Cancer Chemother Pharmacol (1994) 34: 439–443

Cancer Chemotherapy and Pharmacology

The cardioprotector ADR-529 and high-dose epirubicin given in combination with cyclophosphamide, 5-fluorouracil, and tamoxifen: a phase I study in metastatic breast cancer

Bente Sørensen[1], Lars Bastholt[1], Mansoor Raza Mirza[1], Susanne B. Gjedde[2], Preben Jakobsen[3], Henning T. Mouridsen[2], Carsten Rose[1]

[1] Department of Oncology, Odense University Hospital, Odense, Denmark
[2] Department of Oncology, Copenhagen University Hospital, Copenhagen, Denmark
[3] Institute of Pharmacology, Århus University, Århus, Denmark

Received: 5 November 1993/Accepted: 25 March 1994

Abstract. The purpose of this study was to determine the maximal tolerable dose (MTD) of epirubicin and ADR-529 given in combination with cyclophosphamide, 5-fluorouracil, and tamoxifen. A total of 64 breast cancer patients with locally advanced disease or a first metastatic event were included. Using fixed doses of cyclophosphamide, 5-fluorouracil, and tamoxifen, cohorts of ten patients were treated with escalating doses of epirubicin and ADR-529. With the use of protocol criteria specifying evaluation after the first course, the MTD was not reached. Dose reductions carried out due to hematologic toxicity during the first four courses made it impossible to escalate doses of epirubicin beyond 80 mg/m^2 given together with ADR-529 600 mg/m^2. The vascular toxicity of ADR-529 necessitated central venous access in a number of patients. For phase III evaluation of ADR-529 given together with cyclophosphamide, epirubicin, 5-fluorouracil, and tamoxifen (CEF/TAM) we recommend using epirubicin/ADR-529 at 60/600 mg/m^2. Together with evaluation of the cardioprotective properties of ADR-529, we recommend evaluating the impact of ADR-529 on the efficacy of cytotoxic therapy and investigating further the toxicity of ADR-529.

Key words: ADR-529 – Cardioprotection – Metastatic breast cancer

Introduction

Anthracyclines have a wide spectrum of applications in oncology. In the treatment of metastatic breast carcinoma, they are the most active drugs. A number of breast-cancer study groups are currently investigating such important issues as whether or not to use anthracyclines in adjuvant treatment of breast cancer, and, if so, which dose intensity to use. Cardiotoxicity represents a major hazard in these studies.

If anthracyclines are to be included in future adjuvant therapy of patients with breast carcinoma, it is of utmost importance to minimize cardiotoxicity in this potentially curable patient group. Anthracycline-induced cardiotoxicity is believed to be caused by free radicals capable of causing lipid peroxidation of the mitochondrial membranes and endoplasmic reticulum [1]. It appears that the heart is particularly susceptible to free-radical damage because of its lower antioxidant levels as compared with other tissues [2]. Iron is an essential part of these events, being required as a catalyst to initiate hydroxyl radical production.

ADR-529 (formerly ICRF-187) has cardioprotective properties, although its mechanism of action as a cardioprotector has not been fully elucidated. Originally introduced as a possible antitumor agent [3], ADR-529 is a soluble (+)-enantiomer of the insoluble racemate ICRF-159 (Razoxane). Both compounds are bisdioxoperazines derived from ethylenediaminetetraacetic acid (EDTA). ADR-529 is hydrolyzed intracellularly to ICRF-198, a strong chelator of iron [4]. Cardioprotection might therefore be occurring through a reduction in the availability of intracellular iron to form complexes with anthracyclines and, hence, a reduction in the generation of free radicals [5].

The cardioprotective properties of ADR-529 have been examined in a number of different animal models of anthracycline cardiotoxicity. Significant attenuation of cardiotoxicity has been observed in several studies [6–12]. This includes a study showing a similar mechanism of cardiotoxicity induction for doxorubicin and epirubicin as well as equal cardioprotective properties for ADR-529 [13]. The protective effect of ADR-529 against doxorubicin-induced cardiotoxicity in patients with metastatic breast cancer has been established in randomized studies [14–18].

