A Phase I and Pharmacokinetic Study of the Novel Aza-Anthracenedione Compound BBR 2778 in Patients with Advanced Solid Malignancies

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ABSTRACT

BBR 2778 is a novel aza-anthracenedione with no cardiotoxicity in preclinical models. This Phase I dose escalation trial of BBR 2778 was conducted to determine the maximum tolerated dose, the dose-limiting toxicity, and the pharmacokinetic profile of BBR 2778 in patients with advanced solid tumors. BBR 2778 was given in three consecutive weekly 30-min i.v. infusions over a 4-week cycle (cy). Thirty patients (pts) were treated with BBR 2778 at doses ranging from 5 to 150 mg/m^2/week. The dose levels 5, 10, 16.5, 25, 37.5, 75, 112.5, and 150 mg/m^2/week were investigated in 4 pts (9 cy), 3 pts (3 cy), 3 pts (5 cy), 6 pts (9 cy), 1 pt (1 cy), 4 pts (9 cy), 6 pts (18 cy), and 3 pts (4 cy), respectively. The dose-limiting toxicity was neutropenia, typically occurring at day 14. Other toxicities were mild to moderate and were principally thrombocytopenia, lymphopenia, alopecia, nausea, and vomiting and blue coloration of the skin and urine. No significant cardiac toxicity was observed. The plasma dose concentration curve fitted a biexponential profile, with a rapid distribution phase followed by a prolonged elimination phase (mean $t_{1/2,z}$, 12 h). BBR 2778 displayed a large volume of distribution (range, 9.7–29.7 l/kg) with a high plasma clearance rate (0.75–1.31 l/h/kg). Less than 10% of the dose was recovered in urine as unchanged drug. The maximum tolerated dose was 150 mg/m^2/week for 3 weeks, every 4 weeks. On the basis of this study, the recommended dose for Phase II studies is 112.5 mg/m^2/week days 1 and 8 with individual optional administration at day 15, every 4 weeks. Antitumor activity was observed in patients with breast, small cell lung carcinoma, and facial cylindroma. This trial showed that BBR 2778 has a manageable toxicity profile on a weekly schedule. This lead compound of the aza-anthracenedione family shows promising antitumor activity and deserves Phase II investigation in patients with high risk of cumulative cardiotoxicity, such as anthracycline-pretreated breast cancer patients.

INTRODUCTION

Anthracyclines are among the most active cytotoxic agents commonly used in patients with breast cancer, lymphoma, and sarcoma (1). Unfortunately, the use of anthracyclines remains limited by cumulative cardiotoxicity (2). In a recent cohort of 469 patients with advanced breast cancer treated with epirubicin, the risk of cardiotoxicity was 4% at a cumulative dose of 900 mg/m^2 and increased exponentially to 15% at 1000 mg/m^2. The incidence of congestive heart failure was estimated at 7.2%, with one-third of these patients ultimately dying of cardiac failure (3). Parallel to the development of cardioprotective agents such as dexrazoxane (4), efforts have been made to identify new compounds with similar activity and lower toxicity.

