
6	Dau(5)+ADR(100)	0	30	26	4	0	0	0	0.1±0.3
7	Dau(5)+ADR(50)	0	30	23	7	0	0	0	0.2±0.4
8	Dau(5)+ADR(25)	0	30	24	6	0	0	0	0.2±0.4
9	Dau(5)+ADR(5)	0	30	9	16	5	0	0	0.9±0.7

ADR = ADR-529 LpH, Dox = doxorubicin HCl, Dau = daunorubicin

Body weight and weight gain at Week 11 was inhibited by the anthracyclines. The addition of ADR-529 LpH did not completely restore body weight and weight gain to compare with that of the saline control. SGOT was significantly increased in Groups 3 (2.5x), 4 (4.9x), 5 (2.8x), 6 (2.7x), and 7 (2.7x), but only slightly in Group 2 (1.2x) when compared to saline control. SGPT was elevated in Groups 3 (2.5x) and 4 (1.9x). Cholesterol was significantly elevated in Groups 3, (2.3x), 4 (3.2x), 5 (2.4x), 6 (2.4x), and 7 (2.3x) when compared to saline. Group 4 showed a decrease in RBCs, Hb, and Hct (all at $p<0.001$). Albumin was significantly elevated in Groups 2 and 3, compared to the control. Relative and absolute weights of heart, liver, kidneys and spleen were significantly increased in Group 4 vs Group 1. These weights were mostly reversed with the addition of ADR-529 LpH. Doxorubicin treatment alone produced increased karyomegaly (13/30) and single cell necrosis (6/30) in the liver. This group also developed dilatation of cortical tubules (5/26) in the kidneys and increased red pulp hyperplasia (10/26) in the spleen. These lesions also developed in Group 4 but the incidences were not as high. However, a high incidence of glomerulonephritis (17/19) occurred in this group and was completely prevented with the addition of ADR-529 LpH. The other lesions were, for the most part, reduced or completely prevented by ADR-529 LpH.

In conclusion, ADR-529 LpH was effective in protecting mice from the cardiotoxicity produced by daunorubicin or doxorubicin. Hepatic and kidney lesions produced by these anthracyclines were also reduced or prevented by the addition of ADR-529 LpH.

EFFECTIVENESS OF ADR-529 LpH FOR PREVENTING IDARUBICIN-INDUCED CARDIOTOXICITY IN THE BERTAZZOLI MOUSE MODEL.
REPORT # PLS 90-09, P-529-88-003: VOL. 1.13, P. 05-00895

This study evaluated the ability of ADR-529 LpH to protect mice from the cardiotoxic effects of idarubicin. Female ICR Swiss mice were dosed two times per week during Weeks 1, 2, 5, 6, and 7, as indicated in the following table (from Summary Table 5, p. 05-00912). ADR-529 LpH was reconstituted in 1.87% Na lactate. Idarubicin and doxorubicin were dissolved in normal saline. The study was conducted by Adria Labs., Ohio and evaluated as in previous studies.

Results

Gp	Treatment (mg/Kg/inj)	Unsched. Deaths	# Hearts Examined Week-11	Cardiomyopathy Scores 0	1	2	3	4	Mean±SD
1	Saline	0	30	30	0	0	0	0	0
2	ADR(80)	0	30	30	0	0	0	0	0
3	Dox(4)	3	27	0	3	8	14	2	2.6±0.8
4	Ida(1)	3	27	12	9	6	0	0	0.8±0.8
5	Dox(4)+ADR(80)	0	30	8	12	9	1	0	1.1±0.8
6	Ida(1)+ADR(20)	0	30	26	4	0	0	0	0.1±0.3
7	Ida(1)+ADR(10)	0	30	24	6	0	0	0	0.2±0.4
8	Ida(1)+ADR(5)	2	28	15	12	1	0	0	0.5±0.6
9	Ida(1)+ADR(1)	0	30	15	9	6	0	0	0.7±0.8

ADR = ADR-529 LpH, Dox = doxorubicin, Ida = idarubicin

Body weights (relative and absolute) were significantly reduced in Group 3. Group 5 body weights were similar to those of the control. SGOT, SGPT, MCV, and reticulocyte values were significantly increased in Group 3. Group 4 showed increases in SGPT, MCV, and MCH, and decreases in cholesterol, WBCs, RBCs, and lymphocytes, compared to control. These changes were reduced with the addition of ADR-529 LpH. Heart, liver, kidney, and spleen weights (relative and absolute) were all elevated (most all significantly) in Group 4. The addition of ADR-529 LpH ameliorated these changes. In Group 3 most of these organ weights were increased. Combination with ADR-529 LpH likewise ameliorated these changes. Histopathologic changes seen in Group 3 liver consisted of karyomegaly, necrosis (single cell and centrilobular), and cytoplasmic rarefaction. Group 4 developed extramedullary hematopoiesis in the liver and red pulp hyperplasia in the spleen. Many of these changes were prevented or reduced with ADR-529 LpH.

In summary, ADR-529 LpH was capable of reducing cardiotoxicity produced in mice by idarubicin. The greatest protection was observed with a ADR-529 LpH:idarubicin w/w ratio of 20:1. A dose dependent decrease in cardiomyopathy scores occurred with

increasing ratios of ADR 529 LpH:idarubicin. Liver and splenic toxicity was also reduced or prevented with ADR-529 LpH.

EFFECT OF ADR-529 (ICRF-187) ON THE ANTITUMOR ACTIVITY OF DOXORUBICIN IN MOUSE MODELS OF MURINE AND HUMAN CANCER.
REPORT # 203i, PLS 87-08, VOL. 1.16, P. 05-01702

Compounds: Doxorubicin HCl, Adria Lot # 86E02A
ADR-529, Adria Lot # BV-84-206 and 86L46A
Formulations: Solutions in 0.9% saline-solubilized with sonication
Route: IV
Dosage Levels: Doxorubicin (4 through 12 mg/Kg)
ADR-529 (40 through 240 mg/Kg)
Doxorubicin + ADR-529 (various combinations)
Strain: Female Balb/C, BDF1, CDF1, C57BL/6, C3H/He, DBA/2, and ICR Swiss mice were used to maintain passage of the tumors. CDF1 mice were used for all murine leukemias and solid tumor tests. Human tumors were evaluated in CDF1 or or athymic NCr-nu mice.
Tumors: Murine leukemias: P388, L1210, Gross
Murine solid tumors: B16 melanoma, Madison Lung, Lewis Lung
Human: primary breast explant, MX-1 breast tumor, BL/LX5 lung tumor, and BL/BX-7 mammary tumor.

The murine tumor studies were conducted by Adria Laboratories.

conducted the studies on the human tumors. For murine solid tumors and leukemias, antitumor activity was evaluated by comparing medium survival time (MST). Human tumor xenografts and subrenal capsule assays were evaluated by comparing tumor growth; significance was calculated by the Student's t test.

Results

Murine Tumor Models:

Median survival time (MST) and %T/C was increased with doxorubicin, and usually dose related in all of the tumor models studied. ADR-529 also produced increases in MST above controls, which indicated it had slight antitumor activity by itself. Combinations of the two drugs did not result in a decrease in the antitumor activity below what was produced by doxorubicin alone, and a significant increase in MST was at times observed in the combination treated animals when they were compared with the group given the same dose of doxorubicin. Long term survivors were seen in several of the studies, particularly with the Lewis Lung model. There were, however, some toxic deaths occurring in this model, notably in the high dose combination.

Human Solid Tumor Models:

Human Breast Tumor:

When fresh surgical explant of breast tumor from a previously untreated patient was implanted subcapsularly into CDF1 mice, a significant decrease occurred in the growth rate when the animals were treated on a QD1-5 iv adriamycin dose schedule. Sacrifice was on Day 6. Changes in tumor size (Day 6 minus Day 0) in ocular micrometer units (omu) are shown in the following table (from Table 1, p. 05 01727)

Response of Fresh Surgical Breast Explant to Combinations of Adriamycin and ICRF-187 in the 6-Day Subrenal Capsule Assay

Gp	Treatment (mg/Kg/inj)	Average Δ Tumor Size[1] Mean ± SD	Test/Control (%T/C)	Test/Control*** [ΔTS(T)-ΔTS(C)]
1	Control	1.20 ± 0.95	-	-
2	ADR(4)	0.50 ± 0.39*	42	-0.70
3	ICRF(80)	0.85 ± 0.63	71	-0.35
4	ADR+ICRF(4+20)	0.30 ± 0.81*	25	-0.90
5	ADR+ICRF(4+40)	0.10 ± 1.11*	8	-1.10
6	ADR+ICRF(4+60)	0.25 ± 0.40**	21	-0.95
7	ADR+ICRF(4+80)	0.65 ± 0.90	54	-0.55

[1] Change in tumor size (Day 6- Day 0) in omu.
* p<0.05
** p<0.01
*** Delta TS test minus Delta TS control indicates degree of tumor inhibition in omu.
TS = tumor size, ICRF = ICRF-187

Results

There were no unscheduled deaths in the study. Body weight changes were as follows: G1 +1.2%, G2 -14.8%, G3 -9.8%, G4 -26.3%, G5 -15.2%, G6 -15.7%, G7 -14.9%. Maximum decrease in tumor size occurred in Group 5 ($p<0.05$), but tumor size was decrease was more significant in Group 6 ($p<0.01$).

MX-1 Human Breast Tumor:

The following study evaluated the response of MX-1 human breast tumor in an 11 day subrenal capsule assay. A 10x10 ocular micrometer unit (omu) fragment was implanted on Day 0 in athymic NCr-nude mice. There were 10/group in G1-4, and 9/group in G5-7. Doxorubicin was administered at 8 mg/Kg/inj iv on Days 1, 5, and 9. Tumors were measured on Day 11. Changes in tumor size (Day 11 minus Day 0) in ocular micrometer units (omu) are shown in the following table (from Table 1, p. 05 01739).

Response of MX-1 Transplantation Established Human Breast Tumor to Combinations of Adriamycin and ICRF-187 in the 11-Day Subrenal Capsule Assay

Gp	Treatment (mg/Kg/inj)	Average Δ Tumor Size[1] Mean ± SD	Test/Control[2] (%T/C)	Test/Control[3] [ΔTS(T)-ΔTS(C)]
1	Control	22.30 ± 6.48		
2	ADR(8)	0.30 ± 2.43*	1	-22.00
3	ICRF-187(160)	21.75 ± 4.85	98	-0.55
4	ADR(8)+ICRF-187(40)	2.45 ± 2.46*	11	-19.85
5	ADR(8)+ICRF-187(80)	-0.61 ± 2.05*	R6	-22.91
6	ADR(8)+ICRF-187(120)	0.61 ± 3.00*	3	-21.69
7	ADR(8)+ICRF-187(160)	1.11 ± 2.91*	5	-21.19

* $p<0.001$

[1] Change in tumor size (Day 11-Day 0) in ocular micrometer units (omu).

[2] Regression = % regression from original size using the formula (FTS-ITS)/ITSx100.

[3] Delta TS Test minus Delta TS Control indicates degree of tumor inhibition in omu.

Results

Adriamycin was very effective in inhibiting the growth of this tumor model ($p<0.001$), compared to the control. Body weight changes were as follow: G1 +11.4%, G2 -10.9%, G3 +8.6%, G4 -6.5%, G5 -9.8%, G6 -11.3%, G7 -15.1%. ICRF-187 had no antitumor activity by itself; however, in combination with adriamycin, tumor size was reduced significantly ($p<0.001$) with 1:5, 1:10, 1:15, and 1:20 ratios.

BL/LX5 Human Lung Tumor:

The response of BL/LX5 human lung tumor in the 11 day subrenal capsule assay was also evaluated. The tumor (10 X 10 X 10 ocular micrometer units) was implanted on Day 0 in athymic NCr-nude mice, 10/group. Animals were treated Day 1 with the following schedule: There were 9/group in G1-4 and 10/group in G5-7.

Gp	Treatment		
1	Vehicle Control, 0.2 mL/inj	Q4D	(D1,5,9,)
2	Adriamycin, 8 mg/Kg/inj	Q4D	(D1,5,9,)
3	ICRF-187, 160 mg/Kg/inj	Q4D	(D1,5,9)
4	ICRF-187, 40 mg/Kg/inj	Q4D	(D1,5,9)
	Adriamycin, 8 mg/Kg/inj	Q4D	(D1,5,9)
5	ICRF-187, 80 mg/Kg/inj	Q4D	(D1,5,9)
	Adriamycin, 8 mg/Kg/inj	Q4D	(D1,5,9)
6	ICRF-187, 120 mg/Kg/inj	Q4D	(D1,5,9)
	Adriamycin, 8 mg/Kg/inj	Q4D	(D1,5,9)
7	ICRF-187, 160 mg/Kg/inj	Q4D	(D1,5,9)
	Adriamycin, 8 mg/Kg/inj	Q4D	(D1,5,9)

Adriamycin was injected prior to ICRF-187 when administered in combination. The results are presented in the following table (from Table 1, p. 05 01754).

Gp	Average Δ Tumor[1] Size ± SD	%T/C (T/C)[2]	ΔTS(T)-ΔTS(C) (T - C)[3]	FBW/IBW[4]
1	8.50 ± 5.51	-	-	1.04
2	-1.33 ± 3.27*	R13	-9.83	0.83
3	7.39 ± 3.91	87	-1.11	1.04
4	-1.72 ± 3.22*	R17	-10.22	0.86
5	-6.30 ± 3.97*	R61	-14.80	0.81
6	-1.60 ± 1.67	R16	-10.10	0.80
7	-2.25 ± 3.04*	R22	-10.75	0.81

* p>0.001
R regression
[1] Change in tumor size (Day 11-Day 0) in ocular micrometer units.
[2] Regression = Percent regression from original size using the formula (FTS-ITS)/ITS x 100.
[3] Delta TS Test minus Delta TS Control indicates degree of tumor inhibition in omu.
[4] Final Body Weight/Initial Body Weight

There were no unscheduled deaths. Body weight changes were as follows: G1 +3.6, G2 -17.1%, G3 +14.2%, G4 -13.2%, G5 -8.6%, G6 -19.3% G7 -18.4%. ICRF-187 had only a slight effect in reducing tumor size when administered alone. Combinations of ICRF-187 reduced tumor size significantly ($p<0.001$), with the 1:10 ratio showing the greatest change.

BL/BX7 Human Mammary Tumor:

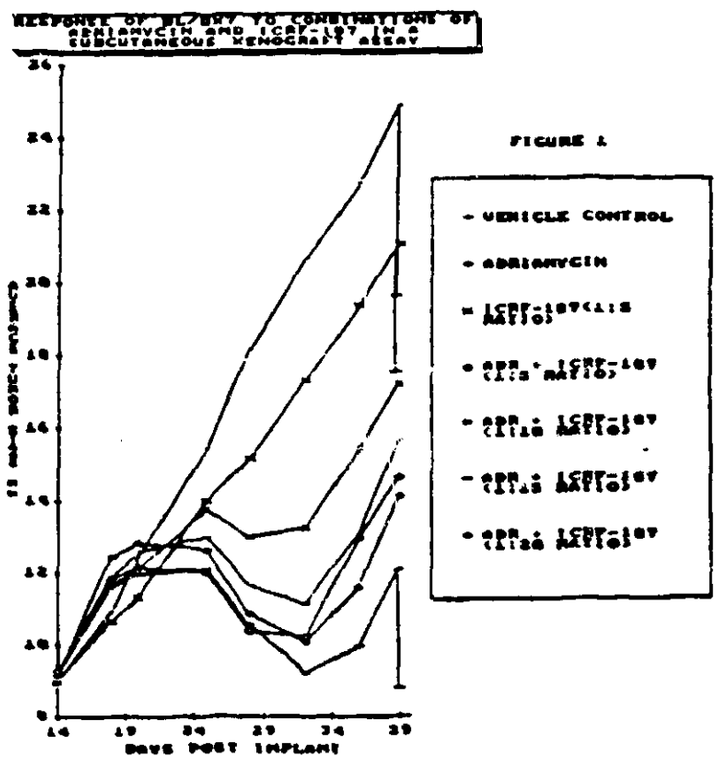
The response of transplantation established BL/BX7 human mammary tumor to combinations of adriamycin and ICRF-187 in a subcutaneous xenograft assay was evaluated in athymic NCr-nu mice. One hundred mice were implanted subcutaneously Day 0 with tumor fragments (800 mm^3) taken from donor animals bearing BL/BX7 tumors which have increased in volume at a growth rate determined appropriate for the model (3-fold in 14 days). Animals were checked for tumor growth twice/week until tumors reach an average of 10 mm diameter. On day 14 the animals with tumors of 10 mm diameter were placed in groups of 10, weighed, and treatment started. Tumors were measured several times during the study. On Day 39 all animals were weighed, sacrificed, and the tumors measured. The treatment and results are presented in the following tables (in part from Table 1, Vol. 1.16, p. 05 01766). Animals were injected iv Q4D on Days 14, 18, and 22. Adriamycin was administered at 8 mg/Kg/inj to Groups 2, 4, 5, 6, and 7.

Group	Treatment
1	Vehicle control, 0.2 mL/inj
2	Adriamycin
3	ICRF-187, 160 mg/Kg/inj
4	Adriamycin + ICRF-187 40 mg/Kg/inj (1:5 ratio)
5	Adriamycin + ICRF-187 80 mg/Kg/inj (1:10 ratio)
6	Adriamycin + ICRF-187 120 mg/Kg/inj (1:15 ratio)
7	Adriamycin + ICRF-187 160 mg/Kg/inj (1:20 ratio)

Tumor Size Measurements (L + M)/2

Gp	Day 14	Day 28	Day 32	Day 36	Day 39
1	8.95±1.99	18.15±4.09	20.65±4.84	22.70±4.62	24.85±4.76
2	9.15±1.14	10.55±2.32	9.20±2.61	9.95±3.20	12.05±3.44
3	8.95±1.42	15.15±1.18	17.30±1.85	19.35±2.35	21.05±2.71
4	9.25±1.33	10.40±3.00	10.21±3.69	12.93±3.76	14.64±3.16
5	9.20±1.68	13.00±3.65	13.35±4.20	15.40±5.22	17.20±5.00
6	9.25±1.47	11.60±3.14	11.10±3.73	13.10±3.69	15.65±4.80
7	9.15±1.18	10.85±2.32	10.06±2.63	11.58±3.33	14.08±3.88

There were 3 deaths in Group 4 (Day 32) and 4 deaths in Group 7 (2 Day 32, 2 Day 36). Body weight loss was 18% in G2, 26% in G4, 10% in G5. 22% in G6, and 29% in G7. Figure 1 (p. 05-01767) shows a graph of the tumor response to treatment and depicts the increase in BL/BX7 tumor growth once treatment is withdrawn. A more rapid tumor growth occurred in all combination groups once drug administration was discontinued. These results may indicate a difference in tumor drug clearance between doxorubicin and ADR-529, errors in tumor measurement, or may not be of any biological significance.



SUMMARY AND EVALUATION:

ICRF-159, the racemic isomer of ICRF-187 (ADR-529), was first evaluated as an antitumor agent by the under IND Both isomers of ICRF-159 were shown to have similar activity in the ip implanted L1210 lymphoid leukemia and the ip implanted P388 lymphocytic leukemia animal models. Marginal activity was also reported with a few other tumor models. No activity was observed against the ip implanted B16 melanocarcinoma and the sc implanted Lewis lung tumor models.

The aqueous solubility of the racemic compound was not high enough to permit sufficient concentrations for delivery of a therapeutic dose to humans; however, the solubility of each isomer in water was about four times greater than that of the racemic drug, and the synthetic yield of the d-isomer was somewhat better than the l-isomer, hence development of the d-isomer was pursued for clinical studies. These early studies with doxorubicin and daunorubicin indicated ADR-529 inhibited the anthracycline-induced cardiotoxicity in mice, rats, hamsters, dogs, and swine, and that the animal results were consistent with what was observed in patients. The emphasis now turned toward the use of ADR-529 as a cardioprotectant with doxorubicin, as the clinical results for ADR-529 as an antineoplastic were not impressive.

It is unclear what the mechanism of ADR-529 cardioprotection is. This is due, in part, to the fact that it is not known for certain how anthracyclines exert their cardiotoxicity. One mechanism explains the anthracycline-induced cardiotoxicity due to the generation of free radicals, which in turn result in lipid peroxidation. Several studies have reported that ADR-529 inhibits free radical formation by chelating Fe(III) and CU(II) ions and interrupts DOX-FE(III) ability to generate hydrogen peroxide and hydroxyl radicals, which then go on to oxidize membrane lipids. Another mechanism suggests histamine release caused by anthracyclines may be responsible for producing cardiotoxicity, and indeed ADR-529 was capable of inhibiting the release of histamine when incubated with rat peritoneal mast cells. Another study has suggested that a change in myocardial alpha c-actin mRNA is involved in the mechanism of action. An additional suggestion on the mechanism is that the drug acts mainly by cheleting intracellular calcium.

Pharmacological studies conducted with ADR-529 indicated the drug had no effect on general behavior at 200 mg/Kg; however, slight hyperirritability/hyperreactivity was produced by 400 mg/Kg in mice. Neuromuscular coordination was not

affected up to 180 mg/Kg, nor was there an interaction with pentylenetarrazol or barbiturate at this dose. At doses of 81 or 200 mg/Kg, no change was produced on hemodynamic effects in anesthetized dogs. At 3200 mg/Kg, mean arterial pressure, cardiac contractability, and heart rate were not effected. Short term hemodynamic responses to norepinephrine, acetylcholine, isoproterenol, or carotid artery occlusion were not effected with 81 or 200 mg/Kg doses of ADR-529. Gastric emptying was not changed significantly with 80 mg/Kg iv, but gastric acid secretion was reduced at 40 mg/Kg iv and higher. No CNS effects were seen in the mouse or rat with iv doses up to 180 mg/Kg. An immunosuppressive effect, indicated by a significant reduction in antibody titers to SRBCs, was observed in mice following ip immunization. At high doses, i.e., 400 mg/Kg iv, significant myelotoxicity was produced in mice.

Cardioprotective studies were conducted with ADR-529 in mice, rats, and dogs by the ip and iv routes. Intraperitoneal studies were also done in hamsters, rabbits, and mini pigs to evaluate cardioprotective activity of ADR-529 against cardiotoxicity induced by doxorubicin, daunorubicin, epirubicin, idarubicin, and mitoxantrone. In mice, most of the studies were conducted using the Bertazzole procedure. In general, the animals were treated with anthracycline, with and without added ADR-529, by various dosing schedules and ratios. Scoring of heart lesions were done blind and randomly, except for controls. The studies showed significant reduced scores in the animals treated with ADR-529, when compared to those dosed only with anthracyclines. In addition, liver and renal damage was also reduced in those animals administered anthracycline plus ADR-529.

In rats, cardiac and renal toxicity was ameliorated significantly with ADR-529:doxorubicin ratios of 5:1 to 20:1. EKG changes (prolongation of QT and ST intervals) produced with doxorubicin administered at 1 mg/Kg/week on a 5-2-5 schedule were significantly reduced with the administration of ADR-529 prior to doxorubicin administration.

Syrian hamsters were evaluated by varying the dose and administration time of ADR-529 in relation to the administration of daunorubicin. Anthracyclines produce lesions in the GI tract, kidneys, and to a lesser degree, in the heart of hamsters. Administration of ADR-529 reduced lethality, weight loss, and these lesions.

Pretreatment with ip doses of 12.5 and 25 mg/Kg ADR-529 significantly reduced the daunorubicin-induced cardiotoxicity in rabbits when evaluated three weeks or three months after

treatment; however, no reduction in mortality occurred in the group administered 25 mg/Kg ADR-529 and 3.2 mg/Kg daunorubicin.

Cardiotoxicity was also reduced in incidence and severity in dogs when ADR-529 was administered by the iv route. Lesions were found to be more severe when ADR-529 was administered 2 hours after doxorubicin, rather than when administered simultaneously. This study indicated ADR-529 should be administered simultaneously with doxorubicin. Dog studies also showed serum iron levels were significantly reduced when doxorubicin was administered alone or in combination with ADR-529. RBCs, WBCs, and Hb were also reduced. Survival was increased in dogs treated with ADR-529 plus doxorubin.

The effect of ADR-529 on the antitumor activity of anthracyclines, cyclophosphamide, 5-FU, cisplatin, cytarabine, methotrexate, bleomycin, and vincristine was evaluated in several animal and human transplanted tumor models. Most of these studies were conducted with doxorubicin. Administration of antineoplastic drugs was chosen to give an antitumor response appropriate for each type of tumor. Median survival times (MST) were calculated according to the procedure and compared with those of the antineoplastic agent in combination with ADR-529. In addition, %T/C, long term survival, and toxic deaths were recorded. Median survival times for combination with ADR-529 were increased; however, there were cases in which the median survival time was decreased. Long term survivors were sometimes observed with the maximum combinations of drugs. On the other hand, there were instances where ADR-529 at specific drug:ADR-529 ratios tended to have no effect or to diminish the efficacy of the drug.

The effect of ADR-529 in combination with doxorubicin was evaluated at ratios of 1:5, 1:10, 1:15, and 1:20 against murine P388 leukemia, L1210 leukemia, Gross leukemia, and murine solid tumors B16 melanoma, Madison lung tumor, and Lewis lung carcinoma. ADR combinations against most tumors resulted in increased activity, based on median survival time, %T/C, and/or long term survivors.

An in vitro approach was taken to evaluate the effect of ADR-529 in combination with doxorubicin on cell survival and cell proliferation of murine sarcoma S180 cells. These studies showed an additive effect on reducing colony formation. A synergistic effect occurred at doses of the two drugs alone which did not reduce colony formation. Cell proliferation was inhibited with the combination when exposure was >24 hours, and cell uptake of doxorubicin was not effected by ADR-529.

Tumor size reduction was also evaluated with doxorubicin in a subrenal capsule assay using fresh surgical explants of human breast tumor, MA1 human mammary tumor, BL/LX5 human lung tumor, or BL/BX7 human mammary tumor. In the presence of ADR-529 administered alone, tumor size reduction was not inhibited. The study with BL/BX7 human mammary tumor, however, showed a more rapid increase in tumor growth in all of the ADR-529:adriamycin ratios studied (1:5, 1:10, 1:15, 1:20) when drug administration was stopped. This increase began about 5 days after the last treatment and continued until the day of sacrifice. These results may indicate the occurrence of tumor protection after drug administration is discontinued, they may reflect a difference in tumor drug clearance between ADR-529 and doxorubicin, errors in tumor measurements, or may express other changes which allow a more rapid tumor increase after drug withdrawal. Tumor ratios of adriamycin to ADR-529 as well as tumor half-live of adriamycin and ADR-529 may be helpful in the interpretation of these results.

In summary, sufficient preclinical data has been presented which demonstrate ADR-529 can provide cardioprotection from doxorubicin-induced cardiomyopathy. Cardioprotection was observed with doxorubicin:ADR-529 ratios of 1:5 to 1:20, which were not always dose related. The extent of cardioprotection was also dependent on the animal species, route of administration, or timing of administration of ADR-529. Protection of other tissues, such as the kidney and liver, were also observed in animals.

The results presented in Report 2031, PLS 87-08, concerning the response seen in the subcutaneous xenograft of the BL/BX7 human mammary tumor in mice after drug withdrawl, have been discussed with Dr. Imondi at Adria. They have agreed to look into this further.

RECOMMENDATIONS:

From the preclinical point of view, based on the pharmacology portion of the application, NDA 20-212 is approvable, provided a suitable scientific explanation is presented which elucidates the more rapid increase in tumor growth observed in the BL/BX7 xenograft after drug treatment is stopped. Additional animal studies may be required to address this problem. Labeling changes will be required and will be discussed in a subsequent review after the BL/BX7 xenograft question has been adequately evaluated.

Almon W Coulter

Almon W. Coulter, Ph.D.

cc:
NDA 20-212
HFD-150/Division File
/ACoulter
/WSchmidt
/GWilliams
/JDeGeorge djd
HFD-151/ECutler
HFD-502/JWeisinger
HFD-340
R/D init JDeGeorge djd 6/29/92
F/T ACoulter

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

NDA 20212

Reviewer: Wendelyn J. Schmidt, Ph.D.
Received by reviewer: 2/12/92
Completed: 4/22/92

Sponsor: Adria Laboratories

Drug Name: ADR-529, Dexrazoxane, Zinecard

Chemical Name: [(+)-1,2-bis(3,5-dioxopiperazin-1-yl)propane]

Indication: cardioprotectant for use with anthracycline chemotherapy "Zinecard is indicated for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration."

Related Drugs and IND/NDAs:

Dosage Forms and Route of Administration: i.v., 250 and 500 mg. lyophilized administered in 10:1 ratio with anthracyclines.