On the basis of the assumption that future adjuvant therapy for breast cancer will consist of more dose-intensive therapy including anthracyclines, we sought to determine the maximal tolerable dose (MTD) of epirubicin and ADR-529 given in combination with cyclophos-

Correspondence to: Lars Bastholt, MD Head of Department, Department of Oncology R, Odense University Hospital, DK-5000 Odense C, Denmark

phamide, 5-fluorouracil, and tamoxifen, which also is a necessary prelude to a phase III trial.

Patients and methods

Patients. For inclusion in the study, patients were required to (1) be under 70 years of age, (2) have a performance status of ≤2 according to WHO guidelines [19], and (3) have experienced a first recurrence after primary therapy for carcinoma of the breast. Furthermore, the patients could have no history of cardiac disease. Cardiac function was evaluated using ECG and multiple ECG-gated radionuclide cineangiocardiography (MUGA) scans, requiring a left ventricular ejection fraction (LVEF) of ≥50%. Finally, patients were required to have normal bone marrow function (WBC, $\geq 3.0 \times 10^9$/l; platelets, $\geq 100 \times 10^9$/l) as well as adequate hepatic and renal function (bilirubin, ≤35 mmol/l; creatinine, ≤130 μmol/l). Consent was obtained from eligible patients after they had received oral and written information about the nature of the study. The protocol was approved by the National Board of Health and by the scientific committees of the participating hospitals.

Cytotoxic therapy. The patients were offered treatment with cyclophosphamide, epirubicin, 5-fluorouracil (CEF), tamoxifen, and ADR-529. Cyclophosphamide, 5-fluorouracil, epirubicin, and tamoxifen were purchased from suppliers, whereas Farmitalia Carlo Erba provided ADR-529 as part of its support of the study. The ADR-529 so supplied is a preservative-lyophilized powder available as 500 mg in a 50-cc amber vial. The powder was reconstituted by adding 50 ml of 1/6 *M* sodium lactate per vial, yielding a reconstituted solution of pH 4.5±0.3. Intravenous infusion of cyclophosphamide and 5-fluorouracil (600 mg/m² each) was followed by escalating doses of epirubicin/ADR-529 (60/600, 80/600, 100/600, 80/800, 100/800, and 100/1000 mg/m²) in cohorts of at least ten patients. This escalation program was chosen to ensure an increasing dose intensity of anthracyclines and an epirubicin/ADR-529 dose ratio ranging between 1:5 and 1:10. ADR-529 was given intravenously over 15 min, followed by epirubicin given intravenously over 15 min. The treatment was repeated every 3 weeks. Tamoxifen (30 mg) was given orally daily throughout the treatment period.

In this study the dose level equal to MTD was defined as the sublevel of epirubicin/ADR-529 at which ≥50% of the patients showed grade 3 stomatitis or grade 4 leukopenia after the first course. Grade 4 leukopenia was defined as a WBC of $< 0.4 \times 10^9$/l lasting for >3 days. WHO criteria [19] were used for grading nonhematologic toxicity. Acute toxicity results were analyzed for each cohort before dose escalation was implemented. WHO criteria [19] were used to measure response in patients with evaluable disease.

A full pharmacokinetic analysis of ADR-529 and epirubicin was performed during the first treatment cycle in all patients. The results will be published in a separate paper, which will also include data on the influence of ADR-529 on the pharmacokinetics of epirubicin.

Dose modifications were implemented due to hematologic toxicity after evaluation of hematologic values on days 7, 10, and 14. Treatment was delayed 1 week if on day 1 of a new treatment series the WBC value was $< 3.0 \times 10^9$/l or the platelet count was $< 100 \times 10^9$/l. On the basis of nadir values the dose of cyclophosphamide and 5-fluorouracil was reduced. If the WBC nadir was $< 1.5 \times 10^9$/l and/or the platelet nadir was $< 75 \times 10^9$/l, the dose was reduced by 25%. The dose reduction was 50% if the WBC nadir was $< 1.0 \times 10^9$/l and/or the platelet nadir was $< 50 \times 10^9$/l. If the nadir counts remained low after the dose of cyclophosphamide and 5-fluorouracil had been decreased, a reduction in the dose of epirubicin was performed according to similar guidelines.