BBR 2778 is a new anthracenedione analogue (Fig. 1) with lower cardiotoxicity in preclinical models (5). BBR 2778 exerts antitumor effects through intercalative interaction with DNA. Like other topoisomerase II inhibitors, BBR 2778 stabilizes the normally transiently bound DNA-protein complex, referred to as a cleavable complex, resulting in the stimulation of topoisomerase II-mediated DNA cleavage (6, 7). *In vitro*, BBR 2778 has been shown to have a broad cytotoxicity profile against human and murine tumor cells, including leukemia and non-small cell lung cancer cells, with IC_{50}s ranging from 2.3 to 6.3 μg/ml (1-h exposure) in human cells (8). The degree of cross-resistance with other topoisomerase II inhibitors has been partially explored, and BBR 2778 was found to be partially cross-resistant in the HT29/Mitox colon cell line, which displays a specific mechanism of resistance to mitoxantrone (9). Using the human tumor cloning assay, BBR 2778 displayed dose-response effects against a wide range of specimens from patients, including breast, ovarian, endometrial, non-small cell lung cancer, and melanoma (10). *In vivo*, BBR 2778 demonstrated antitumor activity comparable with that of mitoxantrone in several murine and human solid tumors and was found to be curative in murine L1210 leukemia and murine YC-8 lymphoma, whereas mitoxantrone and doxorubicin showed only an increase in the survival time (11, 12). Toxicology studies in mice showed reversible myelosuppression (13). Interestingly, in contrast to mitoxantrone, BBR 2778 yielded no delayed cardiotoxic effects at equiactive doses. After repeated i.v. injections in mice, BBR 2778 did not induce any delayed myocardial toxic effects (14), unlike mitoxantrone that caused dose-dependent delayed histopathological myocardial lesions. On the basis of these preclinical results, BBR 2778 was selected for Phase I clinical trials.

This Phase I and pharmacokinetic study evaluates BBR

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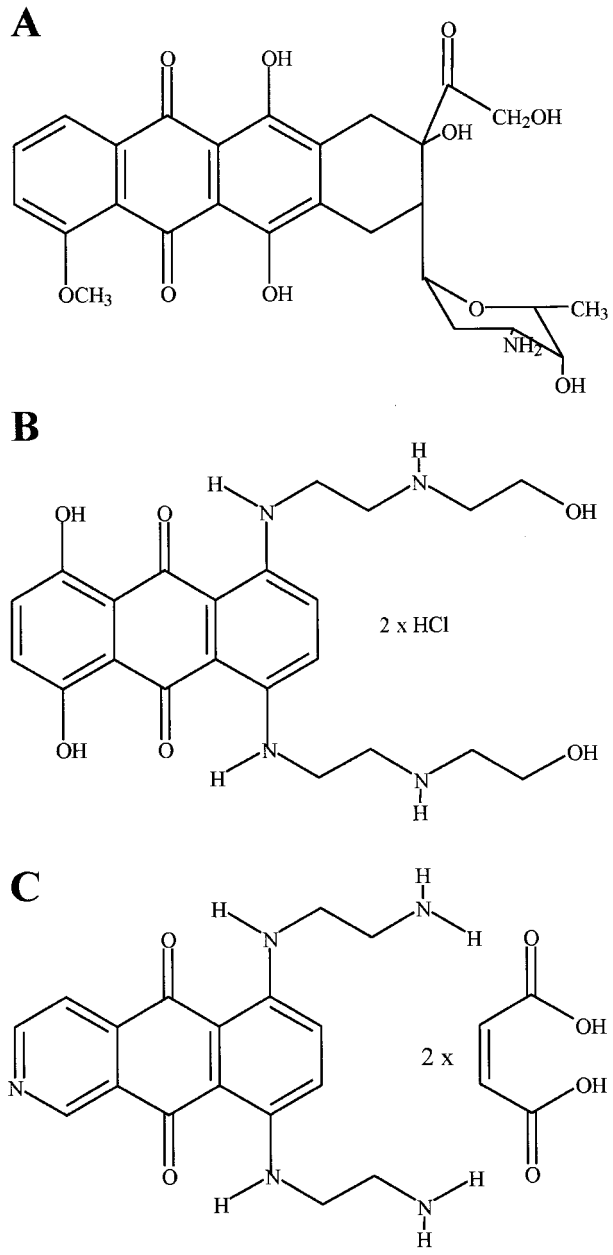

Fig. 1 Chemical structures of doxorubicin (*A*), mitoxantrone (*B*), and BBR-2778 (*C*).