Studies Reviewed in this submission:

I. Pharmacokinetics
   1. Tissue distribution and excretion of ^{14}C-ADR-529 in male mice. Vol 1.55: 15263.
   2. Tissue distribution and excretion of 14C-ADR-529 in the male and female rat. Vol. 1.56: 15304.
   3. Tissue distribution and excretion of 14C-ADR-529 in the male dog. Vol. 1.56: 15427.
   4. Pharmacokinetics of ICRF-187 in the cerebrospinal fluid of subhuman primates. Cancer Treatment Reports. 64(4-5): 734-735. NDA Vol 1.56: 15553.
   5. The enzymatic hydrolysis-activation of the adriamycin cardioprotective agent (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane. Drug metabolism and disposition 19: 74-80. NDA Vol 1.56: 15556.

II. Toxicology
   1. Six week intravenous toxicity study of ADR-529 in the rat. Vol. 1.22: 3726.
   2. Thirteen-week intravenous toxicity study of ADR-529 and epirubicin in the rat with a 6-week recovery. Vol.1.37: 8797.
   3. Effect of ADR-529 on cisplatin toxicity. II. Intraperitoneal administration of ADR-529 in rats twice daily for three consecutive days. Vol. 1.48: 12637.

III. Reproductive Toxicity
   1. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study (including a "behavioral" postnatal evaluation) of ADR-529 and epirubicin administered intravenously to Crl:CD(SD)BR rats. Vol. 1.50: 13498.
   2. Developmental Toxicity (Embryo-fetal toxicity and teratogenic potential) study of ADR-529 and epirubicin administered intravenously to pregnant rabbits. Vol. 1.53: 14470.

IV. Genotoxicity

1. Gene mutation in Salmonella typhimurium on ICRF-187 (Ames Test). vol. 1.54: 14747.
2. Gene mutation test in Salmonella typhimurium on ICRF-187. doxorubicin and their combination, 20:1 ratio (Ames test). Vol. 1.54: 14781.
3. Gene mutation in Salmonella typhimurium with ADR-529 in association with epirubicin, 20:1 ratio (Ames test). Vol. 1.54: 14835.
4. The hepatocyte primary culture/DNA repair assay on ICRF-187. vol. 1.54: 14877.
5. The hepatocyte primary culture/DNA repair assay on ICRF-187 and adriamycin. Vol. 1.54: 14891.
6. DNA repair test in the rat hepatocyte primary cultures with ADR-529 in association with epirubicin, 20:1 ratio. Vol. 1.54: 14906.
7. Metaphase chromosome analysis on ADR-529 in human lymphocytes cultured in vitro. Vol. 1.55: 14962
8. Metaphase chromosome analysis in human lymphocytes treated in vitro with ADR-529 and doxorubicin in a ratio of 20:1. Vol. 1.55: 14985.
9. Micronucleus test in mouse bone marrow cells after i.v. administration of ADR-529. Vol. 1.55: 15029.
10. Micronucleus test in mouse bone marrow cells after i.v. administration of ADR-529 and doxorubicin, 20:1 ratio. Vol. 1.55: 15046.
11. Micronucleus test in mouse bone marrow cells after i.v. administration of ADR-529 and epirubicin, 20:1 ratio. Vol. 1.55: 15079.

Studies reviewed in previous submissions:

1. Pharmacokinetics
   1. Pharmacokinetics of i.v. ^{14}C-ADR-529 in the male rat. (vol. 37.1: 265-292). Serial number 136.
   2. Pharmacokinetics of I.V. ^{14}C-ADR-529 in rabbits. (vol. 44.1, p. 83-122). Serial number 154.
   3. Pharmacokinetic and dose proportionality of ADR-529 in beagle dogs. (vol. 44.1, p. 123-172). Serial number 154.
   4. Plasma protein binding of ADR-529 in rat, rabbit, dog and man. (vol. 44.1, p. 173-212). Serial number 154.
   5. Identification of ADR-529 metabolites in the urine of laboratory animals and man by thermospray (TSP)-HPLC/MS and HPLC-UV methodology. (vol. 44.1, p. 329-428) Serial number 154.
   6. Acute i.v. tolerance of dogs to ADR-529: modification for studying disposition of ADR-529. (vol. 44.1, p. 286-328) Serial number 154.
   7. The effect of ADR-529 on tissue distribution and elimination of 14C-doxorubicin in the mouse. Vol. 37.1: 293-330. Serial number 136.
   8. The effect of ADR-529 on doxorubicin pharmacokinetics in the dog. Vol. 37.1: 331-371. Serial number 136.
   9. A four week intravenous tolerance study of ADR-529 and doxorubicin in dogs: modification to obtain toxicokinetic estimates of drug elimination. (vol. 44.1, p. 249-285) serial number 154.
   10. A four week intravenous tolerance study of ADR-529 and epirubicin in dogs: modification to obtain kinetic estimates of drug elimination. (vol. 44.1, p. 213-248, serial number 154.

II. Toxicology

1. Thirteen-week toxicity study of ADR-529 in the rat with a six week recovery. Vol. 37.5: 001-end. Serial Number 136.
2. Thirteen week intravenous toxicity study of ADR-529 in the beagle dog with a six week recovery period. Vol. 26.1:134-end. Serial number 092.
3. Thirteen week toxicity study of ADR-529 and doxorubicin in the rat with a six week recovery. Vol. 37.7: 1-end. Serial number 136.
4. Thirteen week intravenous toxicity study of ADR-529 and doxorubicin in the beagle dog with a six week recovery period. Vol. 26.2: 273-end. Serial number 092.
5. Thirteen week intravenous toxicity study of ADR-529 and epirubicin in the beagle dog with a six week recovery period. (vol. 44.2, p.1-vol. 3 p. 158) Serial number 154.
6. Precclinical toxicological evaluation of (NSC 169780) IND
7. Acute intravenous toxicity study of ICRF-187 administered to male and female mice.
8. Comparative acute intravenous toxicity study in mice of ADR-529 in M/6 sodium lactate or normal saline.
9. Acute intravenous toxicitiy study of ADR-529 (Lot 87F17Fy in rats and mice [old formulation].
10. Acute intravenous toxicity study of Monsanto ICRF-187 vs. DoneganiICRF-187 administered to rats.
11. Acute intravenous toxicity study of doxorubicin (Adriamycin rapid dissolution) and doxorubicin in combination with ICRF-187 administered to mice.
12. Acute intravenous toxicity studyof doxorubicin (Adriamycin rapid dissolution) and doxorubicin in combination with ADR-529 in mice.
13. Acute intravenous toxicity study of ADR-529/doxorubicin in rats.
14. Acute intravenous tolerance study of ADR-529/doxorubicin in dogs.
15. Six week intravenous toxicity study of ADR-529 in the beagle dog.
16. A four week intravenous tolerance study of doxorubicin and ADR-529 in dogs.
17. Six-week intravenous toxicity study of ADR-529 and doxorubicin in the Beagle dog.
18. Vascular irritation study in rabbits with ADR-529 in 0.9% sodium chloride or M/6 sodium lactate.
19. Human blood compatibility of ADR-529 in M/6 sodium lactate.

III. Reproductive Toxicity

1. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study (including a "behavioral" postnatal evaluation of ADR-529 administered intravenously to Crl:CD(SD)BR rats. Vol. 44.3: 169-402 and vol 44.4: 1-310. Serial number 154.
2. Dosage-range developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ADR-529 administered intravenously to pregnant rabbits. vol. 37.2: 215-277. Serial number 154.
3. Developmental toxicity (embryo fetal toxicity and teratogenic potential study of ADR-529 administered intravenously to pregnant rabbits. Vol. 37.4; 105- 384. Serial number 136.

4. Dosage-range developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ADR-529 and epirubicin administered intravenously to pregnant rats. vol. 37.2: 278-346. Serial number 154.
5. Dosage-range developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ADR-529 and epirubicin administered intravenously to pregnant rabbits. Vol. 37.4: 385-end. Serial number 136.

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

Information in the summaries is also derived from reviews by Drs. Lee-Ham and Coulter. Pharmacology data was reviewed by Dr. A. Coulter.

I. Pharmacokinetics

1. Tissue distribution and excretion of ^{14}C-ADR-529 in male mice. Vol 1.55: 15263.

The study was performed at Adria Laboratories, OH. Four male Swiss white mice/ dose were injected i.v. with 1.0 uCi of ^{14}C-ADR-529 50 mg/kg. Plasma samples were obtained at 5, 10, 15, 30, 45, 60 minutes, 2, 3, 4, 6, 8, 12, 24 48 and 72 hours, tissue distribution was determined at 1, 6, 24 and 72 hours, and urinalysis (radioactivity excreted and metabolite profile) was performed on samples collected between 0-6, 6-12, 12-24, 24-48 and 48-72 hours.

The terminal plasma half-life of ADR-529 in mice was 4.8 hours, with the $AUC_{t\infty}$ equal to 65.7 ug•hr/ml. Volume of distribution was 5.3 L/kg. Sixty percent of the dose was removed in the urine in the first 6 hours. By 72 hours, 89% of the dose was cleared in the urine, while an additional 10% of the dose was cleared in the feces. Of the radioactivity excreted in the urine, 71% coeluted with parental ADR-529, while 27% eluted as a second peak.

At 1 hour, the highest concentration of radioactive compound was in the liver (2.95 fold plasma concentration), followed by kidney (2.16 fold plasma concentration), adrenal (1.56 fold plasma concentration) and plasma. Concentrations in the heart were approximately half those in the plasma. By 24 hours, no radioactivity was detectable in plasma, while levels were highest in skin, liver, fat, large intestine and stomach. Radioactivity was still detectable at 72 hours in the heart, but was most prevalent in fat, muscle, liver, and bone.

2. Tissue distribution and excretion of ^{14}C-ADR-529 in the male and female rat. Vol. 1.56: 15304.

The study was performed at Adria Laboratories, OH. Four Sprague Dawley rats/sex/dose were injected i.v. with 3-5 uCi of ^{14}C-ADR-529 (50 mg/kg). Plasma samples were obtained at 5, 10, 15, 30, 45, 60 minutes, 2, 3, 4, 6, 12, 24, 48 and 72 hours for plasma concentration and concentration in blood components, metabolic stability of the radiolabel was measured by captured CO_2 every 4 hours, biliary clearance was measured for the first 6 hours, and tissue distribution was determined at 1, 6, 24 and 72 hours, and urinalysis (radioactivity excreted and metabolite profile) was performed on samples collected between 0-6, 6-12, 12-24, 24-48 and 48-72 hours.

Radioactivity (0.04% of the total dose) was detectable in the expired air only during the first 4 hours following dosing. Plasma elimination was

triphasic: the distribution half-life of ADR-529 in rats was 17 minutes in males, 20 minutes in females; the second phase was approximately 1 hour in males and females, while the final phase was 67.5 hou in males, 76.4 hours in females. However, approximately 99% of the dose was removed from plasma within the first 8 hours. The AUC_{0-72h} was 101.2 ± 5.4 ug•hr/ml in males, 121.6 ± 3.7 ug•hr/ml in females. Eighty-six percent of the dose was excreted in the urine within the first 12 hours in both males and females. Total urinary excretion over 72 hours was 89% of the dose with another 5% of the dose excreted in the feces. In the urine, 62% of the radioactivity injected onto the HPLC column was identified as parent compound, 2 other metabolites, II and III accounted for 37% and 1% respectively. In bile, where 4% of the total dose was excreted, 27% of the radioactivity was in the parent compound, 70% was unknown II, and 0.7 to 2.2% was unknown III.

At 1 hour post-dose, the highest level of radioactivity was found in the liver (6 fold plasma concentration), kidney (4 fold), and small intestine (2 fold) of both males and females. Plasma protein binding accounted for 22 and 26% of the plasma radioactivity: but, as in vitro experiments showed no protein binding, this was attributed to metabolites of ADR-529. While radioactivity was no longer detectable in plasma at 24 hours, appreciable levels were seen in kidney, large intestine, lung, heart, testis, and whole blood at 72 hours.

3. Tissue distribution and excretion of 14C-ADR-529 in the male dog. Vol. 1.56: 15427.

The study was performed at Adria Laboratories, Columbus, OH. Three male beagle dogs were administered 50 mg/kg 14C-ADR-529 (16 uCi) via the cephalic vein. Blood was sampled at 5, 10, 15, 30, 45, 60 minutes, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hours for plasma kinetics and protein binding. Urine and fecal samples were taken from 0-12, 12-24, 24-48 and 48-72 hours; urine samples were assessed for metabolites. Tissue distribution was assayed at 72 hours.

At the end of 8 hours, the concentration of radioactive compound in the plasma was at the lower limit of detection (2 ug/ml). The half-life in the distribution phase was 4.8 minutes, in the second phase, 1.8 hours. Volume of distribution was 0.80 L/kg, the AUC_{∞} was 159.1 ug•hr./ml. Percentage of radioactive compound bound to plasma was negligible at 1 and 4 hours, 13% of the total radioactivity in plasma at 6 hours. Sixty-five percent of the dose was excreted in the urine within the first 12 hours: a total of 78% of the dose was excreted in the urine by 72 hours. Fecal excretion accounted for an additional 6% of the dose by 72 hours. Urine pH, and therefore metabolic profiles differed in the 3 dogs (pH 6.1 to 8.8) with a range of percent of radioactivity from HPLC column as parent drug from 13.5% to 54.4%. (Untreated dog urine was incubated with ADR-529 and showed a half-life of 2.3 hours.) One other peak was seen. At 72 hours, the highest concentration of radioactivity was seen in the liver, followed by kidney, spleen, muscle, axillary nodes, adrenals, heart, and lung. All other tissues were below the limits of detection.

4. Pharmacokinetics of ICRF-187 in the cerebrospinal fluid of subhuman primates. Cancer Treatment Reports. 64(4-5): 734-735. NDA Vol 1.56: 15553.

Three rhesus monkeys were given 2 hour i.v. infusions of ^{14}C-ADR-529 at 300 mg/sq.m. (25 mg/kg) and the radioactivity in plasma and cerebrospinal fluid determined over 48 hours. Peak CSF levels, reached at the end of the

2 hour infusion. were approximately 10% of plasma levels or 9 ug/ml. At 24 hours. plasma levels of radioactivity had decreased by roughly 90%. and levels of label in the CSF were about 1/3 of the plasma levels.

5. The enzymatic hydrolysis-activation of the adriamycin cardioprotective agent (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane. Drug metabolism and disposition 19: 74-80. NDA Vol 1.56: 15556.

Basically, ADR-529 is metabolized to an open ring form by the action of hydrolases in the liver and kidney, but not in the heart. These hydrolases are most likely DHPase (dihydropyrimidine amidohydrolase).

Overall Summary of Preclinical Pharmacokinetics

A summary of the plasma and excretory parameters for 50 mg/kg i.v. ADR-529 in mice, rats, rabbits and dogs is displayed below. Discrepancies in the 2 rat studies in half-life and AUC exist as data was collected only over the first 12 hours in the second experiment. In all species. the majority of the drug was cleared from the plasma within the first 8 hours. Plasma binding of ADR-529 was less than 10% in all species tested. Most of the drug was removed in the urine (77-89% of the dose) with the exception of the second dog study, (urine pH was frequently high enough to cause degradation of the compound; hence, a lower level of "parent" compound). On the basis of mg/m^2 dose normalized to 1, AUC in mice and rats (0.34-0.44 ug•hr/ml) and in rabbits and dogs (0.16 ug•hr/ml) are similar, although the AUC by mg/kg or mg/m^2 and C_{max} differ by less than a factor of 3 across all species tested.

ADR-529 is metabolized to a polar form by cleavage of a single lactam ring at a time, presumably by a hydroxide catalyzed pathway in the alkaline urine of dogs, interaction with copper or iron complexes of doxorubicin or by DHPase reaction. Metabolites in the plasma were not investigated.

The highest levels of ADR-529 following i.v. injection with 50 mg/kg drug were observed in liver and kidney in mouse, rat and dog: levels were still higher than most other tissues after 72 hours. Other tissues with ADR-529 levels higher than plasma at 1 hour post-injection include adrenal in mouse. and small intestine in rat. After 72 hours, no ADR-529 is detectable in plasma. while radioactivity can still be found in fat, muscle, and bone in mouse. large intestine. lung. testes and whole blood in rat. and spleen, muscle. lymph nodes. adrenals and lung in dog. Detectable levels of ADR-529 are found in mouse. rat and dog in the heart both at 1 hour (less than plasma levels) and at 72 hours (less than liver/kidney, but still detectable).

In combination with doxorubicin and epirubicin, ADR-529 kinetic parameters were not affected. When doxorubicin and ADR-529 were used in combination. doxorubicin kinetic parameters were not changed significantly: however. epirubicin kinetics in combination with ADR-529 were not established.

In the human. the half-life was independent of dose (mean time 3.6 hours). AUC was linear with dose, and correlated with either rat or dog, depending upon which experiment one chooses to believe. In urine, ADR-529 was excreted as either parent drug or polar component, which consisted of mono-ring hydrolysis products (monoacid-monoamines) and diacid-diamine. Volume of distribution is similar to that of dog and rat (which is close to total body water volume. This is further supported by the minimal binding to plasma proteins.

SPECIES	$t_{1/2\beta}$ hours	$AUC_{0-\infty}$ ug•hr/ml	C_{max} (ug/ml)	V_d L/kg	% dose excreted in urine	% parent/ polar metabolite excreted
MICE, ♂ 50 mg/kg	4.8	65.7	104.1	5.3	89.1	55/21
RAT, ♂ 50 mg/kg	67.5	101.2	88.0	---	88.3	62/37
RAT, ♀ 50 mg/kg	76.4	121.6	88.8	---	89.7	62/37
RAT, ♂ 50 mg/kg	0.6	38.5	90.0	1.1	88.1	---
RABBIT, ♂ 50 mg/kg	10.6	87.6	146.3	9.5	86.2	11/89
DOG, ♂ 50 mg/kg	1.8	159.1	136.3	0.8	77.7	31/68
DOG, ♂ 10 mg/kg	1.2	15.2	23.6	1.2	21.9	---
DOG, 25 mg/kg	1.2	40.8	69.8	1.1	29.6	---
DOG, ♂ 50 mg/kg	1.2	77.8	135.9	1.1	22.7	---
DOG, ♂ 100 mg/kg	1.2	162.6	278.7	1.0	35.1	---

Double lines indicate data collected within a single experiment.

III. Toxicology

Acute

In the acute studies with ADR-529 alone, the dose-limiting factor was the solubility of the drug (10 mg/ml). The sponsor stated that the maximum dose for mice and rats was 100 ml/kg i.v. Hence, the doses used were 500 mg/kg and 1000 mg/kg. Vehicles included saline, sodium lactate and phosphate buffers. Deaths were seen only in the mouse and rat at both 500 and 1000 mg/kg, occurred within minutes of dosing, and were accompanied by convulsions, and/or frothing from the nose. Following unexpected death, a necropsy in the rat showed enlarged heart and pulmonary edema, suggesting that death may be due to the large volumes injected; however, the convulsions were seen only in ADR-529 treated animals. There were no abnormal signs after the first hour, or damage seen at necropsy. When saline was used as a buffer, ADR-529 was slightly less toxic to the animals than lactate vehicle. The sponsor suggested that lactate effects were due to hypertonicity.

In dogs, the only notable clinical sign during the 2 week duration of the acute studies was diarrhea within the first 2 days of dosing with 500 or 1000 mg/kg ADR-529. Body weight changes were not significant. AST levels increased 17 fold at 1000 mg/kg; ALT levels were elevated 13 to 50 fold between days 4 and 7. Even at 250 mg/kg AST increased 2 to 5 fold, ALT 2 to 4 fold, although 1 female had an elevated ALT of 28 fold. Changes in hematologic parameters were minimal (<10% change from pre-treatment values).

In the 2 to 4 week acute combination studies, the combination of ADR-529 and either epirubicin or doxorubicin frequently proved more fatal than the anthracycline alone. ADR-529 had no effect on vincristine or cisplatin acute toxicity, as measured by mortality, and, in the case of cisplatin, kidney damage (BUN). As the animals were not followed for more than a month, long term effects of anthracyclines, especially on cardiac tissue could not be assessed.

Signs were either immediate (attributable to ADR-529 in large volumes) or delayed (attributable to anthracycline toxicity). In mice and rats, no behavioral changes occurred which were not present with each drug alone. A single dog (1/2) treated at 40 mg/kg ADR-529 and 2.0 mg/kg doxorubicin was sacrificed-moribund at day 7 with dehydration/prostration; however, no dog treated at 20 mg/kg ADR-529/1 mg/kg doxorubicin showed a biologically significant weight loss or toxic behavioral disturbances other than some anxiety and retching in 2 dogs on the first day of treatment. Hematologic changes in the dogs with ADR-529/doxorubicin treatment occurred within the first 4-10 days following treatment and included decreases in RBC # and related Hct and Hb (max. 10%), decreases in WBC # up to 50%; recovery was complete by day 14. AST and ALT levels were increased by as much as 7 fold from pretreatment values. In one isolated animal, cholesterol and LDH levels doubled at 40mg/kg ADR-529/2 mg/kg doxorubicin. Combining ADR-529 and doxorubicin in the same vial did not significantly alter the acute toxicity of the compounds.

ACUTE TOXICITY STUDY SUMMARY TABLE

SPECIES	DRUG(S)	VEHICLE	LD_{10} (mg/kg)		LD_{10} (mg/m^2)	
			MALE	FEMALE	MALE	FEMALE
ICR MOUSE	ADR-529	SALINE	>1000	>1000	>3000	>3000
ICR MOUSE	ADR-529	LACTATE	>1000	>1000	>3000	>3000
S.D. RAT	ADR-529	SALINE	>1000	>1000	>5900	>5900
ICR MOUSE	ADR-529 + DOX (SINGLE VIAL)	SALINE	31 (26-37)	29 (26-33)	93	87
ICR MOUSE	DOX	LACTATE	16 (14-18)	23 (21-24)	48	69
	ADR-529 + DOX	LACTATE	25 (21-28)	26 (23-29)	75	78
ICR MOUSE	EPI	LACTATE	26 (24-28)	28 (26-30)	78	84
	ADR-529 + EPI	LACTATE	30 (28-33)	34 (31-?)	90	102
ICR MOUSE	CisPt	SALINE	20 (18-22)	---	60	---
	ADR-529 + CisPt	SALINE	19 (17-20)	---	57	---
S.D. RAT	DOX	SALINE	12.5	15	74	88
	ADR-529 + DOX	SALINE	12.0	11.3	71	67
S.D. RAT	ADR-529 + DOX (SINGLE VIAL)	SALINE	12 (11-14)	14 (13-14)	71	83
S.D. RAT	EPI	LACTATE	15	18	88	106
	ADR-529 + EPI	LACTATE	13	14	77	33

Subacute Toxicity

1. Six week intravenous toxicity study of ADR-529 in the rat. Vol. 1.22: 3726.

This study, T-529-88-015, was performed at according to GLP. Drug was from lots 88G18FY and was formulated in sodium lactate buffer. Sprague-Dawley rats, 10/sex/dose were injected once weekly for 6 weeks i.v. with 0, 30, 100, or 300 mg/kg ADR-529.

Measurements and Observations:

Daily: clinical observations
Weekly: body weight,, food consumption
Prior to sacrifice: ophthalmology, hematology/serum chemistry, urinalysis.
Postmortem: necropsy, organ weights, histopathology

Clinical Observations:

No rats died prior to scheduled sacrifice. One MD and 1 HD female had lesions on the tail, but not at the injection site. Weight loss in males and females did not exceed a 10% difference from controls in any dosage group.

Hematology:

RBC number in HD females decreased by less than 10% from control values. White blood cell number decreased to a statistically significant level (34% from controls) in HD females (LD females, 30% decrease, 28% at MD).

Serum Chemistry:

There were no biologically significant changes in serum chemistry.

Urinalysis:

There were no significant changes observed.

Gross Pathology:

No significant changes were seen at necropsy. The absolute and relative weight of the testes decreased from control by approximately 15% at LD, 33% at MD and 47% at HD. Thymic weight in HD females decreased by approximately 20% from control (absolute and relative). Relative weight of the spleen decreased by 22% from control in MD females.

Histopathology:

All of the MD and HD males showed atrophy of the germinal epithelium of the testes, mild at MD, moderate at HD. Other histopathologic findings include 1 male and female HD rats with chronic inflammation of the liver, and 1 HD female with a kidney cyst.

2. Thirteen-week intravenous toxicity study of ADR-529 and epirubicin in the rat with a 6-week recovery. Vol.1.37: 8797.

This study, #T-529-89-003, was performed at according to GLP. The test article used was lot #88N39FY. Twenty-one 4-5 week-old Sprague-Dawley rats/sex/dose were injected once a week for 13 weeks with the following:

1: sodium lactate and saline
2: 5.0 mg/kg/week ADR-529 and 0.25 mg/kg/week epirubicin
3: 10.0 mg/kg/week ADR-529 and 0.5 mg/kg/week epirubicin
4: 20.0 mg/kg/week ADR-529 and 1.0 mg/kg/week epirubicin

5: 20.0 mg/kg/week ADR-529 and saline
6: sodium lactate and 1.0 mg/kg/week epirubicin.
ADR-529 was injected 1/2 hr. prior to epirubicin injection. Fifteen rats/sex/group were sacrificed at 13 weeks, while 6 rats/sex/group were sacrificed 6 weeks later to assess recovery.

Measurements and Observations:
Daily: clinical observations
Weekly: body weight,, food consum
Prior to sacrifice: ophthalmology. /serum chemistry, urinalysis.
Postmortem: necropsy, organ wei ology

Clinical Observations:
Five animals died prior to schedule One group 6 male was sacrificed at study week 9 due to tail trauma ir de injection impossible. A second group 6 male was sacrificed moribund at week 18 with pallor, dyspnea and red nasal discharge; gross pathology showed an enlarged atria and kidneys. One group 5 female was found dead during study week 10 due to pyelonephritis/cystitis. The other group 5 female and the group 3 male were sacrificed during study weeks 3 and 4 due to a persistent and severe infection of a subdermal head lesion. Other clinical observations include sporadic (with time and dose) incidents of alopecia and sores on the tail and head.

Body weight and food consumption:
In males, body weight gain decreased by 12% from control in group 4 beginning on day 15, and continued to be diminished by 11 to 17% through the dosing period. Decreased weight gain was observed in group 3 from day 43 through 57 (10% decrease from control). In group 6, weight gain was decreased by 10 to 17% from day 29 through day 92, then continued to diminish as compared to controls during the recovery period, reaching a maximal decrease of 44% prior to sacrifice. Body weight in females was not altered to a statistically significant level. Although there were sporadic instances of decreased food consumption in the treated groups, there was no consistent correlation with dose or time. with the exception of group 6 during the recovery period (food consumption decreased).

Hematology:
The table below gives some of the statistically significant changes in hematologic parameters at day 92. WBC number was decreased dose dependently to a maximum of 29% and 38% in group 4 males and females at the end of the dosing period. Decreases in WBC # in group 6 for males and females were 14% and 23% (not statistically significant in either case). Platelet and reticulocyte count in males and females in groups 4 and 6 increased at the interim sampling interval. RBC number decreased in a dose dependent fashion, as did hematocrit, and hemoglobin.
At the end of the recovery period. all group 2-4 females had values similar to control (group 6 hemoglobin and RBC number were still decreased). In males, RBC parameters (RBC#, Hct, Hb) were still decreased significantly in both group 4 and 6. All other group 2-4 parameters were not significantly different from control.