Because this study aimed at estimating the MTD, patients were not treated beyond a maximal cumulative epirubicin dose of 1000 mg/m². Treatment was also stopped if the patient showed signs of progressive disease or clinical congestive heart failure or if the patient wished to terminate treatment. Furthermore, a decline of >20% from the baseline LVEF to a value above the lower limit of normal (LVEF, 50%) or a decline of >10% to a value below the lower limit led to treatment termination.

Table 1. Major characteristics of the study group ($n = 64$)

Age:	
Median (range)	53 (30–69) years
Performance status:	
Median (range)	0 (0–2)
Surgery:	
Mastectomy	49
Tumorectomy	4
No operation	11
Previous therapy:	
Adjuvant chemotherapy	21
Hormonal therapy	8
Adjuvant radiotherapy	29
Nor prior therapy	26
Dominant site of disease:	
Soft tissue	22
Bone disease	14
Visceral disease	28
Measurable/evaluable disease	50

Results

Maximal tolerable dose

Between October 1989 and December 1990, 64 patients entered the study. The major characteristics of the patients are shown in Table 1. The median cumulative epirubicin dose was 880 mg/m² (range, 60–1100 mg/m²). Patients received a median of 11 courses (range, 1–21). Table 2 contains the reasons for treatment termination at different dose levels. Six patients showed a decline in LVEF necessitating treatment termination according to the protocol. No case of clinical congestive heart failure was observed. Noncardiac toxicity necessitating treatment termination included treatment refusal due to nausea/vomiting (3), thrombosis in the subclavian vein ascribed to ADR-529 (1), death during therapy due to thrombocytopenia (1), prolonged thrombocytopenia below 100×10^9/l (1), and death during therapy for unknown reasons (1). An investigation into the course of this unexplained death yielded progressive disease as the most likely culprit.

Table 3 displays the nadir values obtained for WBC, platelets, and hemoglobin (Hb) after the first course. With the use of strict protocol criteria for reaching the MTD, this point was not reached in terms of either hematologic toxicity or stomatitis. Figure 1 shows the percentage of patients in each cohort receiving a full dose of all four drugs during the first four courses. Doses were modified during these four courses only due to hematologic toxicity. More than 80% of the patients had no dose modifications as long as the epirubicin dose was kept below 80 mg/m² given together with ADR-529 (600 mg/m²). Over the entire treatment period, a total of eight patients developed febrile neutropenia requiring intravenous antibiotics, including two patients at dose level 80/600 mg/m², three patients at