Table 1 Patient characteristics

No. of patients	30
No. of cycles	54
Median (range)	2 (1–6)
Male/female	16/14
Median age (range)	56 yr (29–75)
WHO performance status	
0	13
1	12
2	5
Prior treatment	
Chemotherapy[a]	13
Chemotherapy[a] and radiation therapy	14
Radiation therapy	3
Immunotherapy	6
Tumor type	
Colorectal carcinoma	2
Gastric carcinoma	2
Ovarian carcinoma	2
Breast carcinoma	2
Lung cancer	
NSCLC[b]	4
SCLC[b]	3
Mesothelioma	1
Sarcoma	5
Melanoma	1
Renal carcinoma	3
Head and neck carcinoma	4[c]
Germ cell tumor	1

[a] Anthracycline-based chemotherapy (n = 14).
[b] NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.
[c] Including one facial cylindroma.

Table 2 Dose escalation scheme

BBR 2778 dose level (mg/m^2/week)	No. of patients	No. of cycles
Initial modified Fibonacci		
5	4	9
10	3	3
16.5	3	5
25	6	9
37.5	1	1
Accelerated dose escalation based on toxicity and PK[a]		
75	4	9
150[b]	3	4
112.5	6	18
Total	30	58

[a] PK, pharmacokinetics.
[b] MTD.

2778 given in a weekly 1-h infusion schedule to patients with solid tumors. The primary objective was to determine the MTD.[2] Secondary objectives were: (*a*) to define the DLT; (*b*) to recommend a dose for further Phase II studies in solid tumors; (*c*) to characterize the pharmacokinetic profile of BBR 2778; and (*d*) to make a preliminary investigation of antitumor activity.

[2] The abbreviations used are: MTD, maximum tolerated dose; DLT, dose-limiting toxicity; CTC, common toxicity criteria.

PATIENTS AND METHODS

Eligibility. Patients with a histologically documented, progressive malignancy for which conventional treatment had failed were eligible for this study. Other eligibility criteria included: (*a*) 18–75 years of age; (*b*) WHO performance status 0–2; (*c*) no radiotherapy, nitrosourea, or mitomycin D within the 6 weeks preceding the study entry; (*d*) no chemotherapy within the 4 weeks preceding the study entry; (*e*) adequate hematopoietic (WBC count ≥4,000/μl, absolute neutrophil count of ≥1500, and platelet count of ≥100,000/μl), hepatic

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Table 3 Hematological toxicities in all therapy cycles

	BBR dose level (mg/m^2/week)							
	5 (9 cy)[a]	10 (3 cy)	16.5 (5 cy)	25 (9 cy)	37.5 (1 cy)	75 (9 cy)	112.5 (18 cy)	150 (4 cy)
Neutropenia								
Grade II							6/18	
Grade III							7/18	1/4
Grade IV							2/18	2/4
Leukopenia								
Grade II						1/9	6/18	2/4
Grade III							8/18	1/4
Grade IV							1/18	
Thrombocytopenia								
Grade II								
Grade III						1/9		
Grade IV								
Anemia								
Grade II						1/9	3/18	
Grade III	2/9[b]				1/1[b]			
Grade IV				1/9[b]				

[a] cy, cycle(s).
[b] Preexisting grade II anemia at study entry.

(normal total bilirubin level of ≤17 μmol/l or ≤9.9 mg/l, and ALT levels ≤1.25 times upper normal limit), and renal function (calculated creatinine clearance, ≥60 ml/min); (*f*) the use of a reliable contraception for women of child-bearing age; (*g*) no acute infectious or cardiac disease; (*h*) the absence of brain metastasis or primary brain tumor; and (*i*) the absence of persistent toxicity related to prior therapy. There was no restriction on the amount of prior anthracyclin/anthracenedione and on the initial heart ejection fraction value of eligible patients. All patients gave written informed consent according to institutional and national guidelines before treatment.