SUMMARY OF MEAN ± S.D. STATISTICALLY SIGNIFICANT* TREATMENT-RELATED HEMATOLOGY CHANGES IN HIGH-DOSE ANIMALS AT COMPLETION OF TREATMENT PHASE

Parameter	Group 1	Group 4	Group 6
Males			
Platelet Count	1027.07±148.71	1314.73±151.01*	1666.36±193.27*
HCT	42.23±1.44	40.25±1.10*	33.81±4.75*
WBC	8.62±0.96	7.30±0.41*	5.76±0.86*
HGB	14.55±0.56	13.50±0.53*	10.86±1.54*
RBC	16.94±0.83	10.54±0.82*	10.09±0.80*
MCHC	34.49±0.86	33.52±0.90*	32.12±0.89*
MCV	49.20±2.83	55.33±2.55*	50.79±1.72*
Reticulocytes	1.95±0.45	4.03±0.95*	6.21±3.27*
%eos	6.09±1.67	6.91±0.83*	5.94±1.56
Segs	18.47±8.76	23.60±6.78	26.86±8.73*
Lymphocytes	79.00±10.41	75.83±7.41	70.07±9.81*
#eos	0.067±0.256	0	0.643±1.080*
Prothrombin	13.97±0.61	13.38±0.58	12.94±0.46*
APTT	24.15±3.95	23.54±3.14	19.81±2.01*
Fibrinogen	223.00±10.42	252.60±30.44	525.71±98.66*
Females			
RBC	7.08±0.28	7.27±0.37*	7.56±0.28
%eos	5.96±1.93	3.67±0.75*	4.57±1.18
Reticulocytes	1.71±0.44	2.51±0.63*	2.41±0.53*
Platelet Count	985.00±129.00	1079.07±172.00	1170.60±97.00*
HGB	14.29±0.30	14.02±0.33	13.79±0.30*
HCT	38.15±0.67	39.07±0.73*	38.39±0.55
MCV	53.40±1.96	56.14±2.21*	54.53±1.67
Prothrombin	14.39±0.45	13.98±0.59	13.43±0.70*

*Statistically different ($p \leq 0.05$) than Group 1 (controls)

Serum Chemistry:

There were no instances in males or females where cotreatment with ADR-529 increased the deviation from control values over that seen with epirubicin alone. In several cases, the ADR-529 appeared to prevent the changes produced by epirubicin: cholesterol and triglyceride levels were increased by 632% and 754% respectively in group 6, but were only increased by 64% and 15% in group 4. Total bilirubin levels were decreased in group 3, 4, and 6 males by 38%, 67%, and 76% respectively. AST and alkaline phosphate levels were approximately half of control in group 6, but were unchanged in group 4 males. Protein levels in group 6 males were decreased by 14% from control levels, while protein in group 4 differed from controls by less than 10%. BUN levels were doubled in group 6 males but did not differ significantly in group 4 males; creatinine levels were unchanged in both groups. Potassium levels in group 4 and 6 males were increased by 8% and 24%. In females, only cholesterol was significantly elevated in group 6, but was unchanged in group 4. In the group 5 males (ADR-529 alone), glucose was elevated by 15% over controls, but returned to control levels during the recovery period.

At final sacrifice, BUN and creatinine levels in group 6 males were increased (1 animal 17 fold increase over control), but were not significantly altered in group 4 males. Other group 6 parameters which were still deranged at recovery included total protein (↓ ♂), albumin (↓ ♂), cholesterol and triglycerides (↑ ♂,♀), A/G ratio (↓ ♂,♀), ALP levels (↓ ♂), and potassium (↑, ♂).

Changes first seen at recovery included the following. AST levels were decreased in groups 4, 5, and 6 males by 39, 31, and 57% as compared to controls. CPK was decreased by 50% in group 4 males, 70% in group 6 males, and 73% in group 6 females. Alpha hydroxybutyrate dehydrogenase levels were decreased by 48%, 34% and 68% in group 4, 5, and 6 males, 30%, 41%, and

73% in group 4, 5, and 6 females. Iron levels were reduced by 64% in group 6 males, 56% in group 6 females.

SUMMARY OF MEAN ± S.E. STATISTICALLY SIGNIFICANT* TREATMENT-RELATED CLINICAL CHANGES IN HIGH-DOSE ANIMALS AT COMPLETION OF TREATMENT PHASE

Parameter	Group 1	Group 4	Group 6
Males			
BUN	14.53±1.60	14.17±1.60	20.57±13.55*
Total Protein	6.31±0.25	5.90±0.16*	5.47±0.35*
Albumin	4.09±0.20	3.77±0.27*	2.61±0.26*
A/G Ratio	1.81±0.13	1.88±0.30	0.87±0.17*
Total Bilirubin	0.245±0.101	0.080±0.004*	0.060±0.000*
Cholesterol	65.27±13.26	107.53±09.30*	477.00±103.50*
Triglycerides	53.07±17.10	62.07±27.56	461.36±225.46*
Potassium	4.49±0.29	4.88±0.31*	5.55±1.53*
AST	107.83±19.43	300.00±23.61	62.29±35.55*
Alk Phos	144.73±39.00	130.27±31.30	76.50±39.05*
Females			
Total Protein	6.29±0.15	6.20±0.32	5.98±0.28*
Albumin	4.34±0.14	4.34±0.25	3.05±0.31*
A/G Ratio	2.20±0.09	2.34±0.26	1.03±0.29*
Cholesterol	76.33±12.43	84.36±10.60	119.53±35.86*

*Statistically different ($p \leq 0.05$) than Group 1 (controls).

Gross Pathology:

Necropsy findings at interim sacrifice (15 animals/sex/dose) were mostly in group 4 and 6 males. Eight group 6 males had pale kidneys, 9 had enlarged kidneys. One group 4 male had a green discoloration of the kidney. One group 6 male had a white discoloration of the liver. One group 6 male had an enlarged spleen. The testis of 15/15 group 4 and 13/15 group 6 males were small. Two group 4 and 5 group 6 males were observed with a small thymus. In the females, 1 group 4 rat had a kidney cyst, and 1 group 4 rat had a deformed liver.

The only finding in group 4 males was small testes at final sacrifice. Group 6 males had 1/6 enlarged adrenal, 1/6 harderian gland discoloration, 1/6 enlarged heart, 5/6 pale/enlarged kidney, 1/6 pale pancreas, 4/6 small prostate, 4/6 small testes, and 4/6 small thymus. In females, 2/6 group 6 rats had pale kidneys.

Organ weights:

Absolute and relative to body thymus weight was elevated in group 5 males by 22% over control. Spleen weight in group 5 males was decreased by 11% (relative and absolute); similar decreases were seen in groups 2-4 while group 6 showed a 26% absolute, 38% relative increase in weight. Changes in relative liver, kidney, adrenal, thyroid, testes, and heart weights in males are shown in the table below. Testes weight decreased dose dependently in groups 2-4. Thymic weight in females decreased dose dependently in groups 2-4 to a maximum of 21% in group 4; group 6 weights were not changed to a statistically significant level.

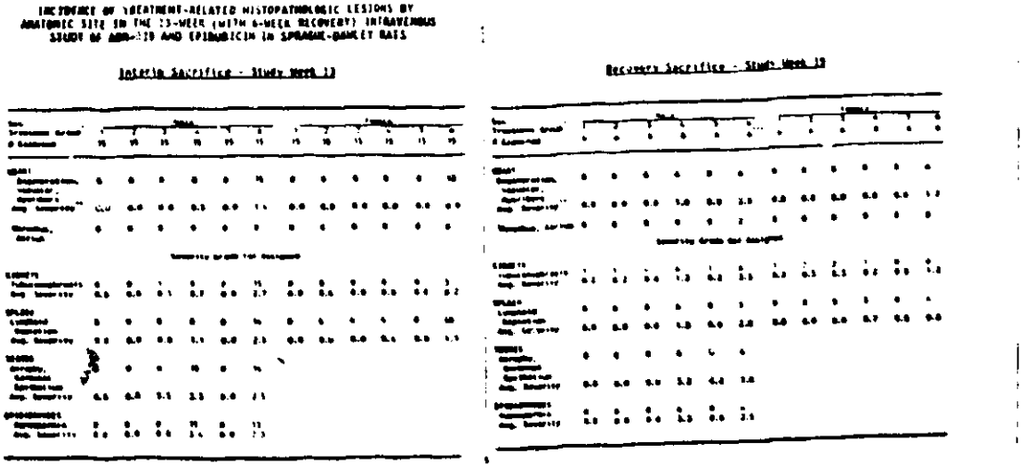
% CHANGE IN ORGAN TO BODY WEIGHT RATIOS AT INTERIM SACRIFICE (MALES).

ORGAN	GROUP 4	GROUP 6
LIVER	10% ↑	46% ↑
ADRENAL	24% ↑	30% ↑
THYROID	---	28% ↑
KIDNEY	14% ↑	91% ↑
HEART	11% ↑	20% ↑
TESTES	56% ↓	48% ↓

At final sacrifice, absolute and relative weights of all organs except testes in males did not differ significantly from control. The absolute weight of adrenal was increased in group 5 males (26%). In females, the absolute weight of the ovaries were decreased in groups 3, 4, and 5 by 23, 28 and 21% respectively. Relative organ weights of these two parameters were not statistically different from controls. In group 6 males the following relative organ weights remained altered: spleen (27% ↑), liver (75% ↑), adrenal (89% ↑), thyroid (49% ↑), kidney (225% ↑), and heart (60% ↑). Testes weight decreased dose dependently to a nadir in group 4 of 60% of control: group 5 decreased by 13%, group 6 by 38%. In females, relative weight of liver was increased by 21% over controls in groups 3 and 4, 56% in group 6. Kidney weight was increased by 38% in group 6 females.

Histopathology:

The major findings at interim sacrifice were in the heart, spleen, testes and epididymis (see table below). Other findings at interim sacrifice included 1/15 group 4 females with lung chronic inflammation/ eosinophilic perivascular infiltration, thymic atrophy (1/15 group 4 and 2/15 group 6 males), chronic periportal inflammation in the liver in 1 group 4 female, and fatty changes in the liver with 1 group 6 male, pancreatic islet hyperplasia in 1/15 group 5 and 6 males, and pancreatic infarct in 1/15 group 6 males. All of these resolved by the end of the recovery period with the exception of thymic atrophy (1/6 group 6 males).



3. Effect of ADR-529 on cisplatin toxicity. II. Intraperitoneal administration of ADR-529 in rats twice daily for three consecutive days. Vol. 1.48: 12637.

The study was not performed according to GLP. Ten male Sprague Dawley rats were treated with 200 mg/kg ADR-529 or saline i.p. followed immediately by 0, or 7-11 mg/kg cisplatin i.v. Additional doses of ADR-529 were administered 4 hours later, and twice daily for the next 2 days. The animals in the ADR-529 alone group showed no clinical abnormalities. In the cisplatin + ADR-529 groups, all the animals but 1 (in the 7 mg/kg group) died within 3-8 days. In the saline and cisplatin groups up to 10 mg/kg, only 1 animal died. No necropsies or clinical chemistry were performed.

Summary and Evaluation of Subchronic Toxicity Studies from this Submission.

In the rat ADR-529/epirubicin study, observations in the ADR-529 alone group (20 mg/kg/week) included 1/21 death from pyelonephritis/cystitis at week 10 and 1/21 sacrificed due to persistent infection of a subdermal head lesion at week 3-4. Males had elevated glucose (15% over control), but this returned to vehicle control levels during the recovery period. No changes in AST or ALT levels were seen, but α-hydroxybutyrate dehydrogenase levels were decreased by approximately 35% in both males and females. Necropsy findings at the end of the treatment phase in ADR-529 rats included a 22% increase in thymic weight, an 11% decrease in spleen weight (both relative and absolute), while at the end of the recovery phase, testes weight (absolute and relative) was decreased by approximately 13%.

There were no toxicities present in groups 2-4 rats which were not present in the group 6 animals. Frequency and severity of damage to the testes was greater in group 4 (high dose ADR-529 and epirubicin) than in the epirubicin alone group. WBC number was more severely depressed in group 4 rats than group 6 rats.

ADR-529 decreased the effects of epirubicin on moribidity, RBC #, cholesterol and triglycerides, AST and alkaline phosphatase, serum proteins, BUN, and K' at the end of treatment; while CPK, alpha hydroxybutyrate dehydrogenase, and iron were less affected in group 4 than group 6 at recovery. These effects were reflected in the diminished changes in liver and kidney weights in group 4 as compared to group 6 rats, and the decrease in hypertrophy and histopathologic findings in the group 4 rats.

Overall Summary of Acute and Subchronic Toxicity of ADR-529

Due to limited solubility (10 mg/ml) of ADR-529, a LD50 level for rodent i.v. administration could not be determined. Only at the maximal dose, 5900 mg/m2, rats convulsed. In the rare animals that died, usually within an hour of injection, an enlarged heart and pulmonary edema were observed, even in lactate vehicle animals, suggesting that hypertonicity effects were involved from the large volumes used (up to 100 ml/kg). With both 6 and 13 weekly doses of ADR-529 in the rat, testicular atrophy (decreased weight, germinal epithelial atrophy and decreased sperm in the epididymis), infrequent (2/20) liver inflammation or deformation, and sporadic decreases in WBC number were observed. No new toxicities were seen with lengthened exposure. In females the NOEL was 115 mg/m2 with 13 weeks exposure; in males, a NOEL was not established.

With acute exposure to ADR-529 in the dog, 1/2 animals died at 40 g/m2. At 10 and 20 g/m2, liver enzymes AST and ALT increased over 10 fold within the first week, then returned to normal by 14 days. With increased length of

exposure at lower doses, liver enzyme elevation was seen only in 1 male at 5 g/m2 and 6 weeks exposure. In the 13 week experiment, no changes in liver enzymes and no dose dependent abnormal liver histopathology were observed. As was seen in the rats, testes weights and germinal epithelial atrophy were observed in all ADR-529 treated males, although there did appear to be some recovery over the 6 week drug hiatus. New toxicities seen with the increased exposure time to 3600 mg/m2 included decreases in red blood cell parameters (#, hematocrit and hemoglobin), gliosis, lymphatic telangiectasis of the heart, extramedullary erythropoiesis and megakaryopoiesis of the spleen, nephrosis, and fibrosis of the kidney. Again, in males a NOEL was not established, while in females, the NOEL was 400 mg/m2. Thus, with acute exposure to high doses of ADR-529, the main target organ for toxicity was the liver, but with longer exposure periods, testes becomes affected. Toxic effects were greater in the male than the female.

In combination with the anthracyclines, the doses of ADR-529 were much lower than those tested singly. In acute studies, the combination of drugs at high doses frequently caused more deaths than the anthracycline alone, the period of observation was too short to determine effects on cardiac tissue. The changes observed in acute and subchronic studies could be largely attributed to anthracycline toxicity. No new toxicities were observed when ADR-529 and either doxorubicin or epirubicin were combined. However, toxicity to both red and white blood cells and the testes was increased in the combination in rats and dogs. Liver and kidney toxicities were mitigated by ADR-529 co-administration: organ weights, serum protein, BUN, creatinine and cholesterol were altered to a lesser degree than that seen with doxorubicin or epirubicin alone. Finally, frequency and severity of cardiac lesions was decreased with the ADR-529/epirubicin combination, and severity decreased with ADR-529/doxorubicin, as evidenced by decrease in vacuolation and decreased alterations in ECG.

III. Reproductive toxicity

1. Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study (including a "behavioral" postnatal evaluation) of ADR-529 and epirubicin administered intravenously to Crl:CD(SD)BR rats. Vol. 1.50: 13498.

This study (T-529-88-017) was performed according to GLP at ADR-529 (lot # SSHO2FY) and epirubicin (20:1) were administered i.v. to 35 presumed pregnant rats/dose on days 7-17 of gestation with 0, 0.5 1.4, and 4.0 mg/kg/day ADR-529 preceding administration of epirubicin (0, 0.025, 0.070, or 0.200 mg/kg/day) by 30 minutes. Twenty-three of the dams were C-sectioned on day 20 of gestation, while the remaining dams delivered normally and were allowed to rear their pups (litters were culled to 8 pups/litter on postparturition day 7). Litters were again culled to 2 males and females/litter at day 21, when the mothers were sacrificed and the F1 generation was allowed to mature, testing for learning and retention in the passive avoidance and watermaze test, then mated. F1 generation females were sacrificed on presumed day 20 of gestation.

Measurements and Observations:

Daily: maternal mortality, weight

Day 29 (C-section): Maternal: gross necropsy, number/placement of implantation, early/late resorptions, live/dead fetuses, and corpora lutea.

Fetal: internal/external/skeletal anomalies/malformations. weight.

Clinical Observations:

One HD non-pregnant dam died on gestational day 12. No gross lesions or unusual behavior were noted prior to death. One LD dam died on day 22 of gestation of presumed delivery complications. Other observations on this dam included 1 pup delivered, 10 still in utero, the remains of a cannibalized pup in the stomach, mottled liver, red vaginal exudate after day 20, and weight loss after day 20. No other unscheduled deaths occurred during the study. During the lactation period, 3 F_0 MD females had masses in the mammary area; in one female, the mass contained an "off-white to green caseous substance" at necropsy, identified as a galactocele.

Body Weight/Food consumption:

The decrement in body weight of the F_0 females in the MD group was 9% as compared to controls over days 7-18, and 41% in the HD females; at HD there was a decrement in weight gain versus controls of 19%. Feed consumption during this period was reduced as well.

Litter Data, 20 day cesarean:

In the dams sacrificed at day 20, there was no statistical difference in the number of females pregnant, the number of corpora lutea, the number of implantations, and the litter size. Two dead fetuses were found in the LD group. Early resorptions actually decreased with increasing dose, while late resorptions were observed in the LD (5) and MD (1) groups only. Overall, fetal body weight was decreased by 11% as compared to controls in the HD group (9% decrease in males, 13% in females.

Incidence of malformations were 1 fetus/1 litter control (threadlike tail), 2 fetuses/2 litters LD (eye bulge depressed, short and edematous body, syndactyly, microphthalmia and anophthalmia, and inward rotation of the hind limbs and a second fetus in another litter with threadlike tail), and 1 fetus/1 litter HD (eye bulge depressed, microphthalmia). One variation was seen in the LD group (the inward rotation of the hind limbs seen in the malformed fetus mentioned previously). Skeletal alterations seen were primarily incomplete ossification of the thoracic or pelvic bones; however, 1 fetus had a sacral hemivertebrae, 1 LD fetus had no caudal vertebrae.

Litter data, term:

In the term delivery group, the days of gestation, number of implantation sites per delivered litter, and number of liveborn pups/litter did not differ. One LD female died during the delivery process (1 fetus cannibalized, 1 fetus in the vagina, and 10 fetuses still in the uterus). Stillborn pups were seen in 1 control dam , 2 MD dams, and 1 HD dam. One control dam had 1 pup die prior to weighing on day 1 3 more males died between days 4 and 7, and the remaining 8 pups died on day 8 post-parturition. One LD pup died between days 1 and 4, 1 MD litter lost 1 male between days 1 and 4, and 1 female between days 4 and 7; while in HD litters, 3 males and 2 females died between days 1 and 4. Body weight in the HD pups was decreased by 15-17% of controls on days 1-21 post parturition. One HD pup had a depressed eye bulge (malformation), while 1 MD pup had a threadlike tail (malformation).

Behavior/mating of the F_1 rats:

Clinical observations in the F_1 generation included chromorhinorrhea in 5/24 LD and HD males, 8/24 MD males; and alopecia in 1/24 control and MD males, 4/25 HD males. During the post-weaning period, 1 control female was found dead at day 22 (little body fat apparent), and 1 HD female was found dead at day 4 (appeared dehydrated). In females, alopecia was observed in 3/24 LD, 4/24 MD and 2/23 HD rats. Only the HD group were slower to achieve surface righting reflex (average day for 50% of the litter to achieve goal 1.5 days control, 3.4 days for HD). No change was seen in time to pinna unfolding, eye opening, acoustic startle, air righting, or pupil constriction. There were no significant differences in the passive avoidance or watermaze learning tests between treated and control rats. In HD males, the testes descended a half day later than in controls, while in females, vaginal patency was observed 2 days later in HD rats than in controls. At the scheduled post-mortem, 4/24 MD and 6/24 HD males had small/purple/dark and/or flaccid testes. One HD female had pale/speckled kidneys.

F_1 generation mating:

Prior to cohabitation (rats were 93-97 days old) body weight in the HD males was still decreased by 7% from controls, in females, a 9% decrease. There were no significant differences between treated and control rats (males or females) in days in cohabitation, or pregnancy rates, despite the fact that HD males had testes weights (both relative and absolute that were decreased by 13% and 9% respectively. During pregnancy, weight gain in HD females was decreased compared to controls, but not to a statistically significant level.

At day 20 of presumed pregnancy (rats without confirmed mating date were excluded from the evaluation summary), there were no dead fetuses or late resorptions. There was a 12% decrease from control in the number of HD corpora lutea, and concomitantly, an 18% decrease in litter size, but these were not statistically significant. There was also an increase in the number of early resorptions in MD (30) and HD (23) offspring as compared to control (17).

In the fetuses examined, 1 MD fetus had a small head with no snout, no apparent eye bulges, pinnae low and placed forward, no oral opening, no facial papillae, and skin thickened on ventral side of neck between pinnae. One control fetus had absent eye bulges.

2. Developmental Toxicity (Embryo-fetal toxicity and teratogenic potential) study of ADR-529 and epirubicin administered intravenously to pregnant rabbits. Vol. 1.53: 14470.

This study, T-529-SS-023, was performed at according to GLP. Drug used was lot #SSHO2FY. Five month old female Hra:(NZW)SPF rabbits (19/dose) were artificially inseminated (day 0) then injected i.v. on days 6 through 18 of presumed gestation with 0, 0.5, 1.5 or 3.0 mg/kg/day of ADR-529 followed 30 minutes later with 0, 0.025, 0.075 and 0.15 mg/kg/day of epirubicin via marginal ear vein. Rabbits were euthanized on day 29.

Measurements and Observations:

Daily: maternal mortality, weight

Day 29 (C-section): Maternal: gross necropsy, number/placement of implantation, early/late resorptions, live/dead fetuses, and corpora lutea.

Fetal: internal/external/skeletal anomalies/malformations, weight.

Maternal observations:

One control group doe aborted on day 18 of gestation. No unusual clinical signs were observed prior to abortion. The aborted litter consisted of 7 fetuses and 2 early resorptions. The only dose-dependent clinical sign was vocalization during injection: 5/19 LD, 6/19 MD and 11/19 HD does. Alopecia and abnormal feces were observed sporadically with dose and time. There were no significant differences in body weight or food consumption between treated and control rabbits. At necropsy, parovarian cysts were observed, but showed no relation to dosage.

Litter data:

When the data for dams with all conceptuses resorbed (2 control, 1 LD, and 1 HD) and does with a single conceptus (1 LD), the number of pregnant rabbits, number of corpora lutea, and number of implantations did not differ significantly between treated and control rabbits. There was 1 dead fetus in the MD group. Although there was not a statistically significant change in litter size, and resorptions, there was a trend toward decreased litter size at HD due to increased number of early and late resorptions (14 resorptions/147 implantations in the control, 18 resorptions/115 implantations in the HD). Neither the percentage of males/litter or the fetal weights differed significantly in the treated versus control groups.

Nearly all the litters in both treated and control groups had at least 1 fetus with an alteration (2 litters at LD and 1 at HD were free of alterations). The number of litters with malformations and fetuses with malformations actually decreased with increasing dose (6/16 litters control, 0/13 LD, 2/15 MD and 1/14 HD). Fetuses with any variation/litter ranged from 41% to 49% with no relationship to dose. Gross external malformations included 1 control fetus with umbilical hernia, 1 control fetus with gastroschisis and agenesis of the pollex; and 1 MD fetus with encephalocele. External variations included 1 control fetus with paws rotated downward and 1 LD fetus with rear limbs rotated medially. One control fetus malformation was persistent truncus arteriosus. Soft tissue variations were lung intermediate lobe agenesis (3 control, 2 LD, 6 MD, and 2 HD fetuses), small intermediate lung lobe (1 control fetus), circumcorneal hemorrhage (2 MD fetuses), moderate dilation of the lateral ventricles of the brain (1 MD), and enlarged aorta (1 MD fetus). Skeletal malformations were in the skull (1 MD fetus with suture enlargement associated with encephalocele), thoracic hemivertebrae (1 control, 1 MD) misaligned caudal vertebrae (2 control), and split ribs (1 MD and 1 HD fetus). Variations observed, mainly irregular ossification in the skull) were increased to a statistically significant level over control (32% of the fetuses) in the LD (44% of the fetuses) and MD (48% of the fetuses). Other variations were incomplete ossification of the sternebrae or pelvic pubes.

Summary of Reproductive Toxicity from this Submission

In rats, the maternal no observable effect level (NOEL) was at the LD level (0.5 mg/kg ADR-529/0.025 mg/kg epi/day), based on maternal weight. The NOEL for the offspring was MD, 1.4 mg/kg ADR-529/0.07 mg/kg epi/day based on low fetal weight, delay of ossification, and testicular anomalies in the HD group (4.0 mg/kg ADR-529/0.2 mg/kg epi/day). There were malformations in the F_1 generation, although there was no clear dose dependence. Despite

the presence of small/purple/flaccid testes in the HD males. there were no significant differences in litter parameters in the F_1 generation.

In the rabbit, with the exception of a dose dependent increase in vocalization during injection. there were no significant signs in the dams (NOEL was below the LD, 0.5 mg/kg ADR-529/0.025 mg/kg epi/day, based on the vocalization. NOEL was 3.0 mg/kg/day ADR-529/0.15 mg/kg/day epirubicin based on other observations). In the offspring, the NOEL based on increased number of resorptions. was the MD, 1.5 mg/kg ADR-529/0.075 mg/kg epi/day. Malformations and variations were not dose-dependent.

From this data. the combinations of ADR-529 and epirubicin tested caused a change in the males' testes in rats at 4.0 mg/kg ADR-529/0.2 mg/kg epirubicin/day. The 1.5 mg/kg ADR-529/0.075 mg/kg epirubicin/day in rabbits was fetotoxic.

Overall Summary of Reproductive Toxicity of ADR-529.

The maternal NOEL level for ADR-529 in rats was 2.0 mg/kg (11.8 mg/m^2); at 8.0 mg/kg, the dams had a decreased body weight gain of greater than 10% of control and a blue-ish bladder mass was observed. The offspring NOEL was 0.5 mg/kg (2.95 mg/m^2). The incidence of variations and malformations increased dose dependently and fetal birth weight was also decreased at the highest dose. Types of malformations observed at MD (2 fetuses) and HD (13 fetuses) included imperforate anus, exencephaly, microphthalmia and anophthalmia, hydrocephalus, and agenesis of the tongue or palate. Incomplete ossification was also seen frequently. The F1 generation animals were slower to mature physically and sexually, and had difficulties in mating (2/23 HD female rats actually became pregnant).

In rabbits, maternal death (1/20), abortion (1/20) and a decrement in weight gain of 12% (not statistically significant), were seen in the HD group (20 mg/kg or 220 mg/m^2). At MD (5 mg/kg), only increased vocalization during the injection was observed; thus, the NOEL in rabbits was 5 mg/kg (55 mg/m^2). In the offspring, an increase in resorptions and malformations was seen at HD. The malformations were mostly skeletal: fused thoracic arches and misaligned. bifid or fused ribs and vertebrae. Therefore, the NOEL in offspring was also 5 mg/kg.

Thus. in rats, ADR-529 is teratogenic at doses below maternotoxic levels (12 mg/m^2). In rabbits. ADR-529 is maternotoxic and fetotoxic at 220 mg/m^2.

In combination with epirubicin, (doxorubicin was not tested, presumably because of its known mutagenicity), the rat maternal toxicity occurred at doses lower than the fetotoxicity (NOEL in dams. 0.5 mg/kg ADR-529/0.025 mg/kg epirubicin; fetal NOEL. 1.4 mg/kg ADR-529/0.07 mg/kg epirubicin). In the rabbit. the only maternal toxicity was vocalization during injection. There was a small degree of fetotoxicity at the 1.5 mg/kg ADR-529 0.075 mg/kg epirubicin. These reproductive toxicities were not surprising in light of the clastogenic effects of ADR-529.

Genotoxicology

Note: all the following experiments were conducted according to GLP.

1. Gene mutation in Salmonella typhimurium on ICRF-187 (Ames Test). vol. 1.54: 1-747.

The study was performed at

. Concentrations of ADR-529 used were 62.5, 125, 250, 500,

or 1000 ug/plate, lot 861.46A, solubility 10-12 mg/ml in water at room temperature. Positive controls were 2-nitrofluorene (2-NF), 9-aminoacridine (9-AA), 2-aminoanthracene (2-AAN), 2-acetylaminofluorene (2-AAF), benzo (a) pyrene (baP), and sodium azide (SAZ). S. typimurium strains used were TA1535, TA100, TA1537, TA1538, and TA98. Metabolic activation was done with the S-9 fraction.

There was no statistical difference between the numbers of revertants/plate in ADR-529 treated and control plates with or without metabolic activation of the test compound. The number of revertants plate with positive controls was approximately 10 fold higher. No inhibition of the growth of the bacterial lawn was seen with concentrations up to 1000 ug/plate of ADR-529.

2. Gene mutation test in Salmonella typhimurium on ICRF-187, doxorubicin and their combination, 20:1 ratio (Ames test). Vol. 1.54: 14781.

The study was performed at

Concentrations of ADR-529 used were 62.5, 125, 250, 500, or 1000 ug/plate, lot 861.46A. Doxorubicin concentrations were 3.12, 6.25, 12.5, 25, and 50 ug/plate. Positive controls were 2-nitrofluorene (2-NF), 9-aminoacridine (9-AA), 2-aminoanthracene (2-AAN), 2-acetylaminofluorene (2 AAF), benzo (a) pyrene (baP), and sodium azide (SAZ). S. typimurium strains used were TA1535, TA100, TA1537, TA1538, and TA98. Metabolic activation was done with the S-9 fraction.