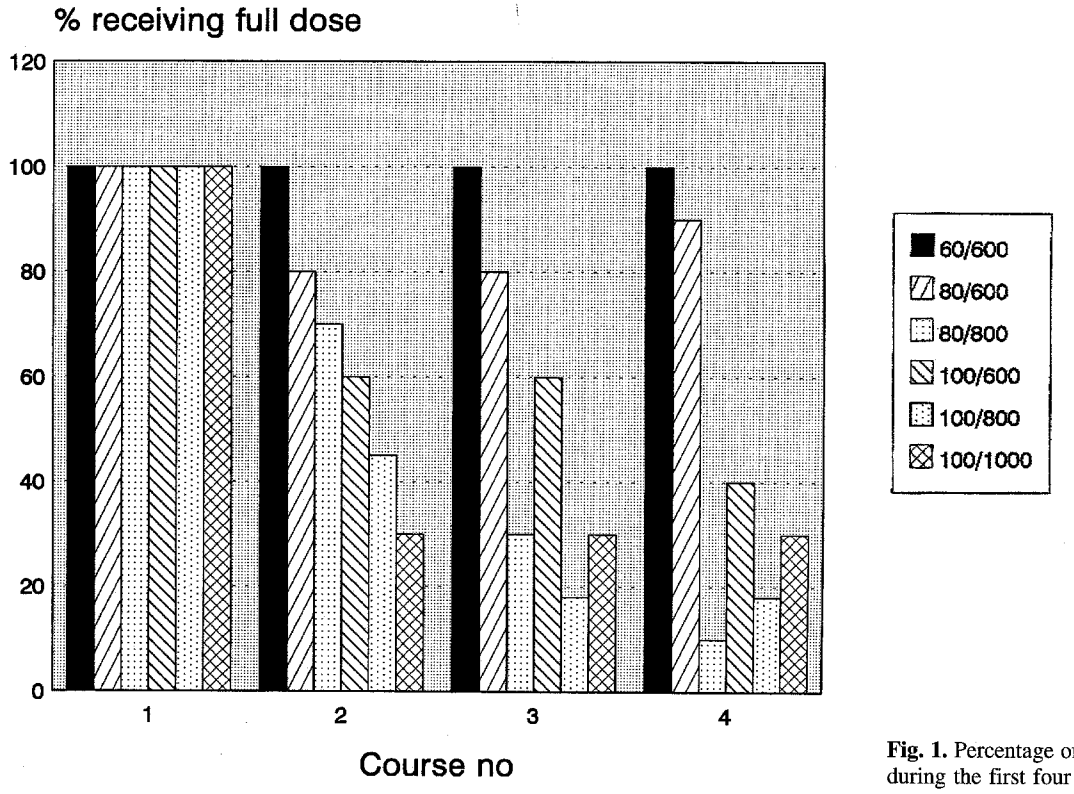

Fig. 1. Percentage of patients receiving a full dose during the first four courses of cytotoxic therapy

Table 2. Reasons for treatment termination

	Epirubicin/ADR-529 (mg/m²)					
	60/600	80/600	100/600	80/800	80/1000	100/1000
Maximal cumulative dose	2	6	6	4	9	6
Progressive disease	7	2	2	1	1	4
Cardiotoxicity	0	1	1	2	0	2
Toxicity	1	1	1	3	1	1
Cumulative dose, mg/m², median (range)	540 (60–1040)	960 (317–1100)	945 (650–1000)	880 (403–1000)	970 (600–1015)	800 (282–1014)

Table 3. Toxicity encountered after the first course of cytotoxic therapy

	Epirubicin/ADR-529 (mg/m²)					
	60/600	80/600	100/600	80/800	80/1000	100/1000
Patients	10	10	10	10	11	13
Evaluable patients	9	10	10	10	11	13
WBC nadir, ×10⁹/l, median (range)	2.7 (1.5–4.1)	0.9 (0.6–3.7)	1.1 (0.6–1.7)	1.5 (0.7–2.0)	1.4 (0.6–3.0)	1.1 (0.4–2.5)
Patients with grade 4 hematologic toxicity	0	0	0	0	0	1
Platelet nadir, ×10⁹/l, median (range)	209 (115–327)	132 (15–318)	147 (78–247)	119 (49–219)	124 (23–244)	115 (73–292)
Hb, nadir, mmol/l (range)	7.0 (5.1–7.8)	6.8 (5.8–8.4)	6.7 (5.2–7.5)	6.7 (6.1–8.5)	6.8 (5.3–8.2)	7.0 (6.0–8.0)
Patients with grade 3 stomatitis	0	1	0	0	0	0

dose level 100/600 mg/m^2, two patients at dose level 80/1000 mg/m^2, and one patient at dose level 100/1000 mg/m^2.

One patient with locally advanced breast cancer received 14 doses of chemotherapy. Although a partial response was obtained, the chemotherapy was stopped due to prolonged thrombocytopenia and no evidence of further remission and was replaced by local radiotherapy. This patient developed secondary myelodysplasia 2 years after the initiation of combination therapy and ADR-529. A chromosome aberration was found in the bone marrow (karyotype 45 XX, –7/46 XX); 6 months later the patient had fulminant progression in her metastatic breast cancer and concomitant transformation of myelodysplasia into acute myeloblastic leukemia. Upon her death an autopsy confirmed these diagnoses.