Study Design. This study was performed according to French drug regulations and compliant with the principles of the Helsinki declaration. The protocol was approved by the ethics committee of the University of Kremlin-Bicêtre, Bicêtre, France. Assessments undertaken prior to treatment included physical exam with vital signs, complete blood cell count with WBC count differential, serum chemistry (sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, urea, uric acid, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, and albumin), calculated creatinine clearance, urinalysis, electrocardiogram, evaluation of left ventricular function by cardiac ultrasound or scintigraphy, chest X-ray, and tumor measurement by ultrasound, computed tomographic scan, and/or magnetic resonance imaging as appropriate. During treatment, patients had weekly assessments, including all of the laboratory tests undertaken at baseline. Physical examination, evaluation of drug-related toxicity according to the National Cancer Institute-CTC version 1 criteria (15), blood hematology, and biochemistry were assessed before each new treatment cycle. Evaluation of left ventricular function was repeated after cycles 2, 4, and 6 as applicable. Response evaluation was performed according to the WHO criteria (16) in patients with measurable disease after every second treatment cycle. Patients were withdrawn from the protocol in case of tumor progression.

Drug Administration. BBR 2778 was supplied by Novuspharma (Monza, Italy) in vials that contained 0 or 50 mg of lyophilized sterile drug. Immediately before infusion, the drug was diluted in 500 ml of normal saline solution. BBR 2778 was administered as an i.v. infusion over 60 min on days 1, 8, and 15, cycles being repeated every 28 days. Individual therapy was continued until evidence of disease progression or the occurrence of a DLT.

Dose Escalation Procedures, MTD, and DLT. The starting dose of BBR 2778 was set at one-tenth of the LD_{10}, *i.e.*, 5 mg/m^2/week. Dose escalation initially proceeded according to a modified Fibonacci scheme; later, an accelerated scheme based on toxicity and pharmacokinetics data were used (see "Results"). The decision to escalate the dose was based on toxicity observed in the first cycle. No dose escalation in individual patients was allowed. Toxicity was graded using National Cancer Institute-CTC criteria. In this study, DLT was defined as any of the following events occurring during the first course of treatment: (*a*) grade III nonhematological toxicity (excluding alopecia, nausea, and vomiting); (*b*) grade IV vomiting; (*c*) grade IV hematological toxicity of >4-day duration; and (*d*) grade III hematological toxicity of >4-day duration and/or associated with neutropenic sepsis. If one of the initial three patients had ≥CTC grade 2, further evaluation of toxicity at this level was performed by treating three additional patients. The MTD was defined as the dose associated with an incidence of DLT in two-thirds of the patients or in more than or equal to two of six patients. The recommended dose was defined as the dose escalation step immediately before MTD.

Pharmacokinetic Study. Starting at the 5 mg/m^2/week dose level, plasma and urine were sampled during the first cycle in 23 patients to determine the total BBR 2778 concentration. Peripheral blood (8 ml) was collected in heparinized tubes immediately before drug administration and 15, 30, 60, and 90 min, then 2, 3, 4, 6, 8, and 24 h from the start of the infusion. Whole-blood samples were immediately centrifuged (1500 × *g* for 15 min), and plasma was frozen at −20°C for subsequent