The highest concentration of DOX inhibited growth of the bacterial lawn. Again, no concentration of ADR-529 tested was statistically different from control, while positive controls were approximately an order of magnitude larger than controls. In the TA1538, TA98 and TA100 strains, DOX increased the number of revertants/plate in a dose dependent fashion to a maximum of 8.5 fold in TA98, 2 fold in TA100, and 3.7 fold in TA1538 at 25 ug. In the 20:1 combination of the two compounds, there was no change from control in TA1535 and TA 1537, but revertants/plate were increased dose dependently in TA1538 (3.2 fold), TA 98 (8.6 fold) and TA100 (2.2 fold) at 500 ug ADR-529/25 ug DOX/plate. With microsomal activation, a similar profile was seen. There did not appear to be either protection or synergism with the combination of ADR-529 and doxorubicin.

3. Gene mutation in Salmonella typhimurium with ADR-529 in association with epirubicin, 20:1 ratio (Ames test). Vol. 1.54: 14835.

The study was performed at

. Concentrations of ADR-529 used were 62.5, 125, 250, 500, or 1000 ug/plate, batch N S001, lot 88/01 and 88/02. Epirubicin concentrations were 3.12, 6.25, 12.5, 25, and 50 ug/plate. Positive controls were 2-nitrofluorene (2-NF), 9-aminoacridine (9-AA), 2-aminoanthracene (2-AAN), 2-acetylaminofluorene (2-AAF), benzo (a) pyrene (BaP), and sodium azide (SAZ). S. typimurium strains used were TA1535, TA100, TA1537, TA1538, and TA98. Metabolic activation was done with the S-9 fraction.

Revertance with epirubicin or ADR-529 alone were not performed in this experiment. With the 20:1 combination, a dose dependent increase in revertant was seen in TA1538, TA98 and TA100 with and without metabolic activation. Inhibition of the bacterial lawn was seen at the highest dose of epirubicin/ADR-529 (approximately 3, 12 and 5 fold increases above control levels respectively). Positive control levels of revertants were about an order

of magnitude larger than control.

4. The hepatocyte primary culture/DNA repair assay on ICRF-187. vol. 1.54: 14877.

The study was performed at ..... The positive control was 2-aminofluorene (2-AF) and the negative control was fluorene: both were dissolved in DMSO. A primary culture of rat hepatocytes was treated with ADR-529 (max. concentration 0.1 mg/ml) for 18 hours, then stained for autoradiography. Concentrations of ADR-529 up to 0.05 mg/ml did not have a statistically different number of grains/nucleus than the control and negative control samples (-10 to -14). The positive control was 45.9 ± 4.4. No cytotoxicity was seen.

5. The hepatocyte primary culture/DNA repair assay on ICRF-187 and adriamycin. Vol. 1.54: 14891.

The study was performed at The positive control was 2-aminofluorene (2-AF) and the negative control was fluorene: both were dissolved in DMSO. The results of the controls were numerically identical to the ones used in the previous study (#4) and were performed on the same date, indicating that these experiments were performed a single time. Eight concentrations of ADR-529/adriamycin (20:1) with the maximum level at 0.1/0.005 mg/ml were tested in triplicate, with the 3 highest concentrations 0.01/0.0005, 0.05/0.001, and 0.1/0.005 mg/mg ADR-529/adriamycin were cytotoxic. The other concentrations did not yield grain counts statistically different from controls. The positive control was the same as the previous experiment.

6. DNA repair test in the rat hepatocyte primary cultures with ADR-529 in association with epirubicin, 20:1 ratio. Vol. 1.54: 14906.

The study was performed at Primary cultures of rat hepatocytes were exposed to ADR-529 and epirubicin (20:1 ratio) for 18 hours at concentrations ranging from 0.1/0.005 to 100/5 ug/ml. Concentrations of ADR-529/epirubicin at and above 10/0.5 were cytotoxic. Concentrations of 1/0.05 and 3/0.15 resulted in increased grain counts (7.7 and 9.7 respectively compared to control levels of ~5.9).

7. Metaphase chromosome analysis on ADR-529 in human lymphocytes cultured in vitro. Vol. 1.55: 14962

The study was performed at Human lymphocytes were cultured with phytohaemagglutinin. ADR-529 in M/6 sodium lactate was added to cultures at 0.1, 0.3, 1.0, 3.0 and 10.0 ug/ml without metabolic activation and 1.0, 3.0, 10, 30 and 100 ug/ml with S-9 fraction activation. Positive controls were mitomycin C and cyclophosphamide with metabolic activation.

In the cells without metabolic activation, there was a dose dependent increase in aberrations/cell beginning at 0.3 ug/ml. The maximal increase in aberrations recorded at 3 ug/ml was a 15 fold increase over controls and was similar to the increase seen with mitomycin C. The number of polyploid metaphases was also increased at 1, 3, and 10 ug/ml with the maximal increase over control a 290 fold increase; no increase was seen with mitomycin C.

With metabolic activation, there was still a dose dependent increase in the aberrations/cell and % of aberrant cells beginning at 10 ug/ml reaching a

maximum of 20 fold (aberrations/cell) to 42 fold (% cells with aberrations) as compared to controls. Cyclophosphamide at 10 ug/ml was increased 76 fold over control. The percentage of polyploid metaphases also increased dose dependently beginning at 3 ug/ml with a maximal increase of 54 fold over control at 100 ug/ml. No change was seen with cyclophosphamide. The aberrations with and without metabolic activation included gaps, breaks, minutes, and interchanges.

8. Metaphase chromosome analysis in human lymphocytes treated <u>in vitro</u> with ADR-529 and doxorubicin in a ratio of 20:1. Vol. 1.55: 14985.

The study was performed at Human lymphocytes were cultured with phytohaemagglutinin. Concentrations of ADR-529 and doxorubicin are listed below. Positive controls were mitomycin C and cyclophosphamide with metabolic activation. Doxorubicin alone was not tested so it could not be determined whether ADR-529 adds to the genotoxicity of doxorubicin.

nanograms/ml			
Without activation		With activation	
ADR-529	+ doxorubicin	ADR-529	+ doxorubicin
62.5	3.12	125	6.25
125	6.25	250	12.5
250	12.5	500	25
500	25	1000	50
1000	50	2000	100
		4000	200

The two highest concentrations of ADR-529 and doxorubicin were cytotoxic in both experiments. Polyploid metaphases were not counted in this experiment. In the cells without metabolic activation, there was a dose dependent increase in aberrations beginning at 62.5 ng/ml ADR-529/3.12 ng/ml DOX with a maximal increase of 13.5 fold (% + gaps) over control at 250 ng/ml ADR-529/12.5 ng/ml DOX. Mitomycin C aberrations were increased by 18 fold over control at 0.1 ug/ml. With metabolic activation, the dose dependent increase began at 500/25 and reached a maximum of 5 fold increase over controls at 1000/50 ADR-529/DOX. Cyclophosphamide showed a 7 fold increase over controls at 10 ug/ml.

9. Micronucleus test in mouse bone marrow cells after i.v. administration of ADR-529. Vol. 1.55: 15029.

The study was performed a . ADR-529 in M/6 sodium lactate was administered i.v. to CD1 mice (5/sex/dose) at 20, 63, and 200 mg/kg. Positive control was 50 mg/kg cyclophosphamide i.p. Marrow samples were taken 24 hours after dosing (at 48 hours, reduction was so severe that scoring was impossible).

The number and percentage of micronucleated polychromic erythrocytes increased, independent of sex, dependent on dose, from 16 fold over control at 20 mg/ml to a maximum of 22 fold over control at 200 mg/kg. Cyclophosphamide controls were increased over control 42 fold. The ratio of polychromic to normochromic erythrocytes did not differ to a statistically significant extent in any treated group, indicating a lack of effect on proliferation.

10. Micronucleus test in mouse bone marrow cells after i.v. administration of ADR-529 and doxorubicin, 20:1 ratio. Vol. 1.55: 15046.

The study was performed at ADR-529

in M/6 sodium lactate was administered i.v. to CD1 mice (5/sex/dose) at 20, 63, and 200 mg/kg; doxorubicin was administered 30 minutes later at 1, 3.15, and 10 mg/kg. Positive control was 50 mg/kg cyclophosphamide i.p. Marrow samples were taken 24 hours after dosing (at 48 hours, reduction in ratio of polychromatic/normochromatic erythrocytes was so severe that scoring was impossible). No doxorubicin alone control was tested.

Micronuclei increased in both sexes 11 fold over control at 20 mg/kg ADR-529/1 mg/kg DOX, 23 fold at 63/3.15 mg/kg and 19 fold at 200/10 mg/kg; however, the ratio of polychromic/normochromic erythrocytes at 200/10 mg/kg was decreased to 0.63, indicating a degree of cytotoxicity at this dose. Cyclophosphamide at 50 mg/kg i.p. increased micronuclei by 28 fold over control.

11. Micronucleus test in mouse bone marrow cells after i.v. administration of ADR-529 and epirubicin, 20:1 ratio. Vol. 1.55: 15079.

The study was performed at ADR-529 in M/6 sodium lactate was administered i.v. to CD1 mice (5/sex/dose) at 20, 63, and 200 mg/kg; epirubicin was administered 30 minutes later at 1, 3.15, and 10 mg/kg. Positive control was 50 mg/kg cyclophosphamide i.p. Marrow samples were taken 24 hours after dosing (at 48 hours, reduction in ratio of polychromatic/normochromatic erythrocytes was so severe that scoring was impossible). No epirubicin alone control was tested.

The difference in increase of micronuclei between males and females was not statistically significant. A 15 fold increase in micronuclei as compared to control at LD, 22 fold at Md and 23 fold at HD was observed; however, at HD the ratio of polychromic to normochromic erythrocytes was reduced by 41% from control, indicating significant cytotoxicity.

Summary of Genotoxicity from this Submission

ADR-529 alone was not genotoxic in the Ames test in Salmonella or in the hepatocyte primary culture repair assay. However, toxicity was seen in the metaphase chromosome analysis and in the mouse micronucleus test (clastogenicity) beginning at 0.3 ug/ml in non-metabolically activated marrow cells, and 20 mg/kg in the mouse micronucleus test. The exact contribution of ADR-529 to anthracycline genotoxicity cannot be precisely determined from the data given as anthracycline alone controls were not run concurrently. While it can be stated that the combination of compounds has a greater toxicity than ADR-529 alone, it cannot be conclusively stated that there is no synergy between ADR-529 and anthracyclines.

Integrated Summary of Preclinical Pharmacology and Toxicology

Mice, rats, rabbits, guinea pigs, and dogs all had evidence of dose dependent cardioprotection from doxorubicin toxicity with ADR-529. Cardioprotection consisted of a decrease in both incidence and severity of cardiac lesions (vacuolization, myofibrillar loss, and changes in ECG) at ADR-529:DOX ratios of 5:1 to 20:1. Protection was observed with ADR-529 administered 30 minutes prior to DOX/EPI, or simultaneously; acute toxicity did not differ between 30 minute preadministration or concurrent administration.

ADR-529 was originally investigated for use as an anticancer agent, but had little activity alone. In the mouse tumor models, there was no decrease in mean survival time with DOX, EPI or IDA coadministration; in some models, some degree of additivity of tumor reduction was noted. However, in the BL BX7 human tumor xenograft, DOX-induced reduction in tumor regrowth was decreased with ADR-529 co-treatment, indicating that ADR-529 may protect specific tumor types. The sponsor has agreed to further investigate the response of BL BX7 tumor to ADR-529 and DOX.

The pharmacokinetics of ADR-529 were not affected by administration of DOX or EPI. Conversely, ADR-529 did not significantly alter the pharmacokinetics of DOX (EPI was not tested). ADR-529 had a plasma half-life of 1-2 hours in dog and human with minimal plasma protein binding; plasma concentrations increased linearly with increasing dose administered. Elimination was primarily via the kidney.

While doses of DOX were approximately 4 fold higher in the efficacy studies than those in the toxicity experiments, the findings were comparable. In acute studies, high dose ADR-529 alone (4000 mg/m^2 in dog) led to elevated AST/ALT levels, yet coadministration of ADR-529 and doxorubicin at lower levels in efficacy studies resulted in decreased toxicity to the liver and kidney. Similar results were included in the 6 week and 13 week combination toxicity studies. The protection in liver and kidney was not unexpected in light of the high concentrations of ADR-529 seen in these organs in tissue distribution studies. While ADR-529 concentrations in the heart never reached the high levels seen in kidney and liver, drug remained detectable (and at a higher level than plasma) from 24 to at least 72 hours post-treatment. Target organs for toxicity were marrow and testes; toxicity to these organs was more severe in combination with DOX and EPI. Toxicity was more severe in males than females; yet, no major differences between pharmacokinetic parameters in male and female rats were observed. NOEL levels with weekly administration of drug for 13 weeks are shown in the following table.

Although ADR-529 was not mutagenic in the Ames assay, clastogenicity was observed in the mouse micronucleus test. Therefore, it was not unexpected that ADR-529 was teratogenic in rats below maternally toxic levels (12 mg/m^2), and fetotoxic in rabbits at maternotoxic levels (220 mg/m^2).

NOEL LEVELS IN RATS AND DOGS WITH 13 WEEKLY DOSES OF ADR-529

TREATMENT	RAT		DOG	
	MALE	FEMALE	MALE	FEMALE
ADR-529	<118 mg/m^2	118 mg/m^2	<400 mg/m^2	400 mg/m^2

A survey of the literature revealed no additional information on the effect of ADR-529 in combination with anthracyclines on tumor response, although 1 paper (Lazo et al., 1978, Cancer Res. 38:2263-2270) described the increased metastatic potential (lung colonization) of B16 melanoma cells treated in vitro with ICRF-159, the racemate of ADR-529.

In conclusion, ADR-529 diminished cardiac damage by DOX in several animal models. Tumor protection was minimal with the exception of the BL BX7 human xenograph, which the sponsor agreed to investigate further. Labelling changes in the preclinical sections include alterations in statements on tumor protection, pregnancy category and description, and carcinogenicity/mutagenicity.

Recommendations

1. The labelling in the "Pregnancy" section currently reads as follows.

Pregnancy - *Pregnancy Category C* - Dexrazoxane was maternotoxic at dosages of 2 mg/kg and embryotoxic and teratogenic at 8 mg/kg when given daily to pregnant rats during the period of organogenesis. Teratogenic effects in the rat included imperforate anus, microphthalmia, and anophthalmia.[33] In rabbits, dosages of 5 mg/kg daily during the period of organogenesis were maternotoxic and dosages of 20 mg/kg were embryotoxic and teratogenic. Teratogenic effects in the rabbit included several malformations as well as agenesis of the gallbladder and of the intermediate lobe of the lung.[34] The possible adverse effects of ZINECARD on fertility in males and females, in humans or experimental animals, have not been adequately studied. ZINECARD should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

It should read something like this:

Dexrazoxane was maternotoxic in rats at dosages of 8 mg/kg (47 mg/m²); teratogenic and embryotoxic effects were seen in the offspring at this dose as well. Teratogenic effects were also seen at 2 mg/kg (12 mg/m²). Teratogenic effects in the rat included imperforate anus, microphthalmia and anophthalmia, exencephaly, hydrocephalus and agenesis of the tongue or palate. In rabbits, doses of 20 mg/kg (220 mg/m²) were maternotoxic, embryotoxic, and teratogenic. At this dose, fetal weight was decreased, whole litters were resorbed, and skeletal malformations were seen as well as agenesis of the gall bladder and intermediate lobe of the lung.

Adverse affects on the fertility on males and females in humans or experimental animals has not been adequately studied; however, the testicular atrophy seen with dexrazoxane administration was increased with doxorubicin cotreatment in both rats and dogs. Fertility was impaired in the male and female rats treated in utero during organogenesis at 8 mg/kg. Zinecard should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Wendelyn J. Schmidt, Ph.D.

cc:
NDA ORIG.
HFD-150
/ECutler
/GWilliams
/JDeGeorge
/ACoulter

PHARMACOLOGIST 45 DAY REVIEW COVERSHEET

NDA NO: 20212
SPONSOR: ADRIA
REVIEWER: A.W. COULTER & W. SCHMIDT
DATE OF REVIEW: 3/26/92
DRUG: ZINECARD (ADR-529)
DRUG CATEGORY: CHEMOPROTECTANT
INDICATION: CARDIOPROTECTANT (↓FREQ, SEVERITY) w/DOX administration
RELATED DRUG: _____
STEREOISOMER? YES X NO ___
DELIVERY SYSTEM: _____

Pharmacology studies submitted in this NDA: 75 Studies - 43 studies concerned with the primary therapeutic effect 18 studies on interactions with anti-tumor drugs, and 14 studies on safety, etc. (in vivo & in vitro)
Pharmacology(primary and secondary) Studies in rat, mouse, dog, hamster, rabbit, mini-pig
Pharmacokinetics(ADME) PK/DISTRIB. RAT, DOG

Toxicology studies submitted in this NDA (studies with asterisks have been previously reviewed)

	Mouse	Rat	Guinea pig	Rabbit	Dog	primate
Single Dose						
I.V.	✓	✓	___	___	✓	___
p.o.	___	___	___	___	___	___
others	___	___	___	___	___	___
Repeated Dose						
p.o.(i.v.) 3 month	___	✓	___	___	✓	___
p.o.(i.n.) 6 month	___	___	___	___	___	___
p.o. 12 month	___	___	___	___	___	___
Carcinogenicity	___	___			___	
Reproductive Toxicity						
Segment I	___	___				
Segment II	___	✓		✓		
Segment III	___	✓				
Dermal toxicity	___	___	___	___		
Ocular toxicity	___	___		___		

Genotoxicity 1. AMES 2. METAPHASE CHROMOSOME 3. MICRONUCLEUS 4. DNA REPAIR

EVALUATION:

- The submission generally acceptable for review:
  yes X No ___
  if no, comment:

- Appropriate studies submitted: Yes X No ___
  if no, comment:

- Target organs of toxicity: MARROW, TESTES; w/ high doses, liver

- Reproductive or development toxicity: Yes — teratogenic in rats (bel mat embryotoxic at maternal tox. dose

- Carcinogenicity studies: NONE
  number of studies: Rat ( ) Mouse ( )
  Historical control data submitted: Yes ( ) No ( )
  Consultation to Biometrics required: Yes ( ) No ( )

- GLP problem: yes ( ) no (X)

- Inactive ingredient, metabolic, impurity or attractable concerns? No

- Other comments:

RECOMMENDATION:

Fileability yes (X)
no ( )
if no, comment:

Division of Oncology and Pulmonary Drug Products
Addendum to Review of Chemistry, Manufacturing and Controls

NDA #: 20-212 **CHEM. REVIEW#:** 5A **REVIEW DATE:** April 21, 1995

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
None			

NAME AND ADDRESS OF APPLICANT: Pharmacia Inc.
7001 Post Road
Dublin, OH 43017

MAILING ADDRESS OF APPLICANT: P.O. Box 16529
Columbus, OH 43216

DRUG PRODUCT NAME:

Proprietary:	ZINECARD for Injection
Nonproprietary/USAN:	Dexrazoxane for Injection
Code Name/#:	ADR-529, ICRF-187, NSC-169780
Chem. Type/Ther. Class:	1-P

PHARMACOL. CATEGORY/INDICATION: Intracellular chelating agent, cardioprotectant for use in conjunction with doxorubicin

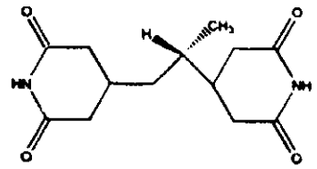
DOSAGE FORM: Sterile lyophilized powder for injection, supplied with a vial of M/6 Sodium Lactate Injection, USP

STRENGTHS: 250 mg, 500 mg

ROUTE OF ADMINISTRATION: Intravenous

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:
(S)-4,4'-(l-methyl-1,2-ethanediyl)bis-2,6piperazinedione
$C_{11}H_{16}N_4O_4$ MW 268.28



CONSULTS:
Environmental Assessment and draft FONSI were reviewed in HFD-150 for completeness and submitted under consult to HFD-102 for final review and sign-off 4/11/95.

REMARKS/COMMENTS:
A Finding of No Significant Impact for Zinecard was finalized 4/20/95 (See Attachment). There are no further deficiencies in the CMC sections.

CONCLUSIONS AND RECOMMENDATIONS: This application is **approved** from a CMC standpoint.

Steven R. Koepke, Ph.D.
Review Chemist

4/25/95

CC:
Orig. NDA
HFD-150 Division File
HFD-150/S. Koepke
HFD-150/E Tolgyesi
HFD-151/CSO/MPelosi
R/D Init. by:
[redacted]Reviews\ndas\n20212r5.00a

FEB-1

APR 21 1995

Division of Oncology and Pulmonary Drug Products
Review of Chemistry, Manufacturing and Controls

NDA #: 20-212 **CHEM. REVIEW#: 5** **REVIEW DATE: April 17, 1995**

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment (BC)	02-Feb-95	03-Feb-95	23-Feb-95
Amendment (BF)	14-Mar-95	16-Mar-95	20-Mar-95
Amendment (BF)	17-Mar-95	20-Mar-95	21-Mar-95
Amendment (BC)	23-Mar-95	24-Mar-95	28-Mar-95
Amendment (BC)	27-Mar-95	29-Mar-95	30-Mar-95
Amendment (AL)	12-Apr-95	13-Apr-95	17-Apr-95

NAME AND ADDRESS OF APPLICANT: Pharmacia Inc.
7001 Post Road
Dublin, OH 43017

MAILING ADDRESS OF APPLICANT: P.O. Box 16529
Columbus, OH 43216

DRUG PRODUCT NAME:

Proprietary:	ZINECARD for Injection
Nonproprietary/USAN:	Dexrazoxane for Injection
Code Name/#:	ADR-529, ICRF-187, NSC-169780
Chem. Type/Ther. Class:	1-P

PHARMACOL. CATEGORY/INDICATION: Intracellular chelating agent, cardioprotectant for use in conjunction with doxorubicin

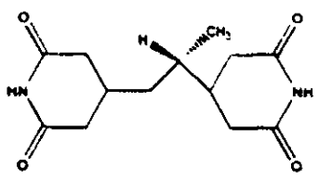
DOSAGE FORM: Sterile lyophilized powder for injection, supplied with a vial of M/6 Sodium Lactate Injection, USP

STRENGTHS: 250 mg, 500 mg

ROUTE OF ADMINISTRATION: Intravenous

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:
(S)-4,4'-(l-methyl-1,2-ethanediyl)bis-2,6piperazinedione
$C_{11}H_{16}N_4O_4$ MW 268.28



CONSULTS:

Environmental Assessment report was prepared by A. Mukherjee, Ph.D. (HFD-102) on 10/21/94. Pharmacia's response to the deficiencies (2/2/95 amendment) was reviewed in HFD-102 and the responses (3/23/95 and 3/27/95 amendments) were reviewed in HFD-150 for completeness and submitted under consult to HFD-102 for final review and sign-off 4/11/95.

Microbiology Consult Report #3 by Paul Stinavage, Ph.D., dated 1/30/95 judged the application approvable, pending on the Applicant's commitment to provided additional test data post-approval.

ESTABLISHMENT EVALUATION REQUESTS:

The CGMP status of the manufacturing facilities was judged acceptable by Compliance on 12/15/94 and 1/25/95. The Chemist requested on 2/9/95 a new 60-day update and the facilities were judged acceptable on 3/20/95.

REMARKS/COMMENTS:
Final package labeling was submitted 4/12/95 and is acceptable. As of the date of this review, no response was received from HFD-102 concerning the Environmental Assessment.

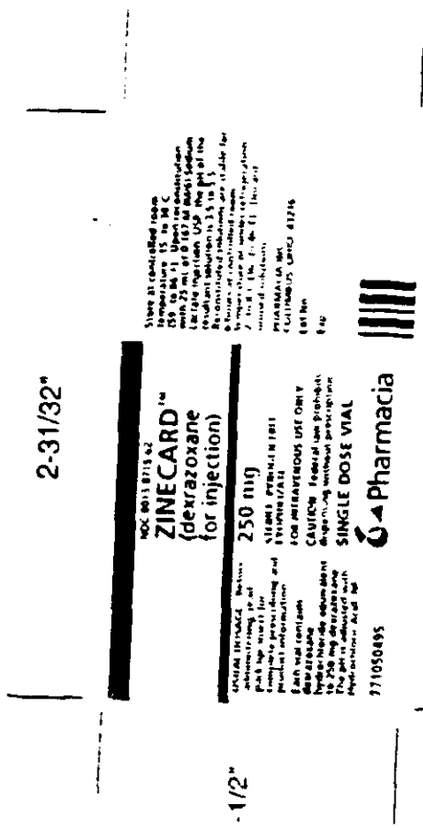
CONCLUSIONS AND RECOMMENDATIONS: This application is approvable from a CMC standpoint pending an acceptable review of the Environmental Assessment from HFD-102.

Steven R. Koepke, Ph.D.
Review Chemist

4/21/95

CC:
Orig. NDA
HFD-150 Division File
HFD-150/S. Koepke
HFD-150/E Tolgyesi
HFD-151/CSO/MPelosi
R/D Init. by:
Reviews\ndas\n20212r5.000

ZINECARD™
(dexrazoxane
for injection)
NDA 20-212
250 mg Vial Label
Part # 771050495



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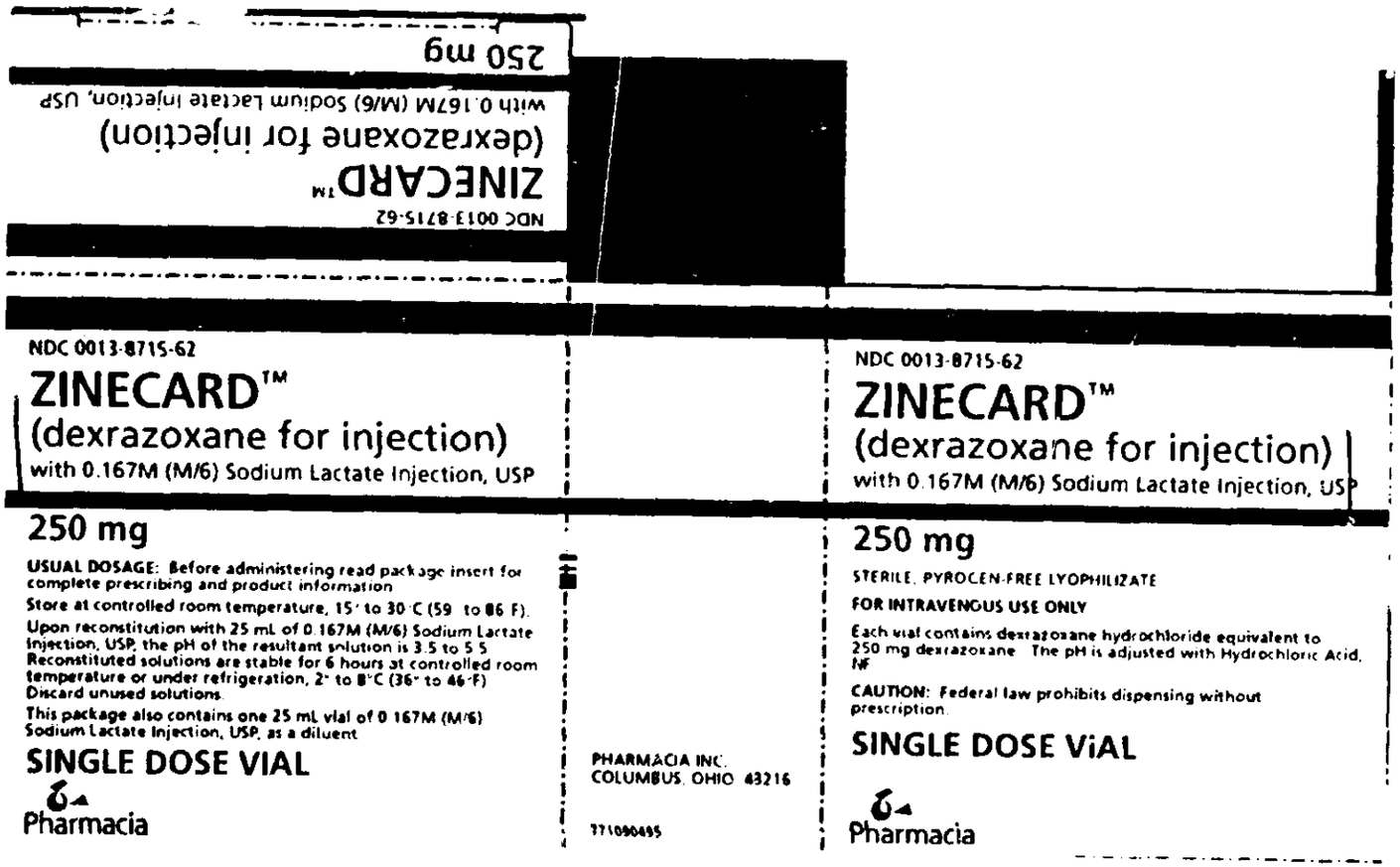


NDC 0013-8715-62

ZINECARD™

(dexrazoxane for injection)

with 0.167M (M/6) Sodium Lactate Injection, USP

250 mg

USUAL DOSAGE: Before administering read package insert for complete prescribing and product information.