A total of 16 patients experienced pain on the injection of ADR-529 in peripheral veins. Clinical signs of phlebitis were observed in eight patients for whom the application of central venous catheters (CVCs) was necessary to continue treatment. In one of these eight patients the first three courses were associated with pain on injection in direct connection with ADR-529. The fourth course was given without a problem via a CVC. The fifth course was associated with pain and swelling in the supraclavicular region and clinical signs of thrombosis in the subclavian vein. Treatment with ADR-529 was stopped, and further chemotherapy with CEF was given without incident.

We observed nausea/vomiting episodes of median WHO grade 2 (range, 1–3) in 39 of 64 patients; furthermore, 5 patients had stomatitis of median WHO grade 2 (range, 1–3).

Efficacy

Evaluation of efficacy data showed a median time to progression of 581 days (95% confidence limits, 541–626 days). Among the 64 patients entered, 50 had evaluable disease. Of these, 23 patients obtained an objective response (complete response, 13 patients; partial response, 10 patients), yielding a response rate of 46% (95% confidence limits, 31.8%–60.7%).

Discussion

The purpose of the present study was to determine the MTD of ADR-529 given in combination with CEF/tamoxifen (TAM). With the use of strict protocol criteria for achieving the MTD (≥50% of patients with stomatitis of WHO grade 3–4 or grade 4 leukopenia), this point was not reached in terms of either hematologic toxicity or stomatitis. However, evaluation of hematologic toxicity during the first four courses showed that in a conventional 3-week schedule, the dose of epirubicin could not be extended beyond 80 mg/m^2 given with ADR-529 (600 mg/m^2). This finding is in accordance with data reported by Hochster et al. [20] for the MTD of ADR-529 given together with doxorubicin.

Pain on injection and phlebitis resulting from the use of peripheral venous access were major complaints. CVCs were applied in patients with phlebitis. Among the patients with a CVC, one developed thrombophlebitis in the subclavian vein. Liesmann et al. [21] reported on a phase I trial in which one patient had pain on injection that was not associated with any objective finding. Similarly, in a phase II study on ADR-529 in advanced adenocarcinoma of the kidney, Brubaker et al. [22] reported that patient developed phlebitis of grade III after single-drug treatment with ADR-529. Clinical studies on ADR-529 and doxorubicin [14–18] or epirubicin [23] have not demonstrated any vascular toxicity. However, due to thorough registration of this toxicity, we feel confident that ADR-529 is responsible for the high frequency of vascular toxicity observed in this study. No unexpected nausea/vomiting or stomatitis was observed.

One patient developed myelodysplasia 2 years after the initiation of cytotoxic therapy. The patient had received no previous adjuvant cytotoxic therapy. Cytogenetic analysis of the bone marrow revealed a loss of chromosome 7 in a number of cells. Therapy-related leukemia secondary to treatment with alkylating agents was often present in association with myelodysplasia and cytogenetic aberrations. Among the most frequent aberrations is a loss of chromosome 5 or 7 or of various parts of their long arms [24]. Dioxopiperazine derivatives have recently been shown to inhibit DNA-topoisomerase II [25]. Leukemias secondary to therapy with drugs targeting DNA-topoisomerase II often present with balanced chromosome translocation [26] and a more overt clinical picture. For these reasons, the leukemia in our patient probably developed secondary to treatment with cyclophosphamide.

Evaluation of efficacy data in phase I studies is difficult. However, the rate of response in patients with evaluable disease and the time to progression for all patients entered were comparable with previously published data [27]. We conclude from the present study that ADR-529 (600 mg/m^2) can be safely given together with CEF/TAM using an epirubicin dose of ≤80 mg/m^2 as based on evaluation of the MTD and other toxicities. Assuming that epirubicin will in the future be included in adjuvant treatment of primary breast carcinomas, there is clearly a need for concomitant use of a cardioprotector. Even though preclinical studies have shown that ADR-529 also has cardioprotective properties against epirubicin-induced cardiotoxicity [13], a controlled phase III trial is needed to establish the role of ADR-529 in CEF combination chemotherapy.