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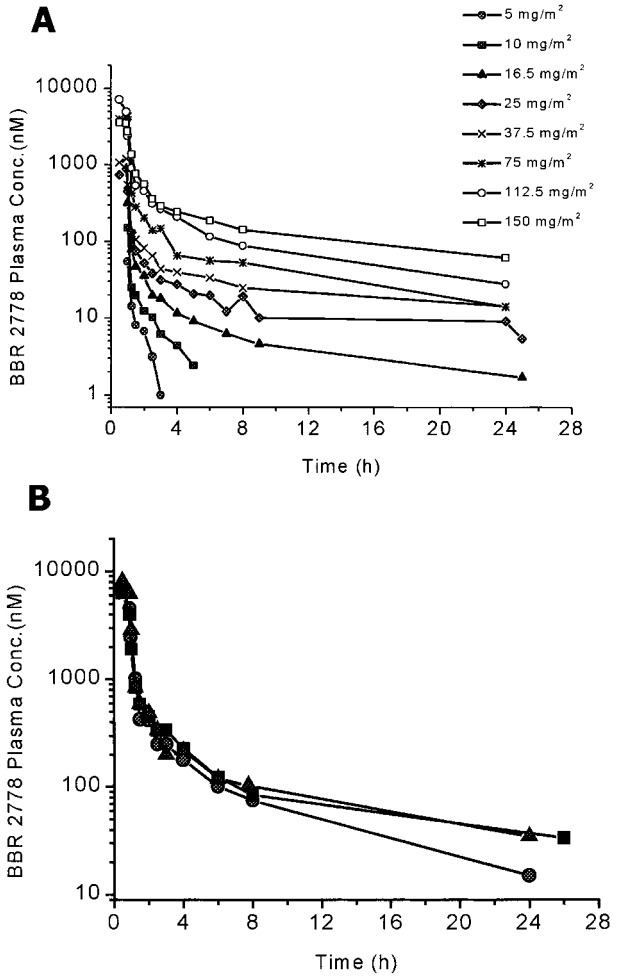

Fig. 2 Concentration × time profile of BBR-2778. *A*, mean plasma concentration-time profiles of BBR 2778 in patients after i.v. infusion of 5, 10, 16.5, 25, 37.5, 75, 112.5, and 150 mg/m^2. *B*, individual plasma concentration-time profiles of BBR 2778 in three patients after i.v. infusion of 112.5 mg/m^2 (recommended dose).

analysis. Twenty-four-h (H24) urine collections were made at baseline (timing, H24 →H0) and during the first treatment day (timing, H0→H4; H4→H8; H8→H24) for the measurement of drug excretion. Total volume of each urine fraction was accurately measured, and 100 ml of those aliquots were stored at −20°C until analysis.

Analytical Methods. BBR 2778 in plasma and urine was determined using validated methods. A column-switching high-performance liquid chromatography method using a Supelco ABZ column (250 × 2.1-mm; 5 μm) as analytical column, and a Supelco Supelguard ABZ column (20 × 4.6-mm; 5 μm) as auxiliary column was developed for the BBR 2778 assay in human plasma. Samples (1 ml), spiked with the internal standard (BBR 2952), were brought to pH 9 with sodium acetate buffer and extracted with dichloromethane (8 ml). After centrifugation, the organic phase was separated, added to 85% formic acid (100 μl), and dried under a nitrogen stream at room temperature. The residue was redissolved in 50 mM of pH 3 potassium phosphate buffer (300 μl). Aliquots ranging from 25 to 100 μl were injected onto the auxiliary column equilibrated with 0.05 M potassium buffer pH 5:acetonitrile:methanol (92:4:4, vol/vol/vol). After we briefly (5 min) washed the auxiliary column, the drug and internal standard were eluted with 0.05 M potassium phosphate buffer pH 3:acetonitrile (93:7, vol/vol) and transferred to the analytical column, where the chromatographic separation occurred. For plasma, the limit of quantitation of the method was 2 ng/ml, and the limit of detection was 1 ng/ml. The response was linear for plasma concentrations ranging from 2 to 250 ng/ml. Accuracy and precision were good; accuracy varied between 98.4 and 100.0%, and precision varied between 5.0 and 10.3%. The method was applicable to urine samples with minor changes. In urine, the response was linear for concentrations ranging from 50 to 5000 ng/ml, the limit of quantitation was 50 ng/ml, and the accuracy and precision ranged from 101.4 to 103.7% and 2.0 to 3.7%, respectively.