Store at controlled room temperature, 15° to 30°C (59° to 86°F).

Upon reconstitution with 25 mL of 0.167M (M/6) Sodium Lactate Injection, USP, the pH of the resultant solution is 3.5 to 5.5. Reconstituted solutions are stable for 6 hours at controlled room temperature or under refrigeration, 2° to 8°C (36° to 46°F). Discard unused solutions.

This package also contains one 25 mL vial of 0.167M (M/6) Sodium Lactate Injection, USP, as a diluent.

SINGLE DOSE VIAL

Pharmacia

PHARMACIA INC.
COLUMBUS, OHIO 43216

771090495

NDC 0013-8715-62

ZINECARD™

(dexrazoxane for injection)

with 0.167M (M/6) Sodium Lactate Injection, USP

250 mg

STERILE, PYROGEN-FREE LYOPHILIZATE

FOR INTRAVENOUS USE ONLY

Each vial contains dexrazoxane hydrochloride equivalent to 250 mg dexrazoxane. The pH is adjusted with Hydrochloric Acid, NF.

CAUTION: Federal law prohibits dispensing without prescription.

SINGLE DOSE VIAL

Pharmacia

ZINECARD™
(dexrazoxane
for injection)
NDA 20-212
250 mg Carton
Part # 771090495

SODIUM LACTATE
INJECTION, USP
NDA 20-212
25 mL Vial Label
Part # 774030395

2-31/32"

1-1/2"

NDC [illegible]

SODIUM LACTATE INJECTION, USP

Each mL contains 18.6 mg of sodium lactate. Sodium Hydroxide NF and/or Hydrochloric Acid NF may be added for pH adjustment.

PHARMACIA INC
COLUMBUS, OHIO 43216

774030395

25 mL SINGLE DOSE VIAL

0.167 Molar (M+)

Isotonic

For Drug Diluent Use Only
Not for use in the treatment of Lactic Acidosis

CAUTION: Federal law prohibits dispensing without prescription

Pharmacia

Store at controlled room temperature 15° to 30°C (59° to 86°F)

Lot No

Exp

4-17/32"

1-5/8"

NDC 0013 8725-89

ZINECARD™
(dexrazoxane for injection)

500 mg

STERILE, PYROGEN-FREE LYOPHILIZATE

FOR INTRAVENOUS USE ONLY

CAUTION Federal law prohibits dispensing without prescription

SINGLE DOSE VIAL

Pharmacia

USUAL DOSAGE: Before administering, read package insert for complete prescribing and product information

Each vial contains dexrazoxane hydrochloride equivalent to 500 mg dexrazoxane. The pH is adjusted with Hydrochloric Acid NF

772050495

Store at controlled room temperature, 15° to 30°C (59° to 86°F). Upon reconstitution with 50 mL of 0.167 M (M/6) Sodium Lactate Injection, USP, the pH of the resultant solution is 3.5 to 5.5. Reconstituted solutions are stable for 6 hours at controlled room temperature or under refrigeration, 2° to 8°C (36° to 46°F). Discard unused solutions.

PHARMACIA INC
COLUMBUS, OHIO 43216

Lot No

Exp

ZINECARD™
(dexrazoxane for injection)
NDA 20-212
500 mg Vial Label
Part # 772050495

NDC 0013-8725-89

ZINECARD™

(dexrazoxane for injection)

with 0.167M (M/6) Sodium Lactate Injection, USP

500 mg

USUAL DOSAGE: Before administering read package insert for complete prescribing and product information.

Store at controlled room temperature, 15° to 30°C (59° to 86°F)

Upon reconstitution with 50 mL of 0.167M (M/6) Sodium Lactate Injection, USP, the pH of the resultant solution is 3.5 to 5.5. Reconstituted solutions are stable for 6 hours at controlled room temperature or under refrigeration, 2° to 8°C (36° to 46°F). Discard unused solutions.

This package also contains one 50 mL vial of 0.167M (M/6) Sodium Lactate Injection, USP, as a diluent.

SINGLE DOSE VIAL

Pharmacia

PHARMACIA INC
COLUMBUS, OHIO 43216

772090495

NDC 0013-8725-89

ZINECARD™

(dexrazoxane for injection)

with 0.167M (M/6) Sodium Lactate Injection, USP

500 mg

STERILE, PYROGEN-FREE LYOPHILIZATE

FOR INTRAVENOUS USE ONLY

Each vial contains dexrazoxane hydrochloride equivalent to 500 mg dexrazoxane. The pH is adjusted with Hydrochloric Acid, NF.

CAUTION: Federal law prohibits dispensing without prescription.

SINGLE DOSE VIAL

Pharmacia

ZINECARD™
(dexrazoxane for injection)
NDA 20-212
500 mg Carton
Part # 772090495

4-17/32"

1-5/8"

NDC 0013 6916 40

SODIUM LACTATE INJECTION, USP

Each mL contains 18 6 mg of sodium lactate
Sodium Hydroxide, NF and/or Hydrochloric Acid, NF may be added for pH adjustment

50 mL SINGLE DOSE VIAL

0 167 Molar (M/6)
Sterile
For Drug Diluent Use Only
Not for use in the treatment of Lactic Acidosis
CAUTION: Federal law prohibits dispensing without prescription

Store at controlled room temperature, 15° to 30°C (59° to 86°F)

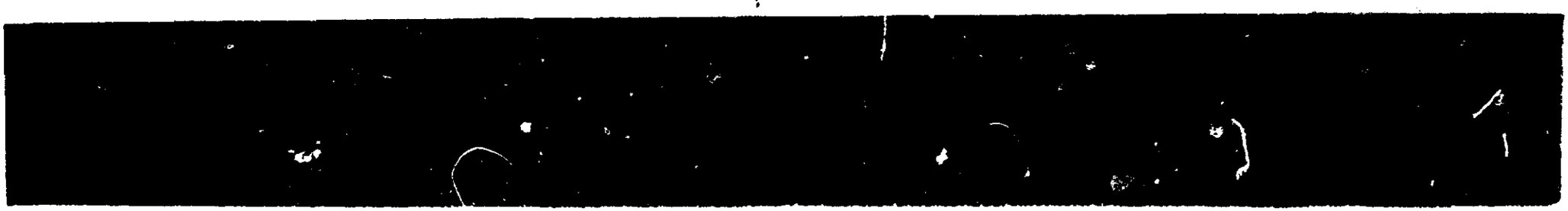
PHARMACIA INC
COLUMBUS, OHIO 43216

Lot No
Exp

774050395

Pharmacia

SODIUM LACTATE
INJECTION, USP
NDA 20-212
50 mL Vial Label
Part # 774050395



DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-212 **DATE REVIEWED:** 2/9/95

REVIEW #: 4 **REVIEWER:** Eva Tolgyesi, Ph.D.

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE
AMENDMENT (BC)	2/02/95	2/03/95

NAME & ADDRESS OF APPLICANT:
Pharmacia Inc.
7001 Post Road
Dublin, OH 43017

MAILING ADDRESS OF APPLICANT:
P.O. Box 16529
Columbus, OH 43216

DRUG PRODUCT NAME
Proprietary: ZINECARD for Injection
Established: Dexrazoxane for Injection
Code Name/#: ADR-529, ICRF-187, NSC-169780
Chem.Type/Ther.Class: 1P

PHARMACOL. CATEGORY/INDICATION: Intracellular chelating agent, cardioprotectant for use in conjunction with doxorubicin

DOSAGE FORM: Sterile lyophilized powder for injection, supplied with a vial of M/6 Sodium Lactate Injection, USP

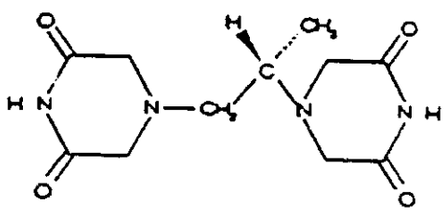
STRENGTHS: 250 mg, 500 mg

ROUTE OF ADMINISTRATION: Intravenous

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA:

Chemically, dexrazoxane is (S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione. The structural formula is as follows:



$C_{11}H_{16}N_4O_4$ M.W. 268.28

CONSULTS:

Environmental Assessment report was prepared by A. Mukherjee, Ph.D. (HFD-102) on 10/21/94. Pharmacia's response to the deficiencies listed in that report (2/2/95 amendment) is under review in HFD-102.

Microbiology Consult Report #3 by Paul Stinavage, Ph.D., dated 1/30/95 judged the application approvable, pending on the Applicant's commitment to provided addtional test data past approval.

ESTABLISHMENT EVALUATION REQUESTS:

The CGMP status of the manufacturing facilities was judged **acceptable** by Compliance on 12/15/94 and 1/25/95. The Chemist requested on 2/9/95 a new "60-day update".

REMARKS:

CONCLUSIONS & RECOMMENDATIONS:

The submitted amendment addressed all chemistry deficiencies in a satisfactory manner. Thus the NDA is **APPROVABLE** pending on:

a. an acceptable ENVIRONMENTAL ASSESSMENT REPORT and a FONSI being issued by Dr. Phil Vincent, Environmental Assessment Officer, HFD-102;
b. revised package insert.

Eva Tolgyesi, Ph.D. 2/9/95

cc:
Org. NDA 20-212
HFD-150/Division File
HFD-150/ETolgyesi
HFD-150/CHoiberg
HFD-151/MPelosi
R/D Init by:
File: C:\WPFILES\N20212R4.000

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-212

DATE REVIEWED: 1/23/95

REVIEW #: 3

REVIEWER: Eva Tolgyesi, Ph.D.

SUBMISSION TYPE	DOCUMENT DATE
AMENDMENT (BL)	12/29/94
AMENDMENT (BZ)	1/06/95

NAME & ADDRESS OF APPLICANT:

Pharmacia Inc.
7001 Post Road
Dublin, OH 43017

MAILING ADDRESS OF APPLICANT:

P.O. Box 16529
Columbus, OH 43216

DRUG PRODUCT NAME

Proprietary: ZINECARD for Injection
Established: Dexrazoxane for Injection
Code Name/#: ADR-529, ICRF-187, NSC-169780
Chem.Type/Ther.Class: 1P

PHARMACOL. CATEGORY/INDICATION: Intracellular chelating agent, cardioprotectant for use in conjunction with doxorubicin

DOSAGE FORM: Sterile lyophilized powder for injection, supplied with a vial of M/6 Sodium Lactate Injection, USP

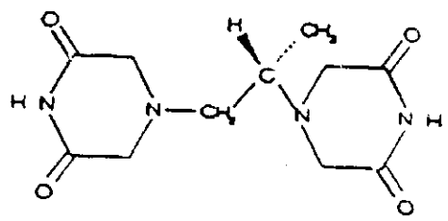
STRENGTHS: 250 mg, 500 mg

ROUTE OF ADMINISTRATION: Intravenous

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA:

Chemically, dexrazoxane is (S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione. The structural formula is as follows:



$C_{11}H_{16}N_4O_4$ M.W. 268.28

CONSULTS:

Statistical Consult Request (HFD-715) had been filed on 8/30/94 and a report was provided on 12/19/94 by Roswitha E. Kelly, evaluating the stability data.

Environmental Assessment report was prepared by A. Mukherjee, Ph.D. (HFD-102) on 10/21/94. The deficiencies were faxed to the Applicant on 10/1/94 and have not been addressed yet by the Applicant.

Microbiology Consult Report (by Paul Stinavage, Ph.D., dated 12/1/94) identified several deficiencies. The Applicant's response to these issues (submitted on 1/6/94) is being evaluated by the Microbiologist.

ESTABLISHMENT EVALUATION REQUESTS:

The CGMP status of all manufacturing facilities was judged **acceptable** by Compliance on 12/15/94.

REMARKS:

CONCLUSIONS & RECOMMENDATIONS:

The information submitted in the amendments is acceptable, with the exception of a few minor deficiencies pertaining to the regulatory specifications for the drug product and labeling (see attached DRAFT LETTER TO APPLICANT, CHEMIST'S PART). The CSO should communicate these deficiencies to the Applicant. Thus the application is still incomplete and inadequate under 505(b)(1)(D) of the Federal Food, Drug and Cosmetic Act. Non-approval is recommended, until these deficiencies as well as those pertaining to microbiology (sterility assurance) and the Environmental Assessment Report had been adequately addressed.

Eva Tolgyesi, Ph.D.
Review Chemist

1/23/95

cc:
Org. NDA 20-212
HFD-150/Division File
HFD-150/ETolgyesi
HFD-150/CHoiberg
HFD-151/MPelosi
R/D Init by:
File: C:\WPFILES\N20212R3.000

1/24/95

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-212 **DATE REVIEWED:** 11/8/94

REVIEW #: 2 **REVIEWER:** Eva Tolgyesi, Ph.D.

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
AMENDMENT (AZ)	8/2/94	8/5/94	8/8/94
AMENDMENT (BC)	9/22/94	9/23/94	9/23/94
AMENDMENT (AC)	9/27/94	9/29/94	9/29/94

NAME & ADDRESS OF APPLICANT:
Pharmacia Inc.
7001 Post Road
Dublin, OH 43017

MAILING ADDRESS OF APPLICANT:
P.O. Box 16529
Columbus, OH 43216

DRUG PRODUCT NAME
Proprietary: ZINECARD for Injection
Established: Dexrazoxane for Injection
Code Name/#: ADR-529, ICRF-187, NSC-169780
Chem.Type/Ther.Class: 1P

PHARMACOL. CATEGORY/INDICATION: Intracellular chelating agent, cardioprotectant for use in conjunction with doxorubicin

DOSAGE FORM: Sterile lyophilized powder for injection, supplied with a vial of M/6 Sodium Lactate Injection, USP

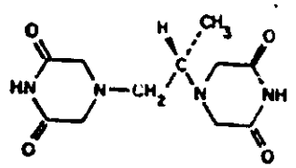
STRENGTHS: 250 mg, 500 mg

ROUTE OF ADMINISTRATION: Intravenous

Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA:

Dexrazoxane [*990*] (dex ray zoks' ane). $C_{11}H_{16}N_4O_4$. 268.27. (1) 2,6-Piperazinedione, 4,4'-(1-methyl-1,2-ethanediyl)bis-, (*S*)-; (2) (+)-(*S*)-4,4'-Propylenedi-2,6-piperazinedione. *CAS-24584-09-6.* INN. *Cardioprotectant.* (Adria) ✦*ADR-529; ICRF-187; NSC-169780*



SUPPORTING DOCUMENTS:

Drug Master Files:

CONSULTS:

Statistical Consult Request (HFD-715) was filed on 8/30/94. No report has been received yet.

Environmental Assessment Consult report was provided by A. Mukherjee, Ph.D. (HFD-102) on 10/21/94. The deficiencies were faxed to the Applicant on 10/1/94.

Microbiology Consult Request (HFD-160) was filed on 9/30/94.

ESTABLISHMENT EVALUATION REQUESTS:

Filed on 9/15/94 and 10/13/94.

REMARKS:

CONCLUSIONS & RECOMMENDATIONS:

Most of the information submitted in the amendments is acceptable, however, there are a few remaining issues, which need to be addressed. Thus the application is still incomplete and inadequate under 505(b)(1)(D) of the Federal Food, Drug and Cosmetic Act. Non-approval is recommended. Draft Letter, Chemist's Part is attached to this review.

Eva Tolgyesi, Ph.D.
Review Chemist
11/8/94

cc:
Org. NDA 20-212
HFD-150/Division File
HFD-150/ETolgyesi
HFD-150/CHoiberg
HFD-151/MPelosi
R/D Init by:
F/T by:
File: C:\WPFILES\N20212R2.000

Cutter

JUN 18 1992

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-212 **DATE REVIEWED:** 5/27/92

REVIEW #: 1 **REVIEWER:** Eva Tolgyesi, Ph.D.

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	2/7/92	2/10/92	2/19/92
AMENDMENT	3/23/92	3/24/92	3/25/92

NAME & ADDRESS OF APPLICANT:
Adria Laboratories
Division of Erbamont, Inc.
P.O. Box 16529
Columbus, Ohio, 43216

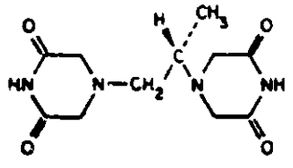
DRUG PRODUCT NAME
Proprietary: ZINECARD for Injection
Established: Dexrazoxane for Injection
Code Name/#: ADR-529, ICRF-187, NSC-169780
Chem.Type/Ther.Class: 1A

PHARMACOL. CATEGORY/INDICATION: Intracellular chelating agent, cardioprotectant for use in conjunction with doxorubicin

DOSAGE FORM: Sterile lyophilized powder for injection supplied with a vial of M/6 Sodium Lactate Injection, USP
STRENGTHS: 250 mg, 500 mg
ROUTE OF ADMINISTRATION: Intravenous
Rx/OTC: Rx

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOLECULAR WEIGHT:

Dexrazoxane [*1990*] (dex ray zoks' ane). $C_{11}H_{16}N_4O_4$. 268.27. (1) 2,6-Piperazinedione, 4,4'-(1-methyl-1,2-ethanediyl)bis-, (*S*)-; (2) (+)-(*S*)-4,4'-Propylenedi-2,6-piperazinedione. *CAS-24584-09-6*. INN. *Cardioprotectant.* (Adria) ✦*ADR-529; ICRF-187; NSC-169780*



SUPPORTING DOCUMENTS:

Drug Master Files:

CONSULTS:

Statistical Consult Request (HFD-715) filed on 3/26/92.
Microbiology Consult Request (HFD-160), filed on 4/13/92.
Environmental Assessment Consult Request (HFD-102) filed on 3/26/92.
Request for Trademark Review was filed on 3/23/92. The Labeling and Nomenclature Committee found the ZINECARD trademark acceptable (4/28/1992).

ESTABLISHMENT EVALUATION REQUESTS were filed on 4/27/92.

REMARKS:

CONCLUSIONS & RECOMMENDATIONS:

The application is incomplete and inadequate under 505(b)(1)(D) of the Federal Food, Drug and Cosmetic Act.

Non-approval is recommended. Draft Letter, Chemist's Part is attached to this review.

Method Validation will be initiated at a later date, after the specifications and tests have been finalized and appropriate Methods Validation Packages have been provided by Adria Laboratories.

cc:
Org. NDA 20-212
HFD-150/Division File
HFD-150/ETolgyesi
HFD-150/JBlumenstein
HFD-151/ECutler
HFD-102/CKumkumian
R/D Init by: 6/8/92
F/T by:
File: B: N20212.CR1

Eva Tolgyesi, Ph.D. 5/27/92
Review Chemist

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

Zinecard®

(dexrazoxane for Injection)

NDA 20-212

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

FINDING OF NO SIGNIFICANT IMPACT
NDA 20-212
ZINECARD (Dexrazoxane for Injection) 250 and 500 mg VIALS

The Food and Drug Administration Center for Drug Evaluation and Research 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, Pharmacia Inc. (formerly called Adria Laboratories) has prepared an abbreviated environmental assessment (21 CFR 25.3la(b)(3)) (attached) which evaluates the potential environmental impacts of the manufacture and use of ZINECARD 250 and 500 mg vials.

Dexrazoxane, a designated orphan drug, is indicated for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration in women with metastatic breast cancer who have received a cumulative doxorubicin dose of 300 mg/m^2 and who, in their physician's opinion, would benefit from continuing therapy with doxorubicin.

Dexrazoxane is chemically (S)-4,4'-(1-methyl-1,2-ethanediyl)bis[2,6-piperazinedione], having a molecular weight of 268.28. It is sparingly soluble in water at pH values typical of environmental samples and slightly soluble in alcohol. The partition coefficient is of the order of 10^3. The pKa values are 2.1, 10.1 and 11.1. The firm has provided a list of impurities and substances introduced into the environment which were determined to be confidential business information.

The firm has provided extensive information and documentation from the Italian officials responsible for environmental pollution prevention at the point source of manufacture of the drug substance. The firm has provided extensive documentation and information regarding environmental pollution prevention at the point source of manufacture of the drug product in including copies of environmental permits, etc., from Federal, State and Local Authorities. Review of the documentation for those sites by FDA resulted in the conclusion that proper pollution prevention measures have been taken.

FDA has determined that environmental fate and effect data are not required as ZINECARD is intended for the treatment of a rare disease or for similarly infrequent use.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured and used without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the drug substance and drug product are expected to minimize occupational exposures and environmental release. Any residues of dexrazoxane or its major metabolites entering the environment as a result of administering the drug to humans are expected to rapidly degrade.

4/19/95 — Steven R. Koepke, Ph.D., Review Chemist
Date

4/19/95 — Eva Tolgyesi, Ph.D., Supervisory Chemist
Date

4/19/95 — Phillip G. Vincent, Environmental Assessment Officer
Date

4/20/95 — Robert A. Jeffussi, Associate Director of Chemistry, CDER
Date

Attachments: Environmental Assessment for Dexrazoxane
Material Safety Data Sheet
Final draft labeling

REDACTIONS MADE
BY APPLICANT

ENVIRONMENTAL ASSESSMENT

Dexrazoxane for Injection

(FOI releasable version)

Prepared for:

Pharmacia Inc.
(formerly Adria Laboratories)

7001 Post Road

Dublin, Ohio 43017

REDACTIONS MADE
BY APPLICANT

CONTENTS

SUMMARY

This Environmental Assessment (EA) has been prepared by Dames & Moore, and revised by Pharmacia Inc. (formerly Adria Laboratories) as part of the New Drug Application (NDA) submitted by Pharmacia for Dexrazoxane for Injection. This product has received designation as an orphan drug. The purpose of the EA is to supply information that will allow the Food and Drug Administration (FDA) to implement the provisions of the National Environmental Policy Act of 1969 (NEPA). This Act (NEPA) requires the FDA to identify actions that may significantly affect the quality of the human environment (21 CFR 25.1(b)(1)). In this EA, the manufacture, distribution, and use of the aforesaid new product are examined from the perspective of resource utilization, environmental releases, and ultimate disposition of the drug substance and all related materials.

As directed in 21 CFR 25.1(b)(3), the approach utilized for preparation of this EA was to identify and then focus on significant issues that will determine the environmental impact of the proposed action. In doing so, it was ascertained that resource utilization during manufacture and distribution of this new drug product will require no irreversible or irretrievable commitment. All raw materials are commodity chemicals. Wastes generated during manufacturing of the bulk drug substance in Italy will be (1) incinerated, (2) sent as hazardous waste to a land fill, or (3) biologically degraded in an onsite treatment plant. These three alternatives are regulated by Italian governmental agencies. Wastes from the manufacturing of the drug product in the United States will be destroyed in EPA-permitted incinerators. Releases of dexrazoxane to the environment will occur only when it is injected and subsequently eliminated. Approximately Confidential of this eliminated material should consist of metabolic degradation products. These releases would be distributed to sewage treatment facilities throughout the United States.

After being released to sewage treatment facilities, dexrazoxane and its metabolites would be hydrolyzed to 1,2-propylenediaminetetraacetic acid (1-methyl-EDTA). This hydrolysis can occur both enzymatically and nonenzymatically, the enzymatic hydrolysis being catalyzed by the extracellular hydrolases that are released by the sludge microflora. The 1-methyl-EDTA, produced by this hydrolysis, would then be released to surface waters that received the effluent.

Other environmental releases of 1-methyl-EDTA arise from its use in photoprocessing and in analytical chemistry. Although the amount of 1-methyl-EDTA used for these purposes is uncertain, its structural homolog, ethylenediaminetetraacetic acid (EDTA), is a major commercial chemical in the United States with an annual production (including sodium salts) that exceeds 33,000 metric tons. The structure of 1-methyl-EDTA differs from the structure of EDTA by only 1 methylene group.

The commercial products in which EDTA is present include laundry detergents, food preparations, photographic chemicals, cosmetics, pharmaceuticals, and fertilizers. The environmental fate and effects of EDTA have been extensively studied and reviewed. As instructed in the Environmental Technical Assistance Handbook (FDA/CFSAN-87/30), the available information on this structurally similar chemical is utilized (wherever appropriate) to predict the environmental fate of dexrazoxane. This was also the methodology used for constructing the fate-and-transport scenarios published in Water-Related Environmental Fate of 129 Priority Pollutants (EPA 440/4-79-029ab).

The environmental processes that could affect the fate and transport of 1-methyl-EDTA in surface water are presented in Table S-1. In surface water, the principal process for degradation of the 1-methyl-EDTA would be photolysis of the metal complexes it formed with dissolved ferric iron. Because photolysis in surface water is a significant degradative process only at shallow depths, the rate determining factors for this degradation would be (1) the availability of sunlight, (2) the mixing of surface water with water from lower depths, and (3) the upward diffusion of metal complexes from these depths. Very slow biodegradation would also contribute to the breakdown of 1-methyl-EDTA. The half-life for the loss of 1-methyl-EDTA from surface water would be similar to the half-life associated with EDTA, estimated to vary from 2 days to 2 months, depending on the season.

If dexrazoxane, its metabolites, or their hydrolysis products are present in sewage sludge that is land-filled or land-farmed, their slow biodegradation would be expected. This biodegradation would proceed via hydrolysis to 1-methyl-EDTA, which would then degrade finally to carbon dioxide, water, and nitrogen oxides.

Atmospheric processes are not relevant to the environmental fate of dexrazoxane and its metabolites. These materials are released only to sewage treatment facilities, and their volatilization from water to the atmosphere is not an operable environmental process. In sewage effluent

and surface water, dexrazoxane, its metabolites, and their further hydrolysis products would all be present as non-volatile, ionic complexes of metal cations.

The anticipated levels of 1-methyl-EDTA in the aquatic environment are several orders of magnitude below all reported toxic effect levels for EDTA. The lowest toxicity value for EDTA is the Toxicity Threshold for green algae of 11 mg/L. Even if it is assumed that sewage effluent is released directly to the environment without dilution, the expected concentration of 1-methyl-EDTA would be much lower than this lowest toxicity level. Therefore, release of 1-methyl-EDTA to the environment via treated sewage should pose no adverse risk to aquatic flora and fauna.

TABLE S-1

Summary of Environmental Fate and Transport of Dexrazoxane

Environmental Process	Summary Statement	Confidence of Data[a]
Photolysis	Based on the photolytic behavior of Fe(+3)-EDTA complexes, photolysis is probably the major process for degradation of dexrazoxane and its metabolites in surface waters.	Medium
Oxidation	Environmental oxidation of dexrazoxane and its metabolites probably occurs, but the process is too slow to be significant.	Medium
Hydrolysis	Hydrolysis is the principal process for breakdown of dexrazoxane and its metabolites to 1-methyl-EDTA.	High
Volatilization	This process is not relevant to the transport of dexrazoxane or its metabolites, because these chemicals will exist as nonvolatile complexes in the environment.	High
Sorption	The calculated distribution coefficient, K_d, indicates that adsorption to environmental adsorbents is unlikely. Moreover, similarly structured EDTA is known to be unadsorbed by sludge or sediment.	High
Bioaccumulation	If dexrazoxane (or its metabolites) became available to aquatic organisms, the estimated bioconcentration factor ( ) indicates that bioconcentration and bioaccumulation would not be expected.	High
Biodegradation	Based on the biodegradation of EDTA, it is expected that 1-methyl-EDTA--the hydrolysis product of dexrazoxane and its metabolites--will slowly be degraded by the microflora of the aquatic and terrestrial environments.	Medium

[a]Levels of confidence are based on criteria discussed in EPA 440/4-79-029ab. 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.

1. Date

December 16, 1991 (revised March 23, 1995 to incorporate responses to FDA questions, submitted in the NDA Amendment dated 2/2/95)

2. Name of Applicant

Pharmacia Inc. (formerly Adria Laboratories)

3. Address

7001 Post Road
Dublin, Ohio 43017

4. Description of the Proposed Action

4.1 Requested approval

The proposed action encompasses synthesis, dosage formulation, and use of the new drug product known as Dexrazoxane for Injection. The drug substance is referred to as dexrazoxane (Figure 4-1). The product has received designation as an orphan drug because the patient population that would use dexrazoxane is much less than Confidential. A copy of the Orphan Drug Approval letter is given in Appendix A-1.

The format of this Environmental Assessment (EA) is arranged as required by 21 CFR 25.31(a). Supporting documents for the items discussed in this EA have been organized as appendices in Section 16. The cited literature also accompanies the EA.

4.2 Need for Action

Dexrazoxane demonstrates statistically significant cardioprotection in combination chemotherapy with ADRIAMYCIN. Thus, with use of dexrazoxane, a patient population for which ADRIAMYCIN is indicated can receive treatment with a decreased risk of toxic symptoms.