Among the clinical studies previously published on doxorubicin-containing regimens and ADR-529, ten Bokkel Huiniuk et al. [28] have reported a difference in the response rate obtained using cyclophosphamide/doxorubicin/5-fluorouracil (CAF) with versus without ADR-529. The difference was not statistically significant. Speyer et al. [17] and Narang et al. [29] could not detect any negative impact of ADR-529 on the efficacy of concomitant combination cytotoxic therapy. In vitro studies performed by Sehested et al. [30] have shown that ADR-529 has an antagonistic effect on anthracycline cytotoxicity. On the basis of these observations, it is important for a phase III study also to determine whether ADR-529 has any impact on the efficacy of concomitant chemotherapy. The study must also evaluate vascular toxicity and the impact of ADR-529 on hematologic toxicity.

References

1. Doroshow JH, Akman S, Chu FF, Esworthy S (1990) Role of the glutathione-glutathione peroxidase cycle in the cytotoxicity of the anticancer quinones. Pharmacol Ther 47: 359–370
2. Doroshow JH, Locker GY, Baldinger J, Myers CE (1979) The effect of doxorubicin on hepatic and cardiac glutathione. Rec Commun Chem Pathol Pharmacol 26: 285–295
3. Creighton AM, Hellmann H, Whitecross S (1969) Antitumor activity in a series of bisdiketopiperazines. Nature 222: 384–385
4. Hassinoff BB (1989) The interaction of the cardioprotective agent ICRF-187, its hydrolysis products (ICRF-198) and other chelating agents with Fe(III) complexes of adriamycin. Agents Actions 26: 378–395
5. Harris RN, Doroshow JH (1985) Effect of doxorubicin-enhanced hydrogen peroxide and hydroxyl radical formation on calcium sequestration by cardiac sarcoplasmic reticulum. Biochem Biophys Res Commun 13: 739–745
6. Agen C, Bernardini N, Danesi R, Torre PD, Costa M, Del Tacca M (1992) Reducing doxorubicin cardiotoxicity in the rat using deferred treatment with ADR-529. Cancer Chemother Pharmacol 30: 95–99
7. Dardir MD, Herman EH, Ferrans VJ (1989) Effects of ICRF-187 on the cardiac and renal toxicity of epirubicin in spontaneously hypertensive rats. Cancer Chemother Pharmacol 23: 269–275
8. Herman EH, El-Hage A, Ferrans VJ (1988) Protective effect of ICRF-187 on doxorubicin-induced cardiac and renal toxicity in spontaneously hypertensive and normotensive rats. Toxicol Appl Pharmacol 92: 42–53
9. Herman EH, Ferrans VJ (1983) Influence of vitamin E and ICRF-187 on chronic doxorubicin cardiotoxicity in miniature swine. Lab Invest 49: 69–77
10. Herman EH, Ferrans VJ (1981) Reduction of chronic doxorubicin cardiotoxicity in dogs by pretreatment with (+–)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane (ICRF-187). Cancer Res 41: 3436–3440
11. Herman EH, Ferrans VJ, Myers CE, Vleet JF van (1985) Comparison of the effectiveness of (+/–)-1,2-bis(3,5-dioxopiperazinyl-1-yl)-propane (ICRF 187) and *N*-acetylcysteine in preventing chronic doxorubicin cardiotoxicity in beagles. Cancer Res 45: 276–281
12. Perkins WE, Schroeder RL, Carrano RA, Imondi AR (1982) Effect of ICRF-187 on doxorubicin-induced myocardial effects in the mouse and guinea pig. Br J Cancer 46: 662–667
13. Alderton PM, Gross J, Green MD (1992) Comparative study of doxorubicin, mitoxantrone, and epirubicin in combination with ICRF-187 (ADR-529) in a chronic cardiotoxicity animal model 1. Cancer Res 52: 194–201