Data Analysis. Individual plasma concentration-time profiles were determined using a model-independent approach (WinNonlin Pro version 2.1; Pharsight Co., Mountain View, CA). The pharmacokinetic parameters analyzed were the maximum plasma concentration (C_{max}), the area under the plasma concentration-time curve (*AUC*), systemic clearance (*CL*), the volume of distribution in the postdistributive phase (V_z), and at steady state (V_{ss}), the renal clearance (CL_R), the terminal half-life ($t_{1/2,z}$), and the mean residence time (*MRT*). The elimination rate associated to the terminal phase, λ_z, was estimated by linear regression of the log plasma concentrations as a function of time in the monoexponential terminal part of the curve. The corresponding half-life, $t_{1/2,z}$, was calculated as $t_{1/2,z} = \ln(2)/\lambda_z$. The area under the curve from the time zero to the last observation point, AUC_{tf}, was estimated using the trapezoidal rule. The AUC to infinity, *AUC*, was calculated by adding to AUC_{tf} the additional term $AUC_{(tf-\infty)} = C_{tf}/\lambda_z$, where C_{tf} represents the observed plasma concentration measured at the last time, t_f, at which BBR 2778 was measurable. The estimates of the systemic clearance, *CL*, the volume of distribution in the postdistributive phase, V_z, and at steady state, V_{ss}, were obtained as: $CL = D/AUC$, $V_z = CL/\lambda_z$, and $V_{ss} = CL \times MRT$. From urine concentration data, we evaluated the fraction of excreted dose, *fe*, over a 24-h interval. The renal clearance, CL_R, was calculated according to: $CL_R = Ae_{(0-24)}/AUC_{(0-24)}$, where $Ae_{(0-24)}$ is the amount of drug excreted unchanged in urine in the 24 h after dosing, and $AUC_{(0-24)}$ is the area under the curve to 24 h calculated by the linear trapezoidal rule. When the concentration at H24 was not available, it was interpolated or extrapolated.

RESULTS

A total of 30 patients, whose characteristics are summarized in Table 1, were treated with BBR 2778 over eight dose levels and a total of 58 cycles (Table 2). All of the patients were evaluable for toxicity. Fourteen of the 30 patients had received both prior radiation therapy and chemotherapy, whereas 13 patients were treated previously with chemotherapy alone. In 14 cases, chemotherapy included anthracyclines (doxorubicin or epirubicin). The median number of cycles administrated to each subject was 2 (range, 1–6). Initially, the following dose levels were investigated: 5, 10, 16.5, and 25 mg/m^2/week, adminis-

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Table 4 Mean (SD) values of pharmacokinetic parameters of BBR 2778 in patients after 1-h i.v. administration of increasing doses

	Dose (mg/m^2)							
	5 (n = 4)	10 (n = 3)	16.5[a] (n = 3)	25 (n = 6)	37.5 (n = 1)	75 (n = 1)	112.5 (n = 3)	150 (n = 2)
C_{max} (nM)	54.4	150.6	318.1	701.4	1193.0	4297.6	7120.8	3852.1
	(8.0)	(55.7)	(128.3)	(385.3)	(0.0)	(0.0)	(914.1)	(478.2)
AUC_{tf} (nM·h)	46.0	127.1	353.2	903.6	1491.4	4847.7	7475.9	6835.7
	(10.2)	(43.7)	(119.6)	(389.3)	(0.0)	(0.0)	(908.3)	(897.9)
AUC (nM·h)			502.6	1074.8	1773.4	5027.3	7882.3	8058.5
			(0.0)	(463.7)	(0.0)	(0.0)	(1037.8)	(2119.7)
$t_{1/2,z}$ (h)			11.23	16.46	13.77	8.89	9.50	11.91
			(0.0)	(5.55)	(0.0)	(0.0)	(2.54)	(6.06)
MRT (h)			10.89	11.42	10.40	3.52	4.56	9.85
			(0.0)	(2.36)	(0.0)	(0.0)	(1.61)	(6.24)
CL (l/h/kg)			1.77	1.31	0.96	0.75	0.81	0.90
			(0.0)	(0.71)	(0.0)	(0.0)	(0.09)	(0.18)
V_z (l/kg)			28.62	29.49	19.06	9.66	10.95	14.66
			(0.0)	(15.22)	(0.0)	(0.0)	(2.17)	(4.77)
V_{ss} (l/kg)			19.23	15.19	9.98	2.65	3.63	8.29
			(0.0)	(9.07)	(0.0)	(0.0)	(1.08)	(3.84)
fe(0–24) (%)	6.93	5.48	7.19	6.09	6.34	8.26	6.02	9.24
	(2.42)	(1.99)	(1.40)	(2.04)	(0.0)	(0.0)	(1.05)	(0.97)
CL_R (ml/h/kg)			142	89	72	64	52	94
			(0)	(36)	(0)	(0)	(9)	(9)