4.3 Locations of Production

Two locations are relevant to the manufacturing of dexrazoxane (Appendices B and C). The bulk drug substance will be synthesized in Confidential by Pharmacia S.p.A. (formerly Farmitalia Carlo Erba), Strada Rivoltana, Rodano (Milan), Italy. After its synthesis, the purified drug substance will be shipped to the United States for preparation as a lyophilized product in sealed vials. The latter process will be carried out at Pharmacia Inc. Oncology Division (formerly Adria-SP), 4272 Balloon Park Road NE, Albuquerque, New Mexico 87109.

FIGURE 4-1
Synthesis of Dexrazoxane

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Confidential Information

4.3.1 Bulk Drug Substance

The chemical reactions that lead to dexrazoxane are depicted in Figure 4-1. Detailed directions for the Confidential are provided in NDA Section No. 3A-Drug Substance, Paragraph 3. As a first step, ...

Confidential Information

..... step.

4.3.2 Drug Product

Dexrazoxane will be prepared as a lyophilized product in sealed vials at Pharmacia Inc. Oncology Division's facility in Albuquerque, New Mexico. Manufacturing information relevant to this operation is presented in NDA Section No. 3B-Drug Product, Paragraph 5. The market forecast for this product is provided in Appendix C-1.

4.4 Locations of Use and Disposal

As prescribed medication, this drug would be administered to and eliminated with its metabolites by patients throughout the United States. The amount that is eliminated, therefore, would enter municipal treatment systems throughout the United States. Unused vials (past the expiration date) which are returned to Pharmacia Inc. Oncology Division (Albuquerque) for transport to EPA-permitted incinerators (Rollins Environmental Services, Baton Rouge, LA and/or ENSCO Inc., El Dorado, AR). Solid waste generated at Pharmacia Inc. Oncology Division would also be transported to the same EPA-permitted incinerators. Street addresses and permit numbers of the latter disposal contractors are provided in Appendix C-1. Copies of the permits are provided in Appendix C-2.

4.5 Environmental Setting of Facilities

4.5.1 Pharmacia S.p.A. (formerly Farmitalia Carlo Erba) (FICE)

The facility is set in an industrial zone located in the Regione Lombardia, Provincia di Milano (Appendix B-1). It forms, with several other chemical production plants, the area known locally as the "Chemical Pole" of Pioltello, Limito and Rodano. Residential development is present

near these manufacturing facilities as well as in adjacent Pobbiano. A large park, Villa Invernizzi, is also nearby, but there is no access to it from the industrial area.

Eight wells, drawing from a depth of 27 to 40 meters, serve as the manufacturing source of non-potable water. Drinking water is supplied by the municipal aqueduct. The facility has (onsite) a complete wastewater treatment plant. Discharge of the effluent to a tributary of the Po River is monitored for constituent limitations, as prescribed by Italian law.

4.5.2 Pharmacia Inc. Oncology Division

The two-story facility is set in the Balloon Field Industrial Park in northeast Albuquerque (Appendix C-1). The site is supplied with public utilities, including natural gas, electricity, water, and sanitary sewers. Surrounding facilities consist of offices for businesses and light manufacturing. Most of the Pharmacia Inc. Oncology Division property is covered by the facility structure and its asphalt parking lot. Landscaped areas to the east and vacant land to the west show no signs of environmental stress. An electronics firm directly east of Pharmacia Inc. Oncology Division uses large quantities of solvents that have contaminated the groundwater beneath its property. None, however, has been detected at Pharmacia Inc. Oncology Division.

Prior to its development as an industrial park, the site and adjoining areas were under cultivation. The soils are classified as gravelly fine sandy loam. The depth to groundwater is 180 feet below the surface. Six city wells are located less than one mile to the south. The Rio Grande River is approximately 0.5 mile west of the facility. Storm water is channeled to an arroyo that feeds into the river. The climate is classified as arid continental; average precipitation is 8 inches.

5. Identification of Chemical Substances that Are Subject to This Proposed Action

5.1 Chemical Substances Used in the Manufacture of the Bulk Drug Substance

Specifications for starting materials, reagents, solvents and auxiliary materials are presented in NDA Section 3A, Paragraph 3, pages 2, and 3-8.

5.2 Drug Substance

5.2.1 Chemical Names

A. (S)-4,4'-(1-methyl-1,2-ethanediyl)bis[2,6-piperazinedione]

B. (+)-(S)-4,4'-propylenedi-2,6-piperazinedione

5.2.2 Established Name

Dexrazoxane (USAN)

5.2.3 Other Names

ADR-529
ICRF-187
NSC-169780

5.2.4 CAS Registry Number

24584-09-6

5.2.5 Molecular Formula

C_{11} H_{16} N_4 O_4

5.2.6 Physical Description

Dexrazoxane is a white to off-white crystalline powder. Its chemical and physical properties are listed in Table 5-1. Density and vapor pressure are not listed for dexrazoxane in Table 5-1 because these properties do not affect the fate and transport of this drug substance or its degradation products.

Density is a colligative property of substances rather than a molecular one. Releases of dexrazoxane and its degradation products will always be at concentrations well below maximum solubility and thus separate, solid phases of these substances will not occur in the environment.

Vapor pressure was not determined because dexrazoxane is a strong chelator of iron (Fe^{+3}) and other environmental cations (Appendix D, Section D.3.4). The resulting coordination complexes are ionic and, therefore, will not volatilize from water or soil.

The log soil adsorption coefficient and the log bioconcentration factor were calculated from the solubility rather than from direct measurements because (1) dexrazoxane will have been hydrolyzed to its degradation products before it is released to surface water (Appendix D,

TABLE 5-1

Chemical and Physical Properties of Dexrazoxane

Molecular formula	$C_{11}H_{16}N_4O_4$
Molecular weight	268.28
Melting point(a)	187-197° C
Solubility in water(a)	12,000 mg/L
Density(b)	ND
Vapor pressure(c)	ND
Log soil adsorption coefficient(d)	1.40
Log bioconcentration factor(d)	0.50
Dissociation constants(a)	
pK_a 1	2.1
pK_a 2	10.1
pK_a 3	11.1
Electromagnetic absorption (>290 nm)(d)	None

(a) New Drug Application, Section 3A-Drug Substance

(b) Not determined for dexrazoxane because density is a colligative property rather than a molecular property. Thus, the density of dexrazoxane (as a separate solid phase) is irrelevant to the behavior of the dissolved molecules. If environmental releases of dexrazoxane or related substances occur, they will always be in the dissolved state at concentrations well below the maximum solubility. This justification for not measuring density is substantiated by 21 CFR 25.1(b)(3).

(c) Not determined for dexrazoxane because it is a strong chelator of iron (Fe^{+3}) and other environmental cations (Appendix D, Section D.3.4). The resulting coordination complexes are ionic and, therefore, will not volatilize from water or soil. This justification for not measuring the vapor pressure is substantiated by 21 CFR 25.1(b)(3).

(d) Appendix D. The log soil adsorption coefficient and the log bioconcentration factor are estimated from the solubility using the relationships described in Lyman et al. (1982). The ultraviolet-visible absorption spectrum is discussed in Section D.3.1.

Section D.3.3) and (2) the degradation products are unlikely to be absorbed or bioconcentrated (Appendix D, Sections D.3.5 and D.3.6).

5.3 Impurities In The Drug Substance

In individual batches of the bulk drug, a small amount of related substances I and II and Intermediate II are found.

5.4 Chemical Substances Used in the Manufacture or Administration of the Drug Product

Dexrazoxane
Water
Nitrogen
Hydrochloric acid
Sodium Lactate Solution, 60%

6. Introduction of Substances into the Environment

6.1 Substances Generated

6.1.1 Synthesis of the Drug Substance

A material balance for the synthesis is provided in Table 6-1. It is based on the Description of the Synthesis, NDA Section 3A-Drug Substance, Paragraph 3, pages 11-14. The disposition of the wastestreams will be carried out as indicated in compliance with Italian environmental law. Certificates of compliance from the State and Local Authorities are provided in Appendix A-2. Applicable laws are listed in Appendix B-2 and discussed in Section 6.3.1.

Air pollution devices are described in Appendix B-1 (and Section 6.2.1) and will be operated to meet required limitations for emission. Solid wastes and chlorinated solvents will be transported and disposed of by a hazardous waste contractor. Non-chlorinated organic liquids will be incinerated onsite, as authorized for the Regione Lombardia. Aqueous wastes are treated onsite as described in Appendix B-1 (and Section 6.2.1). Effluent limitations are also listed in Appendix B-1.

In Step 1 (Table 6-1), the only materials that will be released to the environment are the *Confidential* in the wastewater effluent. *Confidential*, of course, will have undergone *Confidential*. In Step 2, *Confidential* may be released in wastewater effluent, if the sludge culture does not become adapted to them (Appendix D, Section D.3.7). Wastes from *Confidential* would not be released.

TABLE 6-1

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Confidential Information

TABLE 6-1 (cont'd)

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Confidential information

TABLE 6-1 (cont'd)

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Confidential Information

6.1.2 Preparation of the Lyophilized Product

After each production day of Dexrazoxane for Injection, excess liquids will be collected, accounted for and placed in special medical waste drums for incineration. The equipment will then be purged with a decontamination solution that will also be placed in the medical waste drums. Waste contractors and their addresses and permit numbers are listed in Appendix C-1. The equipment will be washed after purging and, during the initial washing, minute amounts of dexrazoxane could be released to the City of Albuquerque wastewater system. Solid wastes that contain dexrazoxane will be incinerated.

6.2 Controls Exercised on Residuals and Emissions

6.2.1 Pharmacia S.p.A.

Air Emissions: In the department that will be synthesizing the bulk drug substance, the existing control equipment for water-soluble organic and inorganic volatiles is a scrubber with a capacity of 12,000 m^3/hr. Water-immiscible organic chemicals will be collected with equipment from another department. This equipment consists of a vapor freezing unit (operated with brine and liquid nitrogen) followed by activated-carbon filtration cartridges. The facility incinerator for non-chlorinated liquid waste is also equipped with a scrubber (6,600 m^3/hr). Further information is given in Appendix B-1.

Wastewater: A complete biological wastewater treatment plant is operated onsite (Appendix B-1). This plant has two sections: one to reduce carbon oxygen demand and the other to treat nitrogenous waste. Nitrogen-concentrated wastestreams are processed to reduce their nitrogen content before treatment to remove residual organic carbon. The resulting wastewater is degassed and clarified before its release as effluent. Biological sludges are thickened and disposed of as solid waste. Effluent limitations are listed in Appendix B-1. Wastestreams that enter the treatment plant do not normally contain high concentrations of manufacturing chemicals. Pretreatment within each department removes most of these chemicals for onsite incineration or disposal as hazardous substances. Groundwater protection is discussed in Appendix B-1.

Solid and Nonaqueous Liquid Waste: Hazardous wastes are stored in drums or tanks in a dedicated storage area (Appendix B-1). Liquids that contain less than 2 percent chlorine are incinerated onsite at a maximum rate of 400 kg/hr. Protocols for the handling of wastes--as well as products and

raw materials--are provided for worker safety (Appendix B-2). All workers wear protective clothing and participate in ongoing safety training (Appendix B-2).

6.2.2 Pharmacia Inc. Oncology Division

Air Emissions: Preparation of the lyophilized drug product in glass vials is carried out in a closed system without airborne emissions (Appendix C-1). Moreover, personal protective equipment is utilized to prevent contact of employees with the product.

Wastewater: After its prior decontamination, the equipment will be washed. The water will be sent by sewer to the wastewater treatment plant operated by the City of Albuquerque under an existing permit (Appendix C-1).

Solid and Nonaqueous Liquid Waste: At each step in the manufacturing process, waste will be collected and contained in closable, labeled containers (Appendix C-1). The containers will then be taken to a designated collection area and manifested for incineration as a Non-RCRA Listed Special Medical Process Waste. Wastes from the decontamination of equipment are similarly managed. The contractors for incineration are Rollins Environmental Services (Baton Rouge, LA) and ENSCO Inc. (El Dorado, AR). Employee education is provided through lectures and hands-on training. Material Safety Data Sheets are attached to Appendix C-1.

6.3 Compliance of Proposed Action with Applicable Emission Requirements

6.3.1 Pharmacia S.p.A.

The facility is in compliance with applicable emission requirements. The name of the company official responsible for this compliance is provided in Appendix B-1. The relevant laws for air (Legge 615/66 and DPR 322/71) and for wastewater (Legge 319/76 and Legge 650/79) are listed in Appendix B-2. Effluent limitations for wastewater are tabulated in Appendix B-1. Summary statements of the relevant laws (in English) are provided in Appendix B-3 as well as a discussion of Italian environmental laws and regulations, the responsible governmental agencies, and their administration of these laws.

Appendix B-4 is the Memorandum of Understanding between the U.S. Environmental Protection Agency and the Ministry of Environment of Italy.

This memorandum provides for the exchange of information, sponsorship of conferences, and the initiation of joint research and development projects. The memorandum is the only environmental agreement between the United States and Italy.

6.3.2 Pharmacia Inc. Oncology Division

Pharmacia Inc. Oncology Division does not hold and has not been required to hold any air emission permits for its facility in Albuquerque. Moreover, the facility is not required to have a RCRA hazardous waste permit. Pharmacia Inc. Oncology Division, however, is a conditionally exempt large quantity generator as defined in 40 CFR 261.5. The EPA permit number is NMD982552945. The number of the wastewater discharge permit from the City of Albuquerque is 2055A-4. Copies are provided in Appendix C-2.

6.4 Effect of the Proposed Action on Compliance with Current Emission Requirements

6.4.1 Pharmacia S.p.A.

If the proposed action is approved, manufacturing of the drug substance at the facility in Rodano, Italy, would be carried out within the limitations of current permits. The Italian laws and regulations relevant to the synthesis of dexrazoxane are listed in Appendix B-2. Effluent limitations for wastewater are tabulated in Appendix B-1.

6.4.2 Pharmacia Inc. Oncology Division

At Pharmacia Inc. Oncology Division in Albuquerque, preparation of the drug product will not result in hazardous emissions or releases. Manufacturing will be conducted with existing equipment under current permits.

6.5 Amount of Dexrazoxane and Related Substances Entering the Environment

The potential routes by which dexrazoxane and related substances could enter environments of concern are (1) use and elimination of the drug by human patients in the United States and (2) release in effluent from the drug synthesis in Rodano, Italy. Preparation of the drug product in Albuquerque, New Mexico, will be conducted with only possible trace releases of the drug substance to the environment (Section 6.1.2). Essentially all of the release of dexrazoxane, its metabolites, and their degradation products will occur via human patients and will be transported to publicly owned sewage systems.

6.5.1 Human Elimination

To conservatively (i.e., maximally) estimate the amount of dexrazoxane and its metabolites at a wastewater treatment plant, it is assumed that the bulk requirement for the United States will be the amount of the drug that is used by the U.S. population. For example, in the fifth year post approval, Confidential is forecasted as the bulk requirement for dexrazoxane (The revised forecast, dated December 1994, given in Appendix C-1, for the fifth year of production is now Confidential. Therefore the following calculated amounts of emitted substances are in excess of what is now anticipated). It is also assumed (conservatively) for this calculation that the molecular weights of metabolites and degradation products are the same as the molecular weight of dexrazoxane.

The 1990 Census gives the population of the United States as 250,378,000. Typical minimum and maximum flow rates for wastewater treatment systems are set by Federal and State agencies and range from 280 to 1,500 L/person/day (Metcalf & Eddy, Inc., 1979). The drug (plus metabolites) concentration at the treatment plant can be estimated from the following equation:

$$C = \frac{(A)(10^{12} ng/kg)}{(V)(2.5 \times 10^{8} persons)(365 days/yr)}$$

where:

C = Concentration of drug (plus metabolites) at the treatment plant.

A = Mass of drug injected per year (kg/yr).

V = Volume of wastewater entering a typical treatment plant (280 to 1,500 L/person/day).

The combined concentration of dexrazoxane and metabolites estimated for a wastewater treatment plant would vary from Confidential. Because Confidential percent of injected dexrazoxane is metabolized, the concentration of unchanged drug would be about Confidential of the calculated estimate.

If no adsorption or degradation of dexrazoxane and its metabolites occurs in the wastewater treatment plant, their combined concentration as the effluent enters the receiving surface water can be calculated from the following equation:

$$C' = \frac{(C)(Q_p)}{Q_r + Q_p}$$

where:

- C' = Diluted combined concentration of dexrazoxane and metabolites in the river that receives the effluent (assuming no adsorption or degradation).
- C = Concentration of drug (plus metabolites) at the treatment plant.
- Q_p = Wastewater flow from the treatment plant (m^3/sec).
- Q_r = Surface water flow rate (m^3/sec).

Using the maximum flow rate in the wastewater system, 1,500 L/person/day, Q_p for a treatment plant serving about 1 million people is 17.3 m^3/sec. The flow rate of a typical river (e.g., Potomac River) is 10,000 m^3/sec (Linsley et al., 1975). These flow rates give a value for C' (i.e., combined concentration of drug and metabolites in the receiving surface water) of Confidential (or a dilution factor of Confidential). Using the minimum flow rate in a wastewater system, 280 L/person/day, the corresponding combined concentration of drug and metabolites in the receiving surface water would remain the same (from a dilution factor of Confidential). Because of the variations in treatment plant capacity and in the rates of surface water flow, the dilution factor at wastewater treatment plants throughout the United States may vary (depending on geographic location) from about 10^{-7} to essentially no dilution (i.e., settling ponds or intermittently dry drainage channels).

In Section 7 (and in the discussion of Appendix D), dexrazoxane and its metabolites are considered to be degraded to Confidential (Figure D-1) before leaving a sewage treatment facility. Even if some dexrazoxane survived in the sewage effluent, it would be hydrolyzed in the receiving surface water within a few days (Appendix D). Therefore, the estimated concentration that was calculated for dexrazoxane and its metabolites in surface water should be regarded as the concentration of Confidential expected near an effluent outfall.

An equivalent method for calculating concentrations of drugs released to the aquatic environment has been given in Interim Guidance to the Pharmaceutical Industry for Environmental Assessment - Compliance Requirements for the FDA (PMA, 1991). In this method, the annual production of the drug (lbs/year) is multiplied by 8.9×10^{-9} to obtain the Maximum Expected Emitted Concentration (MEEC) in ppm (or mg/L). This relationship is developed as follows:

$$ppm = (A)(B)(C)(D)(E)(F)$$

where:

A = Pounds/year of production
B = Year/365 days
C = Day-person/150 gallons
D = 1/(246 Million persons as the population of the United States)
E = Gallons/8.34 pounds
F = One million

Using Confidential as the United States requirement for dexrazoxane in the fifth year post approval, the value calculated for MEEC is Confidential. This concentration is within the range of concentrations calculated by the preceding method for a wastewater treatment plant. Subsequent dilution in surface water would occur after the effluent was released.

6.5.2 Manufacturing Effluent from Pharmacia S.p.A. (Rodano, Italy)

From Table 6-1, approximately Confidential (Figure 4-1) and Confidential could be sent by sewer to the onsite wastewater treatment plant from the synthesis of Confidential of purified dexrazoxane. During the fifth year post approval, Confidential could be discharged. The concentration of this material at the treatment plant in the fifth year can be obtained as follows:

$$C = \frac{(R)(10^{-3})\ m^3/L)(10^9\ \mu g/kg)}{(365\ days/yr)(S)}$$

where:

C = Concentration of Confidential resulting from the discharge of the manufacturing wastestream.

R = Mass of Confidential discharged per year.

S = Flow rate at the onsite treatment plant (Appendix B-1).

The calculated concentration at the treatment plant would be Confidential. However, as pointed out in Section 6.2.1, pretreatment of wastestreams removes most organic chemicals before the wastestreams are discharged. Therefore, the calculated concentration should be regarded as a worst-case concentration. If the sludge culture does not become adapted to the complete degradation of this waste material, 1-methyl-EDTA (IV) (Figure D-1) could be released in the effluent (as discussed in Appendix D). Release of the effluent to a tributary of the Po River would, of course, further dilute the concentration.

7. Fate of Emitted Substances in the Environment

Detailed information on environmental processes is provided in Appendix D, Environmental Fate and Transport of Dexrazoxane (ADR-529) and Related Substances. The presentation of these processes (in Appendix D) follows the format developed for Water-Related Environmental Fate of 129 Priority Pollutants (USEPA, 1979). The predicted concentrations of substances entering the environment as a result of the proposed action are based on these processes and on the emissions discussed under preceding Section 6, Introduction of Substances into the Environment.

7.1 Air

Atmospheric processes are not relevant to the environmental fate of dexrazoxane and its metabolites. These materials are released only to sewage treatment facilities, and their volatilization from water to the atmosphere is not an operable environmental process. In sewage effluent and surface water, dexrazoxane, its metabolites, and *Confidential* would all be present as *Confidential*.

7.2 Water

The concentration of dexrazoxane (plus its metabolites) after entering a typical sewage treatment facility has been estimated to range from about Confidential (Section 6.5). After being released to sewage treatment facilities, dexrazoxane and its metabolites would be hydrolyzed to 1,2-propylenediaminetetraacetic acid (1-methyl-EDTA)(IV) (Figure D-1). This hydrolysis would occur both enzymatically and nonenzymatically, the enzymatic hydrolysis being catalyzed by the extracellular hydrolases that are released by the sludge microflora. The 1-methyl-EDTA, produced by this hydrolysis, would then be released to surface waters that receive the effluent. The potential concentration of this degradation product in surface water near the effluent outfall is estimated to be Confidential. In urban areas where photoprocessing laboratories are located, the release of 1-methyl-EDTA in their effluent may increase this concentration (Pavlostathis et al., 1991).

In surface water, the principal process for degradation of the 1-methyl-EDTA would be photolysis of the metal complexes it formed with dissolved ferric iron. Because photolysis in surface water is a significant degradative process only at shallow depths, the rate determining factors for this degradation would be (1) the availability of sunlight, (2) the mixing of surface water with water from lower depths, and (3) the upward diffusion of metal complexes from these depths. Very slow biodegradation would also contribute to the breakdown of 1-methyl-EDTA. The half-life for the loss of 1-methyl-EDTA from surface water would be similar to the half-life associated with EDTA (V) (Figure D-1). As stated in Section D.3.1, Appendix D, the maximum half-life of EDTA in the Neckar River in Germany was calculated to vary from 2 days to 2 months, depending on the season. The products of photodegradation would be more susceptible to biodegradation.

7.3 Soil

If dexrazoxane, its metabolites, or their hydrolysis products are present in sewage sludge that is landfilled or landfarmed, slow biodegradation would be expected. This biodegradation would proceed via hydrolysis to 1-methyl-EDTA, which would then degrade initially to intermediates similar to those observed for EDTA (Table D-1, Appendix D) and finally to carbon dioxide, water, and nitrogen oxides.

8. Environmental Effects of Released Substances

As discussed in Section 7.2, complete hydrolysis of the amide bonds of dexrazoxane (or its metabolites) produces 1,2-propylenediaminetetraacetic acid (1-methyl-EDTA)(IV) (Figure D-1). The structure of this substance differs from the structure of ethylenediaminetetraacetic acid (EDTA)(V) (Figure D-1) by only 1 methylene group. EDTA (V) is a major commercial chemical and, therefore, its environmental effects have been extensively studied. Because it is expected to affect the environment in a manner almost identical to the degradation product of dexrazoxane, information on the environmental effects of EDTA has been used to evaluate the potential impact of the release of dexrazoxane.

In Section 6.5, the amount of 1-methyl-EDTA that could potentially be released to the environment was estimated from the fifth year United States requirement of Confidential of dexrazoxane (Appendix C-1). The calculated potential concentration of 1-methyl-EDTA in sewage is Confidential. Based on typical dilution levels found in North American rivers, surface water concentration of 1-methyl-EDTA in the vicinity of the sewage outfall is estimated as Confidential. These concentrations are conservative (i.e., maximal), because it is assumed that no further degradation of 1-methyl-EDTA would occur. However, as indicated in Section 7.2 and Appendix D, further degradation would indeed occur.

Many toxicity tests for EDTA have been conducted with a variety of aquatic flora and fauna. Several of the most applicable have been summarized here to provide an overview of the general levels of toxicity of EDTA, in comparison to levels of 1-methyl-EDTA expected in surface waters. Table 8-1 summarizes the results of all studies discussed.

Jancovic and Mann (1969) investigated the effects of EDTA on a large selection of aquatic fauna. Species included tube worms, two freshwater amphipods--*Crangon allmanni* Kin., and *Atremia salina* L., sandhoppers, saltwater copepods, guppies, eels, and rainbow trout. The investigators concluded that the lethal level of EDTA was greater than 200 mg/L.

Bringmann and Kuhn (1977) determined the 24-hour aqueous LC_0, LC_{50}, and LC_{100} of 173 hazardous substances for *Daphnia magna*. Lethal concentrations of EDTA were 310, 635, and 1,250 mg/L, respectively.

Bishop and Maki (1980) evaluated the potential for bioconcentration of several chemicals in order to compare the kinetic and plateau methods

TABLE 8-1

EDTA Toxicity Data

Species Tested	Test Results	Reference
Tube worms, freshwater amphipods, sandhoppers, saltwater copepods, guppies, eels, and rainbow trout	Lethal concentration of EDTA is > 200 mg/L	Jancovic and Mann, 1969
Daphnia magna	LC_0 = 310 mg/L, LC_{50} = 625 mg/L LC_{100} = 1,250 mg/L during 24-hr. test.	Bringmann and Kuhn, 1977
Bluegills	BCF approximately equal to zero.	Bishop and Maki, 1980
Freshwater fathead minnow	LC_{50} = 59.8 mg/L during 96-hr. static toxicity test.	Curtis and Ward, 1981
Bluegills	LC_{50} = 159 mg/L during 96-hr. static toxicity test.	Verschueren. 1983
Entosiphon sulcatum (protozoa)	Toxicity Threshold = 36 mg/L	Verschueren, 1983
Uronema parduczi Chatton-Lwoff (protozoa)	Toxicity Threshold = 17 mg/L	Verschueren, 1983
Scenedesmus quadricauda (green algae)	Toxicity Threshold = 11 mg/L	Verschueren, 1983
Microcystis aeruginosa (algae)	Toxicity Threshold = 76 mg/L	Verschueren, 1983
Pseudomonas putida (bacteria)	Toxicity Threshold = 105 mg/L	Verschueren, 1983

for calculating bioconcentration factors (BCFs). Duplicate tests on bluegills were conducted using short-term kinetic experiments and individual 28-day plateau tests. BCF values for EDTA were essentially zero.

In a more recent study, Curtis and Ward (1981) investigated the toxicity of 40 industrial chemicals, including EDTA, to freshwater fathead minnows. Tests were conducted using 96-hour static toxicity methods. Based on the results, EDTA was determined to have an LC_{50} of 59.8 mg/L.

The results of a number of toxicity tests conducted on aquatic flora and fauna are presented in Verscheuren (1983). Toxicity Thresholds (Cell Multiplication Inhibition Test) were provided for two types of protozoa--*Entosiphon sulcatum* (36 mg/L), and *Uronema parduczi* Chatton-Lwoff (17 mg/L); algae--*Scenedesmus quadricauda* (11 mg/L), and *Microcystis aeruginosa* (76 mg/L); and bacteria--*Pseudomonas putida* (105 mg/L). Results were also reported for one, 96-hour static toxicity test for bluegills. For the latter, an LC_{50} of 159 mg/L and a no-effect concentration level of 100 mg/L were listed.

It can be seen (by comparing the toxicity data presented in Table 8-1 to the expected concentrations in sewage and surface water) that the anticipated levels of 1-methyl-EDTA in the environment are several orders of magnitude below all reported toxic effect levels for EDTA. The lowest toxic effect level in Table 8-1 is the Toxicity Threshold for green algae of 11 mg/L. If it is assumed that sewage effluent in the United States is released directly to the environment without dilution, the maximal concentration (Confidential) of 1-methyl-EDTA is 6 orders of magnitude lower than this lowest toxicity level. At Rodano, Italy, the concentration of 1-methyl-EDTA that could be released from the onsite treatment plant would be Confidential. Again, this is a concentration almost 3 orders of magnitude lower than this toxic level. Therefore, release of 1-methyl-EDTA to the environment via sewage effluent should pose no adverse risk to aquatic flora and fauna.

9. Use of Resources and Energy

The proposed action does not require a large commitment of resources. The raw materials that are used in the manufacture of the drug substance are listed in Section 5.1. All of these materials are commercially available. Manufacturing of dexrazoxane will be a very minor activity at Pharmacia S.p.A. in Rodano, Italy. Consumption of resources and energy at

this facility should not be measurably affected. Estimated use of energy at Pharmacia Inc. Oncology Division's facility in Albuquerque, New Mexico, is provided in Appendix C-1.

Because no adverse environmental effects are expected from the degradation products of dexrazoxane, no threatened or endangered species can be affected.