14. Milliard JA, Speyer JL, Hanson K, Shaikh BS, Chang A, Ryan K (1992) Prevention of chronic Adriamycin cardiotoxicity with the bidiozopiperazine dexrazoxane (ICRF-187, ADR-529, Zinecard) in patients with advanced or metastatic breast cancer (abstract). Proc Am Soc Clin Oncol 11: 91
15. Rosenfeld CS, Weisberg SR, York RM, Jones SE, Spicer DV, Khojasteh A (1992) Prevention of Adriamycin cardiomyopathy with dexrazoxane (ADR-529, ICRF-187) (abstract). Proc Am Soc Clin Oncol 11: 62
16. Speyer JL, Green MD, Kramer E, et al. (1988) Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. N Engl J Med 319: 745–752
17. Speyer JL, Green MD, Zeleniuch-Jacquotte A, et al. (1992) ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. J Clin Oncol 10: 117–127
18. Weisberg SR, Rosenfeld CS, York RM, Jones SE, Spicer DV, Khojasteh A (1992) Dexrazoxane (ADR-529, ICRF-187, Zinecard) protects against doxorubicin induced chronic cardiotoxicity (abstract). Proc Am Soc Clin Oncol 11: 91
19. World Health Organization (1979) Handbook for reporting results of cancer treatment. WHO, Geneva
20. Hochster HS, Liebes L, Wadler S, et al (1992) Pharmacokinetics of the cardioprotector ADR-529 (ICRF-187) in escalating doses combined with fixed-dose doxorubicin. J Natl Cancer Inst 84: 1725–1730
21. Leismann J, Belt R, Haas C, Hoogstraten B (1981) Phase I evaluation of ICRF-187 (NSC-169780) in patients with advanced malignancy. Cancer 47: 1959–1962
22. Brubaker LH, Vogel CL, Einhorn LH, Birch R (1986) Treatment of advanced adenocarcinoma of the kidney with ICRF-187: a Southeastern Cancer Study Group trial. Cancer Treat Rep 70: 915–916
23. Basser R, Duggan G, Rosenthal M, Sheridan W, Fox R, Mihaly G (1992) Optimal dose ratio of ADR-529 (ICRF-187) and epirubicin (abstract). Proc Am Soc Clin Oncol 11: 123
24. Pedersen-Bjergaard J, Philip P, Larsen SO, Jensen G, Byrsting K (1990) Chromosome aberrations and prognostic factors in therapy-related myelodysplasia and acute nonlymphocytic leukemia. Blood 76: 1083–1091
25. Tanabe K, Ikegami Y, Ishida R, Andoh T (1991) Inhibition of topoisomerase II by antitumor agent bis-(2,6-dioxopiperazine) derivatives. Cancer Res 51: 4903
26. Pedersen-Bjergaard J (1992) The dioxopiperazine derivatives, their leukemogenic potential and other biological effects. Leuk Res 16: 1057–1059
27. Ejlertsen B, Pfeiffer P, Pedersen D, et al (1993) Decreased efficacy of cyclophosphamide, epirubicin and 5-fluorouracil in metastatic breast cancer when reducing treatment duration from 18 to 6 months. Eur J Cancer 29A: 527–531
28. Bokkel Huiniuk WW ten, Schreuder JE, Dubbleman R, et al (1992) ICRF 187 protects against doxorubicin induced cardiomyopathy. Ann Oncol 3 [Suppl 3]: 114
29. Narang PK, Hochster HS, Reynolds RD, et al (1992) Does the cardioprotectant dexrazoxane (DZR) affect doxorubicin (DOX) kinetics/dynamics (abstract)? Proc Am Soc Clin Oncol 11: 126
30. Sehested M, Jensen PB, Sørensen BS, Holm B, Friche E, Demant EJF (1993) Antagonistic effect of the cardioprotector (+)-1,2-bis(3,4-dioxopiperazinyl-1-yl)propane (ICRF-187) on DNA breaks and cytotoxicity induced by the topoisomerase II directed drugs daunorubicin and etoposide (VP-16). Biochem Pharmacol 46: 389–393