The State of New Mexico does not regard any property in the nearby vicinity of Adria's facility in Albuquerque to have historical or archaeological importance (Appendix A-3).

10. Mitigation Measures

Controls on emissions at the Rodano facility are described in Appendix B-1. Controls and waste minimization at Pharmacia Inc. Oncology Division are described in Appendix C-1. Compliance of the proposed action with applicable emission requirements is discussed in Section 6.3. Occupational safe handling and spill mitigation are practiced at both facilities, as indicated in Appendices B-1 and C-1. Expired vials of dexrazoxane may be returned to Pharmacia Inc. Oncology Division for transport to an EPA-permitted incinerator. The waste contractors are listed in Appendix C-1.

11. Alternative to the Proposed Action

No potential adverse environmental impacts have been identified for the proposed action. Very little release of dexrazoxane and its metabolites to sewage systems would be expected and, before entering a surface water environment, degradation to 1-methyl-EDTA would occur. This substance is also released by the photoprocessing industry (Appendix D, Section D.2). Because no adverse environmental impacts are expected, alternatives to the proposed action are not being considered. If this drug is not produced, the statistical risk to patient populations receiving ADRIAMYCIN chemotherapy will not be reduced.

12. Preparer

Norman W. Gabel
Ph.D., Organic Chemistry, University of Chicago, 1961
M.S., Biochemistry, University of Illinois, 1957
B.S., Chemistry, University of Illinois, 1955

Twenty years experience in chemical research. Twelve years experience in environmental chemistry. Recognized as an expert on environmental fate and transport of organic chemicals in the EPA Office of Waste Programs (Enforcement) Expert Witness Database. Management and direction of a major USEPA program (EPA Contract 68-01-5791) on the assessment of exposure and risk from manufacture, distribution, use, and disposal of industrial and consumer products. Co-author (and Project Manager) of Water-Related Environmental Fate of 129 Priority Pollutants, Vol. I and II, EPA-440/4-79-029. Co-chairman and organizer of the Symposia on Characterization and Cleanup of Chemical Waste Sites, American Chemical Society, Division of Industrial and Engineering Chemistry, Part 1-Dallas, Texas, April 1989, Part 2-Washington, D.C., August 1990. Member of the Selection Committee for Environmental Science and Engineering Fellowships (Post-Doctoral and Advanced) awarded by the American Association for the Advancement of Science, Directorate for Science and Policy Programs. Resume is provided at end of Appendix D.

13. Certification

The undersigned certifies that the information presented herein and provided to Dames & Moore (preparer) by Adria Laboratories (applicant) is true, accurate, and complete to the best of our knowledge.

Signature _____ Date 12/20/91

Title Vice President, Development

Donald E. Hagman, Ph.D.

The undersigned certifies that the information presented is true, accurate, and as complete as provided to the best of the knowledge of Dames & Moore for preparation in accordance with 21 CFR 25.31(a).

Signature Norman W. Gabel Date 12-19-91

Title Senior Chemist / Principal-in-Charge

13. Certification (continued)

The undersigned certifies that the information presented in this revised Environmental Assessment (EA) for Dexrazoxane for Injection is a combination of the EA information provided in the original NDA submitted on February 7, 1992, or in the NDA amendment submitted February 2, 1995.

Signature Martin J Williamson Date 3/23/95
(Martin J. Williamson)

Title Assistant Director, Regulatory Submissions

Twenty years experience in chemical research and methods development for pharmaceuticals, pesticides, polymers and foodstuffs. Eight years experience in Regulatory Affairs including responsibility for the preparation of chemistry, manufacturing and controls sections of IND, NDA, ANDA and AADA submissions, and answering questions from Regulatory Authorities. Thirteen scientific publications. Candidate for USP Committee of Revision (1990). Professional affiliations include American Association of Pharmaceutical Scientists, Regulatory Affairs Professionals Society and Royal Society of Chemistry (UK). Received B.Sc. (Honors) in Chemistry in 1961 and Ph.D. in Chemistry in 1965, both from Exeter University (UK).

14. References

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15. Data Summary Charts and Test Results

This section not normally required for abbreviated Environmental Assessments filed for Orphan Drugs under 21 CFR 25.31a(b)(3)

16. Appendices

A-1: Letter of Approval of Orphan Drug Status for ZINECARD

A-2: Certificates for Compliance with State and Local Authority Environmental Limits for Pharmacia's Drug Substance Manufacturing Plant, Rodano, Italy

A-3: Letter from the Office of Cultural Affairs, Historic Preservation Division, State of New Mexico

A-4: Material Safety Data Sheet (MSDS) for Dexrazoxane

B-1: Environmental Information from Pharmacia S.p.A., Rodano, Italy

B-2: Environmental Laws and Regulations Pertinent to the Facility in Rodano, Italy

B-3: Environmental Laws and Regulations of Italy

B-4: Memorandum of Understanding between the U.S. Environmental Protection Agency and the Ministry of Environment of Italy

C-1: Environmental Information from Pharmacia Inc. Oncology Division, Albuquerque, New Mexico

C-2: Copies of Environmental Permits for Hazardous Material Transportation, Regulated Waste Activities, Wastewater Discharge and Incinerators used by Pharmacia Inc. Oncology Division

D: Environmental Fate and Transport of Dexrazoxane (ADR-529)

APPENDIX A-1

Letter of Approval of Orphan Drug Status for ZINECARD

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Orphan Drug Approval Letter
Confidential Information

APPENDIX A-2

Certificates for Compliance with State and Local Authority Environmental Limits for Pharmacia's Drug Substance Manufacturing Plant at Rodano, Italy

Attachment 1	Unita' Socio Sanitaria Locale N. 58 authorization for the Rodano Plant to discharge water (original language and English translation).
Attachment 2	Lombardy Region Regional Assembly authorization for the incinerator at the Rodano Plant (original language and English translation).
Attachment 3a	Pharmacia's (Farmitalia Carlo Erba's) application for authorization for the prevention of atmospheric pollution at the Rodano Plant (original language and English translation).
Attachment 3b	Erba Biochimica's application for authorization for the prevention of atmospheric pollution at the Rodano Plant (original language and English translation).
Attachment 4	Pharmacia's (Farmitalia Carlo Erba's) report to Unita' Socio Sanitaria Locale N. 58 regarding pilot plant activities (original language and English translation).

ATTACHMENT 1 (Original language)

USSL authorization for the Rodano plant to discharge waste water

BY APPLICANT

UNITA' SOCIO - SANITARIA LOCALE N. 58

U.O TUTELA DELLA SALUTE NEI LUOGHI DI LAVORO
Prot. N. 1057/89/CC/tb

RICEVUTO
-8 MAR. 1989
Risp.

M. 22.02.89

risposta alla nota N. ____

del ____

OGGETTO:

Autorizzazione allo scarico SOCIETA' ERBA BIOCHIMICA s.r.l. Richiesta del 19.12.1988 nostro protocollo 10320.

Spettabile Ditta
ERBA BIOCHIMICA s.r.l.
Via Romagnoli 6
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e p.c. Ill.mo Signor
SINDACO
del Comune di
20090 RODANO

Vista la domanda della SOCIETA' ERBABIOCHIMICA s.r.l. con Sede in MILANO - Via Romagnoli 6 - a firma del Procuratore Dirigente, Ing. A. Fossatelli, volta ad ottenere l'autorizzazione allo scarico dei reflui dell'insediamento di RODANO - Strada Rivoltana km.6/7 - nel Fontanile Gola;

Visto il parere espresso dal Servizio Igiene Pubblica, Ambientale e Tutela Salute Luoghi Lavoro;

Si autorizza la SOCIETA' ERBABIOCHIMICA s.r.l. in via provvisoria a scaricare i suoi reflui nel Fontanile Gola/Rodano nei limiti di qualità e quantità specificati nella domanda (Tabella A per le sole acque industriali);

Si ritiene comunque che il Fontanile Gola non sia recapito idoneo per reflui industriali di qualsiasi natura; ci si riserva quindi di indicare un recapito alternativo per le acque in uscita dal depuratore sulla scorta del giudizio dei Servizi Tecnologici della Provincia da noi espressamente investiti della problematica.
La Ditta è invitata a comunicare preventivamente al Servizio Igiene Pubblica, Ambientale e Tutela Salute Luoghi Lavoro di questa U.S.S.L. 58 la data di effettiva

./.

entrata in funzione del depuratore affinchè possano essere tempestivamente predisposti i controlli di Legge.

Il Presidente
(Renato Turri)

REDACTIONS MADE
BY APPLICANT

ATTACHMENT 1 (English translation)

USSL authorization for the Rodano plant to discharge waste water

REDACTED BY APPLICANT

UNITA' SOCIO - SANITARIA LOCALE N.58

Prot. N. 1657/89/GG/rb
reply to note N .....
of .....

dated 22.02.92

SUBJECT:

Authorization for waste discharge by Erba Biochimica following the application of 19.12.1988 and our protocol 10320

ERBA BIOCHIMICA s.r.l.
Via Romagnoli, 6
MILANO 20146

c.c. The Mayor,
Municipality of
RODANO 20090

Taking into consideration the application made by Erba Biochimica s.r.l. with Head Office in Milan - Via Romagnoli 6 - signed by the Legal Representative, Ing. A. Fossatelli, for the authorization for discharge of wastes from the plant at Rodano, Strada Rivoltana Km 6/7 into the stream Fontanile Gola.

Taking into consideration the opinion expressed by the Service of Public Health, Environment and Protection of Health in the Workplace.

The authorization is given for the Company Erba Biochimica s.r.l. to provisionally discharge its wastes into the stream Fontanile Gola/Rodano within the qualitative and quantitative limits specified in the Application (Table A for idustrial aqueous wastes).

It is considered however that the Fontanile Gola is not the ideal stream for industrial wastes of any nature; and the right is therefore reserved to indicate an alternative site for the waters discharged from the purification plant on the recommendations of the Provincial Technological Services which have been expressly commissioned by us to investigate the matter.

./.

REDACTED BY APPLICANT

UNITA' SOCIO - SANITARIA LOCALE N.58

The Company is requested to communicate to the Service of Public Health, Environment and Protection of Health in the Workplace of this USSL 58 before implementation, the effective date of operation of the purification plant so that the controls required by the may be carried out.

The President

(Renato Turri)

(Signed)

REDACTIONS MADE
BY APPLICANT

ATTACHMENT 2 (Original language)

Authorization from the Region of Lombardy for the incinerator at Rodano

REDACTIONS MADE
BY APPLICANT

REGIONE LOMBARDIA · LA GIUNTA ·

DELIBERAZIONE NR. W : 5458 SEDUTA DEL 12 FEB. 1991

Presidente: ~~Giuseppe GIOVENZANA~~ UGO FINETTI

Presenti gli Assessori regionali:

~~Ugo FINETTI Vice Presidente~~
Roberto BISCARDINI
Claudio BONFANTI
Vittorio CALDIROLI
Sergio CAZZANIGA
Michele COLUCCI
Luciano FORCELLINI
Serafino GENEROSO
Ferruccio GUSMINI
Giancarlo MORANDI
Andrea PARINI
Giovanni ROSSI
Piero SAROLLI
Antonio SIMONE
Giovanni VERGA
Francesco ZACCARIA

Con l'assistenza del Segretario: Giuseppe DI GRIGNO

Su proposta dell'Assessore:

Ambiente, Ecologia

OGGETTO:

L.R. 94/80, D.P.R. 915/82 e D.P.R. 203/88. Autorizzazione alla Ditta Antibioticos S.p.A. - ex Erba Biochimica S.p.A.- di Rodano (Mi), autorizzata con delibera di G.R. n° IV/36988 del 25.10.1988, per il progetto definitivo dell'impianto di termodistruzione realizzato dall'azienda e per la variazione dei quantitativi annui di rifiuti speciali e/o tossici e nocivi inceneriti, rifiuti originati esclusivamente nell'insediamento di Rodano da parte della Ditta Antibioticos e Farmitalia Carlo Erba.

ESECUTIVA
COMMISSIONE DI CONTROLLO
N° 2236 | 2662
del 26 | 03 | 1991

ATTACHMENT 2 (English translation)

Authorization for the Region of Lombardy for the incinerator at Rodano

REDACTED
BY APPLICANT

TRANSLATION OF LETTER

LOMBARDY REGION REGIONAL ASSEMBLY

DELIBERATION N V/ 5458 Date of sitting 12 FEB 1991

President: UGO FINETTI

Regional assessors present:

Roberto BISCARDINI
Claudio BONFANTI
Vittorio CALDIROLI
Sergio CAZZANIGA
Michele COLUCCI
Luciano FORCELLANI
Serafino GENEROSO
Ferruccio GUSMINI
Giancarlo MORANDI
Andrea PARINI
Giovanni ROSSI
Piero SAROLLI
Antonio SIMONE
Giovanni VERGA
Francesca ZACCARIA

With the assistance of the Secretary: Giuseppe DI GIOIA

On the proposal of the Assessor:

Environment, Ecology

SUBJECT:

Regional law 94/80, Presidential Decrees 915/82 and 203/88 Authorization for the company Antibioticos S.p.A - formerly ERBA BIOCHIMICA S.p.A. - of Rodano (MI), approved by deliberation G.R. n° IV/36988 of 25.10.1988 for the definitive project of a plant for thermodestruction prepared by the company and for an amendment of the annual quantity of hazardous/toxic wastes which may be burnt, wastes which originate exclusively from the Rodano plant from the companies Antibioticos and Farmitalia Calo Erba.

EXECUTIVE
CONTROL COMMISSION

N° 2236 / 2662
of 04/03/1991

REDACTIONS MADE
BY APPLICANT

ATTACHMENT 3a (Original language)

Rodano plant data submitted to CRIAL

REDACTED BY APL

STABILIMENTO DI RODANO
STRADA PROVINCIALE
20090 RODANO MILANO

FARMITALIA CARLO ERBA

REGIONE LOMBARDIA
LA GIUNTA
11 LUG 1989
PROTOCOLLO GENERALE

DATA 10 Luglio 1989

VS. RIF.

NS. RIF.

TEL. DIRETTO

Alla
REGIONE LOMBARDIA
SETTORE AMBIENTE ED ECOLOGIA
SERVIZIO PROTEZIONE ARIA
VIA FABIO FILZI 22
20124 MILANO

OGGETTO: DOMANDA DI AUTORIZZAZIONE AI SENSI DEL DPR N. 203 DEL 24 MAGGIO 1988 PER LA PREVENZIONE DELL'INQUINAMENTO ATMOSFERICO, EX ART. 12.

Il sottoscritto	ROSSI ING. ENRICO
nato a	Piacenza
il	24.09.1940
domiciliato per la carica in	Rodano, Via Rivoltana Km 6/7
nella Sua qualità di	Procuratore della Società Farmitalia Carlo Erba s.r.l. per le attività dello Stabilimento sito nell'insediamento produttivo di Rodano Via Rivoltana km 6/7 tel. 02/95320031
con sede legale	via Imbonati n. 24 MILANO tel. 02/69951

C H I E D E :

ai sensi dell' articolo 12 del DPR n. 203 del 24.5.1988, l'autorizzazione per l' esercizio degli impianti esistenti, siti nello Stabilimento FARMITALIA CARLO ERBA in Comune di

RODANO via Rivoltana km 6/7

./.

GRUPPO ERBAMONT

BY APPLICANT

A tal fine allega alla presente richiesta la Relazione Tecnica e 7 fascicoli comprensivi di:

- descrizione dei cicli produttivi;
- indicazioni relative alle emissioni generate ed alle tecnologie adottate per contenere l' inquinamento atmosferico;
- progetti di adeguamento delle emissioni;
- programma tempificato dei lavori di adeguamento.

Distinti saluti.

FARMITALIA CARLO ERBA S.r.l.
STABILIMENTO - RODANO

Allegati :

- Relazione Tecnica composta da 51 pagine.
- 7 Fascicoli :

- Fascicolo " 1 FICE" composto da 106 pagine
- Fascicolo " 2 FICE" composto da 78 pagine
- Fascicolo "3 FICE" composto da 160 pagine
- Fascicolo " 4 FICE" composto da 55 pagine
- Fascicolo " 5 FICE" composto da 42 pagine
- Fascicolo " 6 FICE" composto da 43 pagine
- Fascicolo " 7 FICE" composto da 38 pagine

dove la Società Farmitalia Carlo Erba s.r.l. viene indicata con la forma abbreviata FICE.

REDACTIONS MADE
BY APPLICANT

ATTACHMENT 3a (English translation)

Rodano plant data submitted to CRIAL

TRANSLATION OF LETTER

FARMITALIA CARLO ERBA

10 July 1989

To:
REGION OF LOMBARDY
DEPT OF ENVIRONMENT AND ECOLOGY
OFFICE OF AIR CONTROL
VIA FABIO FILZI 22
20124 MILANO

SUBJECT: APPLICATION FOR AUTHORIZATION ACCORDING TO PRESIDENTIAL DECREE N. 203 OF MAY 24 1988, ARTICLE 12, FOR THE PREVENTION OF ATMOSPHERIC POLLUTION.

The undersigned	ING. ENRICO ROSSI
born in	Piacenza
on the	09.24.1940
domiciled for work in	Via Rivoltana Km 6/7, Rodano
in his capacity as	Attorney for the Company Farmitalia Carlo Erba s.r.l, for the activities of the plant sited at Via Rivoltana Km 6/7, Rodano. Tel: 02/95320031
with legal Head Office at	Via Imbonati n.24, MILAN Tel: 02/69951

R E Q U E S T S :

in accordance with Article 12 of Presidential Decree n. 203, of 5.24.1988 the authorization for use of the existing equipment, sited in the FARMITALIA CARLO ERBA plant at

Via Rivoltana Km 6/7, RODANO

contd.

With this aim is attached to this request the Technical Report and 7 fascicles which include :

- description of the production work;
- data relating to the emissions generated and to the technology employed to contain atmospheric pollution;
- projects for reducing the emissions;
- time and event schedule for the implementation of the projects.

Faithfully yours.

FARMITALIA CARLO ERBA S.r.l.
RODANO PLANT

(signed) Enrico Rossi

Enclosures :

- Technical Report consisting of 51 pages.
- 7 Fascicles :

- Fascicle " 1 FICE" comprising 106 pages
- Fascicle " 2 FICE" comprising 78 pages
- Fascicle " 3 FICE" comprising 160 pages
- Fascicle " 4 FICE" comprising 55 pages
- Fascicle " 5 FICE" comprising 42 pages
- Fascicle " 6 FICE" comprising 43 pages
- Fascicle " 7 FICE" comprising 38 pages

in which the Company Farmitalia Carlo Erba is indicated in the abbreviated form as FICE.

ATTACHMENT 3b (Original language)

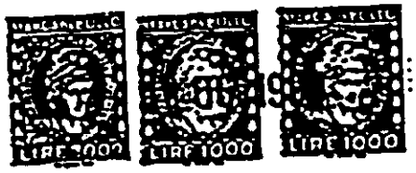
Rodano plant data submitted to CRIAL

REDAC...
BY AF...

ERBA BIOCHIMICA

ERBA BIOCHIMICA
...
...

VIA ROMAGNOLI 6
...MILANO
TELEFONO (02) 6998.1 (CENTRALINO)
TELEX 330420 LARK I POR ERBA BIOCHIMICA
TELEX 340160 SOFT I / POR ERBA BIOCHIMICA
TELEFAX (02) 6998.4400/4383

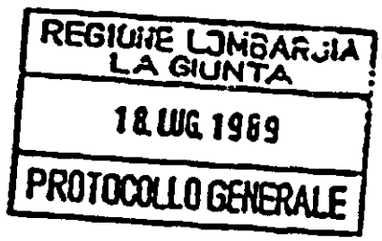


DATA 18 luglio 1989

VS. RIF.

NS. RIF.

TEL. DIRETTO.



Alla
REGIONE LOMBARDIA
SETTORE AMBIENTE ED ECOLOGIA
SERVIZIO PROTEZIONE ARIA
VIA FABIO FILZI 22
20124 MILANO

OGGETTO: DOMANDA DI AUTORIZZAZIONE AI SENSI DEL DPR N. 203 DEL 24 MAGGIO 1988 PER LA PREVENZIONE DELL'INQUINAMENTO ATMOSFERICO, EX ART. 12.

Il sottoscritto	ROSSI ING. ENRICO
nato a	Piacenza
il	24.09.1940
domiciliato per la carica in	Rodano, Via Rivoltana Km 6/7
nella Sua qualità di	Direttore di Stabilimento
dell'insediamento produttivo	ERBA BIOCHIMICA
sito nel	Comune di RODANO Via Rivoltana km 6/7 tel. 02/95320031
della Società	ERBA BIOCHIMICA S.p.A.
con sede legale	via Romagnoli n. 6 MILANO tel. 02/69981

C H I E D E :

ai sensi dell' articolo 12 del DPR n. 203 del 24.5.1988, l'autorizzazione per l' esercizio degli impianti esistenti, siti nello Stabilimento ERBA BIOCHIMICA in Comune di

RODANO via Rivoltana km 6/7

./.

ERBA BIOCHIMICA

foglio n. 2

A tal fine allega alla presente richiesta la relazione tecnica ed 11 fascicoli comprensivi di:

- descrizione dei cicli produttivi;
- indicazioni relative alle emissioni generate ed alle tecnologie adottate per contenere l' inquinamento atmosferico;
- progetti di adeguamento delle emissioni
- programma tempificato dei lavori di adeguamento;

Distinti saluti.

[illegible]

Allegati:

- Relazione Tecnica composta da 51 pagine + 43 allegati.
- 11 Fascicoli :

. Fascicolo " 1 EBC"	composto da 105 pagine	+	152 allegati
. Fascicolo " 2 EBC"	composto da 109 pagine	+	14 allegati
. Fascicolo " 3 EBC"	composto da 159 pagine	+	55 allegati
. Fascicolo " 4 EBC"	composto da 43 pagine	+	49 allegati
. Fascicolo " 5 EBC"	composto da 137 pagine	+	30 allegati
. Fascicolo " 6 EBC"	composto da 45 pagine	+	14 allegati
. Fascicolo " 7 EBC"	composto da 33 pagine	+	9 allegati
. Fascicolo " 8 EBC"	composto da 18 pagine	+	4 allegati
. Fascicolo " 9 EBC"	composto da 18 pagine		
. Fascicolo "10 EBC"	composto da 62 pagine		
. Fascicolo "11 EBC"	composto da 27 pagine		

dove la Società Erba Biochimica S.p.A. è indicata con la forma abbreviata EBC.

ATTACHMENT 3b (English translation)

Rodano plant data submitted to CRIAL

TRANSLATION OF LETTER

ERBA BIOCHIMICA

18 July 1989

To:
REGION OF LOMBARDY
DEPT OF ENVIRONMENT AND ECOLOGY
OFFICE OF AIR CONTROL
VIA FABIO FILZI 22
20124 MILANO

SUBJECT: APPLICATION FOR AUTHORIZATION ACCORDING TO PRESIDENTIAL DECREE N. 203 OF MAY 24 1988, ARTICLE 12, FOR THE PREVENTION OF ATMOSPHERIC POLLUTION.

The undersigned	ING. ENRICO ROSSI
born in	Piacenza
on the	09.24.194( )
domiciled for work in	Via Rivoltana Km 6/7, Rodano
in his capacity as	Plant General Manager
of the manufacturing facility	ERBA BIOCHIMICA
sited in	Municipality of RODANO Via Rivoltana Km 6/7. Tel: 02/95320031
of the Company	ERBA BIOCHIMICA S.p.A
with legal Head Office at	Vi Romagnoli n.24, MILAN Tel: 02/69981

R E Q U E S T S :

in accordance with Article 12 of Presidential Decree n. 203, of 5.24.1988 the authorization for use of the existing equipment, sited in the ERBA BIOCHIMICA plant at

Via Rivoltana Km 6/7, RODANO

contd.

REDACTED
BY APPLICANT

ERBA BIOCHIMICA

With this aim is attached to this request the Technical Report and the relevant attachments which include :

- description of the production work;
- data relating to the emissions generated and to the technology employed to contain atmospheric pollution;
- projects for reducing the emissions;
- time and event schedule for the implementation of the projects.

Faithfully yours.

ERBA BIOCHIMICA
RODANO PLANT
The General Manager
Ing. Enrico Rossi

(signed) Enrico Rossi

Attachments:

- Technical Report consisting of 51 pages + 43 attachments
- 11 Fascicles :

- Fascicle -	1	EBC- comprising	105 pages	+	152 attachments
- Fascicle -	2	EBC- comprising	109 pages	+	14 attachments
- Fascicle -	3	EBC- comprising	159 pages	+	55 attachments
- Fascicle -	4	EBC- comprising	43 pages	+	49 attachments
- Fascicle -	5	EBC- comprising	137 pages	+	30 attachments
- Fascicle -	6	EBC- comprising	45 pages	+	14 attachments
- Fascicle -	7	EBC- comprising	33 pages	+	9 attachments
- Fascicle -	8	EBC- comprising	18 pages	+	4 attachments
- Fascicle -	9	EBC- comprising	18 pages		
- Fascicle -	10	EBC- comprising	62 pages		
- Fascicle -	11	EBC- comprising	27 pages		

in which the Company Erba Biochimica S.p.A. is indicated in the abbreviated form as EBC.

ATTACHMENT 4 (Original language)

Report for the USSL (Local Department of Health) relevant to the Plant activities

REDACTED
BY APPLICANT

VIALE E. BEZZI 24
20146 MILANO ITALIA

TELEFONO (02) 4855 1 (CENTRALINO)
TELEX ...
C/C POSTALE N. ...
CASELLA POSTALE ...

ERBAMONT ITALIA

DATA 18 Marzo 1992

Spettabile
U.S.S.L. n. 58
U.O.T.S.L.L.
Via Don Gnocchi, 2

20064 - GORGONZOLA (MI)

VS. RIF.

NS. RIF. LM/tg

TEL. DIRETTO

Raccomandata R.R.

Oggetto : Attività del Laboratorio Pilota

Trasmettiamo, in allegato, la relazione tecnica riepilogativa delle attività del nostro La boratorio Pilota, relative al 2° semestre 1991.

La documentazione allegata alla presente riveste carattere di estrema riservatezza.

Distinti saluti.

ERBAMONT ITALIA N. V.

All. / Relazione tecnica n. 11 pagine

ERBAMONT ITALIA NV
SEDE LEGALE ROTTERDAM (OLANDA)
CAPITALE HFL ...
CAMERA DI COMMERCIO DI ROTTERDAM N. ...
SEDE AMMINISTRATIVA
VIALE E. BEZZI 24 - 20146 MILANO
TRIBUNALE MILANO RS N. ...
VOL. ... - FASC. 30 - CCIAA ...
COD. FISC. E PART. IVA N. ...

GRUPPO ERBAMONT

RISERVATO

NOTA PER UNITA' SANITARIA LOCALE N. 58 RIGUARDO LE ATTIVITA' DELL'IMPIANTO PILOTA PRESSO IL REPARTO SVILUPPO CHIMICO AREA "G" E AREA "Z" DELLO STABILIMENTO DI RODANO NEL PERIODO 2° SEMESTRE 1991.

LAVORAZIONI ESEGUITE

Nel periodo preso in considerazione sono state studiate le operazioni di sintesi e le rispett[illegible]à di conduzione relative ai seguenti prodotti:

1. ADR 529

Confidential Information

1. ADR 529

Confidential Information

1.1. ADR 529 grezzo

Confidential Information

REDACTIONS MADE BY APPLICANT

RISERVATO

1.2. ADR 529 cristallizzato

Confidential Information

ATTACHMENT 4 (English translation)

Report for the USSL (Local Department of Health) relevant to the Plant activities

REDACTED BY APPLICANT

TRANSLATION OF LETTER

FARMITALIA CARLO ERBA

March 18, 1992

To:
U.S.S.L. n. 58
U.O.T.S.L.L.
Via Don Gnocchi, 2

20064 GORGONZOLA (MI)

Registered Letter

Subject: Pilot Plant Activities

We are enclosing the technical report summarizing the activities of the Pilot Plant Department relevant to the 2nd half of the 1991 year.

The enclosed documentation has to be considered as highly confidential.

Best regards

Encl. /Technical Report - number of pages: 11

REPORT FOR THE USSL N. 58 (LOCAL DEPARTMENT OF HEALTH) RELEVANT TO THE ACTIVITIES OF THE PILOT PLANT IN THE CHEMICAL DEVELOPMENT DEPARTMENT - AREAS G AND Z - OF THE RODANO PLANT DURING THE PERIOD JULY-DECEMBER 1991.

PRODUCTION OPERATIONS CARRIED OUT

In the current period the synthesis of the following products were studied and set up:

1. ADR 529 (Dexrazoxane)

Confidential Information

REDACTIONS MADE
BY APPLICANT

1. ADR 529 (Dexrazoxane)

Confidential Information

1.1. CRUDE ADR 529

Confidential Information

1.2. ADR 529 CRYSTALS

Confidential Information

REDACTIONS MADE BY APPLICANT

APPENDIX A-3

Letter from the Office of Cultural Affairs, Historic Preservation Division, State of New Mexico

REDACTIONS MADE
BY APPLICANT

BRUCE KING
GOVERNOR

STATE OF NEW MEXICO
OFFICE OF CULTURAL AFFAIRS
HISTORIC PRESERVATION DIVISION

VILLA RIVERA, ROOM 101
228 EAST PALACE AVENUE
SANTA FE, NEW MEXICO 87503
(505) 827-6320

THOMAS W. MERLAN
DIRECTOR

HELMUTH J. NAUMER
CULTURAL AFFAIRS OFFICER

September 3, 1991

Ms. Lucy E. Archamboult, RHSP
Senior Environmental Scientist
Prindle Hinds
Environmental, Inc.
7208 Jefferson St. NE
Albuquerque, NM 87109

Dear Ms. Archamboult:

We have received a request from your office to review our site files for cultural resources reported in the area of 4272 Balloon Park Road NE in Albuquerque, New Mexico.

No known archaeological or historical resources are identified in this area. This consultation satisfies the requirements under Section 106 of the National Historic Preservation Act and its implementing regulations 36CFR800.

Thank you.

Sincerely,

for Thomas W. Merlan
State Historic Preservation Officer

TWM/DWC: 32378

RECEIVED SEP - 4 1991

REDACTIONS MADE
BY APPLICANT

APPENDIX A-4

Material Data Safety Sheet for Dexrazoxane

REDACTIONS MADE
BY APPLICANT

Pharmacia

MATERIAL SAFETY DATA SHEET

Date Prepared: March 20, 1995

The information below is believed to be accurate and represents the best information currently available. However, we make no warranty, expressed or implied, with respect to such information, and we assume no liability resulting from its use.

This material safety data sheet is intended for use by personnel who handle this material as part of their job responsibilities. It does not address the therapeutic use of this material. Information concerning the therapeutic use of this material should be obtained from formulated product package inserts and other appropriate references.

DOT Shipping Classification: NA

SECTION 1 - MATERIAL IDENTIFICATION

COMMON NAME:	Dexrazoxane or ADR 529	HAZARD DETERMINATION UNDER OSHA HAZCOM STD:	RTECS# TL6390000 Hazardous –
CHEMICAL NAME:	2,6-piperazinedione, 4,4'-(1-methyl-1,2-ethanediyl)bis-,(S)(+)		
FORMULA:	$C_{11}H_{16}N_4O_4$	CHEMICAL FAMILY:	Piperazine
USE:	Cardio-protective agent		
MANUF. NAME:	Pharmacia S.p.A. (Anthracyclines)	EMERGENCY TELEPHONE NO.:	(614) 764-8100
ADDRESS:	Strada Rivoltana Rodano (Milan), Italy		
DISTRIBUTOR:	Pharmacia S.p.A. (Anthracyclines)		
ADDRESS:	Strada Rivoltana Rodano (Milan), Italy	INFORMATION CALLS:	(614) 761-6284

SECTION 2 - HAZARDOUS COMPONENTS

PRINCIPAL HAZARDOUS COMPONENT(S) Chemical & Common Name(s)	CAS NO.	WT %	HAZARD	EXPOSURE LIMIT
Dexrazoxane	24584-09-6	99 +	NA	Undetermined

SECTION 3 - PHYSICAL/CHEMICAL CHARACTERISTICS AND FIRE/EXPLOSION HAZARD DATA

BOILING POINT: NA	MELTING PT: 191-197°C	SPECIFIC GRAVITY (H_2O=1): NA	VAPOR PRESSURE (mmHg): NA
PERCENT VOLATILE BY VOLUME (%): NA	VAPOR DENSITY (Air=1): NA	EVAPORATION RATE (H_2O=1): NA	
SOLUBILITY IN WATER:	Slightly soluble (10-12 mg/mL)	REACTIVITY IN WATER:	Hydrolyzes slowly
APPEARANCE & ODOR:	White, odorless, crystalline powder		
FLASH PT. & METHOD:	NA	FLAMM. LIM. IN AIR % BY VOL:	UPPER: NA LOWER: NA
EXTINGUISHER MEDIA:	Water or other Class A extinguishing agent	AUTO IGNITION TEMP:	NA
SPECIAL FIRE FIGHTING PROCEDURES:	Firefighters and others who may be exposed to vapors or products of combustion should wear a self-contained breathing apparatus and full protective clothing. Equipment should be thoroughly cleaned after use.		

NA = Not Applicable

REDACTIONS MADE
[illegible]

Dexrazoxane
March 20, 1995

MATERIAL SAFETY DATA SHEET

SECTION [illegible] REACTIVITY HAZARD DATA

STABILITY	UNSTABLE		CONDITIONS TO AVOID: None
	STABLE	X	

INCOMPATIBILITY (Materials to avoid): None

HAZARDOUS DECOMPOSITION PRODUCTS: None

HAZARDOUS POLYMERIZATION: Will not occur	CONDITIONS TO AVOID: None

SECTION 5 - HEALTH HAZARD DATA

PRIMARY ROUTE OF ENTRY: Inhalation, ingestion, absorption through the skin or accidental needle puncture injury.

THRESHOLD LIMIT VALUE: Undetermined

SIGNS AND SYMPTOMS OF EXPOSURE

1. ACUTE OVEREXPOSURE: Undetermined

2. CHRONIC OVEREXPOSURE: Undetermined

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE: Undetermined

CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: No

NATIONAL TOXICOLOGY PROGRAM	YES		I.A.R.C. MONOGRAPH	YES	
	NO	X		NO	X

OSHA	YES		OSHA NUISANCE DUST PERMISSIBLE EXPOSURE LIMIT:	Total 5 mg/cu.m Respirable 5 mg/cu.m
	NO	X		

ACGIH THRESHOLD LIMIT VALUE: Total 10 mg/cu.m
Respirable 5 mg/cu.m

OTHER EXPOSURE LIMIT USED: LD_{50} (i.v. - mice) > 1000 mg/kg LD_{50} (i.v. - rats) > 500 mg/kg
LD_{50} (i.v. - dogs) > 250 mg/kg

EMERGENCY FIRST AID PROCEDURES:
1. INHALATION [illegible] move from area, call a physician
2. EYES Rinse immediately with water or saline, call a physician
3. SKIN Wash with soap and water, rinse
4. INGESTION Call a physician

REDACTED BY APPLICANT

MATERIAL SAFETY DATA SHEET

SECTION 6 - CONTROL AND PROTECTIVE MEASURES

RESPIRATORY PROTECTION (Specify Type)	NIOSH/MSHA-approved for protection from aerosols.				
VENTILATION:	General	LOCAL EXHAUST:	Mechanical	MECHANICAL: (General)	NA
OTHER:	NA		SPECIAL:	NA	
EYE PROTECTION:	Standard safety glasses				
PROTECTIVE GLOVES:	Yes				
OTHER PROTECTIVE CLOTHING OR EQUIPMENT:	NA				

SECTION 7 - PRECAUTIONS FOR SAFE HANDLING AND SPILL/LEAK PROCEDURES

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING	The properties of Dexaminoxane have not been fully investigated. Use due caution in handling. Avoid contact with skin and clothing. Avoid breathing dust.
OTHER PRECAUTIONS	None
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	For spills of powder, use NIOSH/MSHA-approved dust mask. For spills of solutions, use NIOSH/MSHA-approved mask for protection. Wear standard safety glasses and gloves. For dry spills, sweep material into plastic bag and dispose of in accordance with procedures for hazardous waste disposal in most local, state, and federal regulations. For spills of solutions, swab with absorbent wipes, paper towel, cellulose sponge or the like; place absorbent material into plastic bag and dispose of in accordance with procedures for hazardous waste disposal in most local, state, and federal regulations. Scrub the surface with a detergent and rinse with water.
WASTE DISPOSAL METHODS	Deactivate with dilute sodium hydroxide solution. Dispose of in accordance with procedures for hazardous waste disposal in most local, state, and federal regulations.

APPENDICES B, C, and D

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Confidential Information

REDA
BY AP

FINDING OF NO SIGNIFICANT IMPACT
NDA 20-212
ZINECARD (Dexrazoxane for Injection) 250 and 500 mg VIALS

The Food and Drug Administration Center for Drug Evaluation and Research 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, Pharmacia Inc. (formerly called Adria Laboratories) has prepared an abbreviated environmental assessment (21 CFR 25.3la(b)(3)) (attached) which evaluates the potential environmental impacts of the manufacture and use of ZINECARD 250 and 500 mg vials.

Dexrazoxane, a designated orphan drug, is indicated for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration in women with metastatic breast cancer who have received a cumulative doxorubicin dose of 300 mg/m^2 and who, in their physician's opinion, would benefit from continuing therapy with doxorubicin.

Dexrazoxane is chemically (S)-4,4'-(1-methyl-1,2-ethanediyl)bis(2,6-piperazinedione), having a molecular weight of 268.28. It is sparingly soluble in water at pH values typical of environmental samples and slightly soluble in alcohol. The partition coefficient is of the order of 10^3. The pKa values are 2.1, 10.1 and 11.1. The firm has provided a list of impurities and substances introduced into the environment which were determined to be confidential business information.

The firm has provided extensive information and documentation from the Italian officials responsible for environmental pollution prevention at the point source of manufacture of the drug substance. The firm has provided extensive documentation and information regarding environmental pollution prevention at the point source of manufacture of the drug product in New Mexico, including copies of environmental permits, etc., from Federal, State and Local Authorities. Review of the documentation for these sites by FDA resulted in the conclusion that proper pollution prevention measures have been taken.

FDA has determined that environmental fate and effect data are not required as ZINECARD is intended for the treatment of a rare disease or for similarly infrequent use.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured and used without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the drug substance and drug product are expected to minimize occupational exposures and environmental release. Any residues of dexrazoxane or its major metabolites entering the environment as a result of administering the drug to humans are expected to rapidly degrade.

4/11/95
Date

Steven R. Koepke, Ph.D., Review Chemist

4/11/95
Date

Eva Tolgyesi, Ph.D., Supervisory Chemist

Date

Phillip G. Vincent, Environmental Assessment Officer

Attachments: Environmental Assessment for Dexrazoxane
Material Safety Data Sheet
Final draft labeling

M. pellon

JUN 30

CONSULTATIVE REVIEW TO HFD-150
DIVISION OF MEDICAL IMAGING, SURGICAL, AND DENTAL DRUG PRODUCTS
MICROBIOLOGIST'S REVIEW #4
29 June 1995

A. 1. NDA 20-212

Product Name: Zinecard (Dexrazoxane for Injection), 250 mg, 500 mg single use vial.

APPLICANT: Pharmacia Inc. Oncology Division
4272 Balloon Park Road
Albuquerque, New Mexico 87109

2. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Lyophilized powder in vial supplied with 25 mL or 50 mL vial of M/6 Sodium Lactate Injection, USP; intravenous.

3. METHOD(s) OF STERILIZATION:

4. PHARMACOLOGICAL CATEGORY AND / OR PRINCIPAL INDICATION: Zinecard for injection is indicated for the prevention of cardiomyopathy associated with doxorubicin administration.

5. DRUG PRIORITY CLASSIFICATION: 1 P

B. 1. INITIAL APPLICATION DATE: 10 April 1992

2. DOCUMENT DATE: 5 June 1995

3. DOCUMENT RECEIVED FOR REVIEW: 29 June 1995

C. REMARKS: The 5 June 1995 document addresses the applicant's commitment to provide product sterile filtration data resulting from Microbiologist's Review #3.

micro

D. CONCLUSIONS: The submission fulfills the applicant's commitment to provide data that adequately demonstrate the efficacy of filters of either construction for sterile filtration of the subject product.

30 June 1995

Paul Stinavage, Ph.D.

cc: Orig. NDA 20-212
HFD-150/CSO/M. Pelosi
HFD-160/Consult File
Drafted by: P. Stinavage
R/D initialed by P. Cooney

Difussion for PHC 6-30-95

DF

DEC - 1 1994

CONSULTATIVE REVIEW TO HFD-150
DIVISION OF MEDICAL IMAGING, SURGICAL, AND DENTAL DRUG PRODUCTS
MICROBIOLOGIST'S REVIEW NO. 2
1 December 1994

A. 1. NDA 20-212

Product Name: Zinecard (Dexrazoxane for Injection), 250 mg, 500 mg single use vial.

APPLICANT: Pharmacia Inc. Oncology Division
4272 Balloon Park Road
Albuquerque, New Mexico 87109

2. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Lyophilized powder in vial supplied with 25 mL or 50 mL vial of M/6 Sodium Lactate Injection, USP; intravenous.

3. METHOD(s) OF STERILIZATION:

4. PHARMACOLOGICAL CATEGORY AND / OR PRINCIPAL INDICATION: Zinecard for injection is indicated for the prevention of cardiomyopathy associated with doxorubicin administration.

5. DRUG PRIORITY CLASSIFICATION: 1 P

B. 1. INITIAL APPLICATION DATE: 10 April 1992

2. AMENDMENT DATE: 27 September 1994

3. AMENDMENT RECEIVED FOR REVIEW: 9 November 1994

C. REMARKS: The document is an amendment to an, as yet, unapproved NDA. The amendment provides for the use of a different and larger lyophilizer originally reviewed. This new lyophilizer is not only in a different room but, is on a different floor than the subject of the original review. This amendment applies only to the lyophilized component of this two component drug product; the manufacturing location and process for the Sodium Lactate Injection (diluent) is unchanged.

In light of the fact that this amendment represents an entirely new process area, an inspection of the area should be requested by the consulting Division.

D. CONCLUSIONS: The submission is not recommended for approval.

Paul Stinavage, Ph.D.

cc: Orig. NDA 20-212
HFD-150/CSO/M. Pelosi
HFD-160/Consult File
Drafted by: P. Stinavage, 11/28/94
Revised by: P. Stinavage, 12/01/94
R/D initialed by P. Cooney, 12/01/94

RAC 12/1/94

Cuttel
9-18-92

CONSULTATIVE REVIEW TO HFD-150
DIVISION OF MEDICAL IMAGING, SURGICAL, AND DENTAL DRUG PRODUCTS
MICROBIOLOGIST'S REVIEW NO. 1
August 26, 1992

A. 1. NDA No.: 20-212

Product Name: Zinecard (Dexrazoxane for Injection), 250 mg, 500 mg single use vial.

APPLICANT: Adria Laboratories
Division of Erbamount, Inc.
P.O.Box 16529
Columbus, Ohio 43216

2. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Lyophilized powder in vial supplied with 25 mL or 50 mL vial of M/6 Sodium Lactate Injection, USP; intravenous.

3. METHOD(s) OF STERILIZATION:

4. PHARMACOLOGICAL CATEGORY AND / OR PRINCIPAL INDICATION: Zinecard for injection is indicated for the prevention of cardiomyopathy associated with doxorubicin administration.

5. DRUG PRIORITY CLASSIFICATION: 1 P

B. 1. INITIAL APPLICATION DATE: 04-10-92

2. ORIGINAL APPLICATION RECEIVED FOR REVIEW: 05-06-92

3. AMENDMENT DATES: 05-29-92, 08-18-92

4. AMENDMENTS RECEIVED FOR REVIEW: 06-01-92, 08-26-92

C. REMARKS: The chemist requesting the consult review specifically asked for evaluation of the following areas as part of the microbiology review:

a. manufacturing process for the drug product

b. validation of the process, sterility, and LAL methods

c. manufacturing process for Sodium Lactate Injection diluent

d. validation of the sterilization process and sterility test for Sodium Lactate Injection

SEP 23 1992

D. **CONCLUSION** and **RECOMMENDATION**: Recommend approval for sterility assurance and microbiological quality for both the filled lyophilized Zinecard (Dexrazoxane for Injection) drug product and the sterilized M/6 Sodium Lactate Injection, USP diluent accompanying the drug product.

Carol K. Vincent 9-8-92

9/8/92

cc:
Orig. NDA 20-212
HFD-150/Tolgyesi
HFD-160/Consult file/C. K. Vincent
Revised by: CKVincent/08-10-92/08-26-92
R/D Init by: PHCooney/08-03-92

Statistical Review and Evaluation

DATE: DEC 19 1994

NDA#: 20-212

APPLICANT: Pharmacia

NAME OF DRUG: Zinecard (Dexrazoxane for Injection)

DOCUMENTS REVIEWED: One Volume Dated 08/25/94

I. Background

This volume is a desk copy from the sponsor containing stability information, in particular pages 03 00486-00514 from Volume 3 and pages 03 02091-02107 from Volume 7 from the original 1992 NDA submission, as well as answers to questions 21 and 25 from the 8/2/94 amendment and updated stability data. Dr. Tolgyesi (HFD-150) has requested the Division of Biometrics to review these items of interest.

II. Sponsor's Results

The sponsor requests a 36 months expiration dating period based on 4 years data of dexrazoxane potency. They also concluded that the reconstituted and diluted product is stable for six hours when stored at room temperature.

For primary stability data the sponsor submitted four years data of dexrazoxane potency (90-110 % LC) of three batches of 250 mg/vial and of another three batches of 500 mg/vial strength at various temperatures inculding 30 degrees Celsius. As supportive evidence they also submitted data of ten 500 mg/vial lots studied up to four years at 24 degrees Celsius and one 250 mg/vial lot studied for two years.

The sponsor's statistical analyses focus on each batch individually. The expiration dating period is formed by creating a line with a slope that is the lower 95 % confidence limit of the original regression slope and intersecting this line with the lower specification limit. They do not compute expiration dating periods for positive slopes. They conclude that there was insufficient decomposition to project an expiration dating period in 15 of the 17 batches on stability. Two batches projected an expiration dating period of over eight years even after adjusting the intercept down to 100 % to remove overage. They also conclude that the Arrhenius model could not be applied as there was no significant amount of degradation at higher temperatures and many

stat

lines had positive slopes. Their statistical methods were only applied to the potency data of dexrazoxane content.

III. Reviewer's Results

This reviewer applied the statistical methods currently used in the Division of Biometrics to estimated expiration dating periods for the dexrazoxane potency, the sodium lactate diluent, the potency of the reconstituted product, and the potency of the product further diluted with sodium chloride or dextrose in either 5 mg/mL or 1.3 mg/mL strength. The Division's analysis first tests whether batches of a given strength can be pooled in their slope and in their intercept estimates. An expiration dating period will be computed based on a single regression line, on parallel lines, or on individual lines depending on the results of these preliminary analyses. Also, it is practice with a product that has both upper and lower specification limits to use the two-sided 95 % confidence limits around the regression line(s). The earliest intersection of one bound with the upper or lower specification limit is the estimated expiration dating period for this group of batches. The specification limits of all potency measures were taken as 90-110 % label claim (LC) except for sodium lactate, which had specifications of 95-110 % LC. This reviewer's findings are summarized in the attached Table 1.

The three batches each of dexrazoxane 250 mg/vial and dexrazoxane 500 mg/vial stored at 30 degrees C for four years supported an extrapolated expiration dating period of at least seven years. A single batch of dexrazoxane 250 mg/vial stored at 24 degrees C for two years also supported an extrapolated expiration dating period of seven years. An additional ten batches of dexrazoxane 500 mg/vial were stored between two to four years at 24 degrees C. One batch supported an extrapolated expiration dating period of 71 months, the remaining batches of at least seven years. The program calculates maximum expiration dating periods of up to 84 months; it is possible that exact expiration dating periods could be actually longer.

The 1/6 M sodium lactate diluent batches had only nine months data. The four batches used in the 25 mL fill volume had estimated expiration dating periods between 11 and 36 months. The four batches used in the 50 mL fill volume had estimated expiration dating periods between 16 and 36 months. These batches were stored at 30 degrees C.

The reconstituted product and its additional diluents were all stored for eight hours at various temperatures. This reviewer used the 30 degrees Celsius data in her analyses. Two batches of dexrazoxane 500 mg were reconstituted in 1/6 M sodium lactate. Regression analysis on these data showed that the reconstituted product would remain within 90-110 % LC for at least five hours.

The reconstituted product was further diluted with sodium chloride or with dextrose, each at 5 mg/mL and at 1.3 mg/mL strengths. The product diluted in 5 mg/mL sodium chloride is expected to remain within specifications for 12 hours. If 1.3 mg/mL sodium chloride is used as further diluent, the product is expected to remain within specifications for at least eight hours. The product in the 5 mg/mL dextrose diluent can be expected to remain within specification for 11 hours, whereas in the 1.3 mg/mL diluent only five hours are supported.

One batch of 250 mg dexrazoxane was reconstituted in 1/6 M of sodium lactate and stored for eight hours at 30 degrees C. The data were so variable and sparse that the confidence bounds fell outside the specification limits at all times. Therefore, this reviewer assigned no expiration dating time for this batch. The same situation was encountered when the product was further diluted with either 5 mg/mL sodium chloride or with 5 mg/mL dextrose. When 1.3 mg/mL strength of the further diluents were used the data stabilized and the product can be expected to remain within specifications for seven hours when sodium chloride is the diluent and for five hours when dextrose is the diluent.

The reconstituted product of 500 mg dexrazoxane had two batches, the 250 mg product only one batch used to establish an expiration dating time. According to the FDA Guidline (Guidline for Submitting Documentation for the Stability of Human Drugs and Biologics, 1987, p. 25) at least three batches of each strength and package type are required in setting expiration dating periods. The results of a single batch are unreliable and insufficient in representing the behavior of the product over time in general.

Question 21 had requested updated stability data which were analyzed here. Question 25 dealt with the reconstituted product which was also analyzed by this reviewer.

IV. Summary and Conclusion

The sponsor's analyses were not appropriate. It is standard procedure to test for poolability of batches and use the two sided 95 % confidence bounds when a product has both upper and lower specification limits.

This reviewer analyzed the potency of the dexrazoxane content, of the sodium lactate, of the dexrazoxane content in the product reconstituted with 1/6 M sodium lactate, and then further diluted with two strengths of either sodium chloride or dextrose. The findings are summarized in Table 1.

Dexrazoxane stored at 30 degrees C can be expected to remain within specifications for at least seven years. The sodium lactate diluent had only nine months data available for anlysis. Both the four batches for the 25 mL fill volume and the four batches for the 50

mL fill volume regressed to only individual lines and the estimated expiration dating periods were as low as 11 months. The reconsituted product and its further diluents were stored at 30 degrees for eight hours. There were two batches of the 500 mg dexrazoxane product reconstituted in 1/6 M sodium lactate. This reconstituted product can be expected to remain within specifications for five hours. When further diluted with either sodium chloride or dextrose (each in either 5 mg/mL or 1.3 mg/mL strength), the product again can be expected to remain within specifications for at least five hours. Only one batch of 250 mg dexrazoxane was reconstituted in 1/6 M sodium choride. The large variablity and sparsity of data caused the 95 % confidence bounds to fall outside the specification limits at all time. This reviewer therefore assigned no expiration time to this batch. Further dilution with sodium chloride or dextrose at 5 mg/mL resulted in the same findings. Dilution with 1.3 mg/mL sodium chloride or dextrose stabilized the data to estimate a storage time of at least five hours. Setting expiration times based on only one batch is not statistically reliable in general. According to FDA Guideline, at least three batches of each strength of the reconstituted product should be analyzed to establish the length of time the product may be expected to remain within specifications when reconstituted and further diluted.

Roswitha E. Kelly

Concur: for Karl K. Lin, Ph.D. 12/16/94

cc: HFD-150/NDA 20-212 Original
HFD-150/Dr. E. Tolgyesi
HFD-150/Dr. Ochikian
HFD-710/C [illegible]con
HFD-715/[illegible]. Fairweather
HFD-71[illegible]r. K. Lin
HFD-715/R. Kelly
HFD-715/DRU 2.2.1 Zinecard for Injection, Pharmacia
HFD-715/RKELLY/12/16/94/wp-Zinecard.rev

Table 1:

Product	Batch	Act. Time (mos)	Intercept	Slope	Exp. Date (mos)
Dexrazoxane 250 mg/vial (30 C)	90A10FY	48	100.60	-0.026	84
	90A11FY	48	100.67	-0.026	84
	90B10FY	48	99.93	-0.026	84
Dexrazoxane 500 mg/vial (30 C)	90A12FY	48	99.94	-0.016	84
	90A13FY	48	101.02	-0.016	84
	90B11FY	48	100.19	-0.016	84
Dexrazoxane 250 mg/vial (24 C)	91K06FY	24	100.46	+0.024	84
Dexrazoxane 500 mg/vial (24 C)	91A30FY	36	100.01	+0.087	71
	89B49FY	48	101.38	-0.011	84
	89C14FY	48	100.98	-0.018	84
	89D01FY	48	100.65	-0.022	84
	89D18FY	48	99.85	-0.015	84
	91D23FY	36	101.100	-0.033	84
	90F04FY	48	100.26	+0.003	84
	91G04FY	24	99.17	-0.023	84
	90N04FY	36	98.71	+0.013	84
	88N45FY	48	101.38	-0.065	84

Sodium Lactate (25 mL Fill Vol) (30C)	91A18FL	9	97.95	+0.065	22
	90N15FL	9	98.67	-0.003	36
	90N16FL	9	99.47	-0.018	26
	90N17FL	9	97.99	-0.145	11
Sodium Lactate (50 mL Fill Vol) (30C)	91A05FL	9	97.75	-0.033	23
	91A06FL	9	99.23	-0.190	16
	91A22FL	9	98.07	+0.038	36
	91A25FL	9	98.07	-0.020	21
Dexrazoxane (500mg) reconst. in 1/6 M Sod.Lac. (30 C)	90A13FY	8hrs	100.79	-0.974	7hrs
	92B03FY	8hrs	97.66	-0.974	5hrs
Sodium Chloride Diluent (5mg/mL)	for 90A13FY	8hrs	99.39	-0.501	12hrs
	for 92B03FY	8hrs	99.39	-0.501	12hrs
Sodium Chloride Diluent (1.3mg/mL)	for 90A13FY	8hrs	97.98	-0.813	8hrs
	for 92B03FY	8hrs	100.78	-0.813	10hrs
Dextrose Diluent (5mg/mL)	for 90A13FY	8hrs	98.97	-0.382	11hrs
	for 92B03FY	8hrs	98.97	-0.382	11hrs
Dextrose Diluent (1.3mg/mL)	for 90A13FY	8hrs	100.42	-1.147	5hrs
	for 92B03FY	8hrs	100.48	-0.561	10hrs

Dexrazoxane (250mg) reconst. in 1/M Sod.Lact. (30 C)	90A10FY	8hrs	96.55	+0.152	---
Sodium Chloride Diluent (5mg/mL)	for 90A10FY	8hrs	99.34	-0.145	---
Sodium Chloride Diluent (1.3mg/mL)	for 90A10FY	8hrs	100.36	-0.974	7hrs
Dextrose Diluent (5mg/mL)	for 90A10FY	8hrs	99.26	-0.131	---
Dextrose Diluent (1.3mg/mL)	for 90A10FY	8hrs	98.56	-.0697	5hrs

Food and Drug Administration
Center for Drug Evaluation and Research
Oncologic Drugs Advisory Committee
Meeting #34, June 18-19, 1992
Grand Ballroom, Pooks Hill Marriott
Bethesda, Maryland

AGENDA

Thursday, June 18, 1992

9:00 a.m. **CLOSED SESSION**

12:00 noon **LUNCH**

1:00 p.m. **OPENING REMARKS**
I. Craig Henderson, M.D.
Advisory Committee Chairman

R.W. JOHNSON PHARMACEUTICAL RESEARCH INSTITUTE PRESENTATION
NDA 20229 Leustatin™ Injection (2-chloro-deoxy-b-D-adenosine), for treatment of patients with hairy cell leukemia

Miguel Conde, M.D - FDA Reviewer
Charles Schiffer, M.D.- Advisory Committee Reviewer
Sandra Horning, M.D.- Advisory Committee Reviewer

COMMITTEE DISCUSSION

Friday, June 19, 1992

9:00 a.m. **OPENING PUBLIC HEARING**
One hour is allocated, however, the meeting will proceed immediately if the open public hearing does not last that long.

10:00 a.m. **ADRIA LABORATORIES PRESENTATION**
NDA 20212 Zinecard™ (Dexrazoxane for Injection), for preventing/reducing the incidence and severity of cardiomyopathy associated with doxorubicin administration

Grant Williams, M.D.- FDA Reviewer
Clare Gnecco, Ph.D. - FDA Statistician
Kathleen Pritchard, M.D.- Advisory Committee Reviewer
David Ahmann, M.D.- Advisory Committee Reviewer

12:00 noon **LUNCH**

1:00 p.m. **COMMITTEE DISCUSSION**

ADJOURNMENT

AC Meeting Agenda.

END

T. SMITH

J.H.M. RESEARCH &DEVELOPMENT, INC. 5776 SECOND STREET.N.E. WASH. DC 20011