[a] For the 16.5 mg/m^2 dose level, n = 1 for *AUC*, *AUC/D*, $t_{1/2,z}$, *MRT*, V_z, and V_{ss} values.

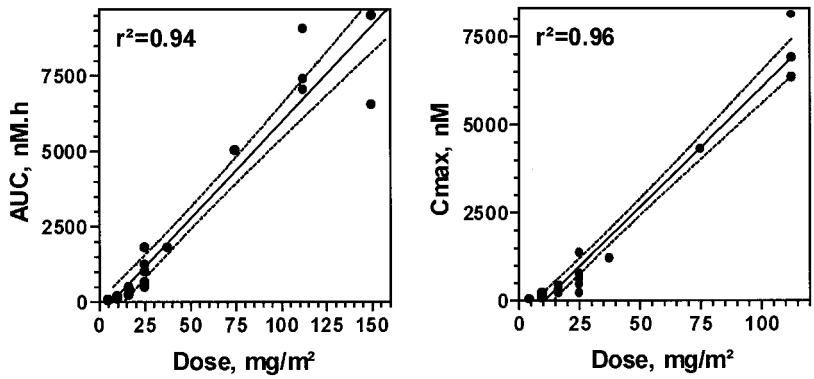

Fig. 3 Pharmacokinetic parameters of BBR 2778. *Left panel*, plot of mean concentrations according to dose. *Right panel*, plot of AUC values according to dose.

tered 3 consecutive weeks of 4. Subsequently, the absence of toxicity and the low plasma concentrations observed prompted us to dose escalate using an accelerated scheme, doubling the dose and including only one patient at each dose level until a grade ≥II toxicity occurred. Thus, the next dose levels were 37.5, 75, and 150 mg/m^2/week, 3 consecutive weeks of 4. Because the MTD occurred at the dose level of 150 mg/m^2/week, the recommended dose was sought via de-escalation to the intermediate level of 112.5 mg/m^2/week. A dose-dependent myelosuppression was the principal toxicity of this regimen.

Hematological Toxicity. Myelosuppression was the most common toxicity observed with BBR 2778. Neutropenia was the DLT of this regimen. Hematological toxicity is summarized in Table 3. There was no evidence of cumulative toxicity in patients treated repeatedly at the same dose level. The nadir of neutrophil count was typically observed on day 14. The duration of grade III–IV neutropenia was <7 days, and the neutrophil count was normalized by day 28. Only mild hematological toxicity was observed up to the 75 mg/m^2/week dose level. At the dose of 150 mg/m^2/week, two episodes of dose-limiting neutropenia, including grade III neutropenia lasting for >4 days and grade IV neutropenia associated with sepsis, were observed in cycle 1. Therefore, this dose was considered to be the MTD. In addition, the third chemo-naive patient treated at 150 mg/m^2/week presented a grade IV febrile neutropenia during cycle 2. At the dose of 112.5 mg/m^2/week, one patient presented a grade IV febrile neutropenia at cycle 1 without evidence of sepsis, and another patient had asymptomatic grade 4 neutropenia lasting <4 days. Given that the incidence of DLT was only one of six patients, this dose level was defined as the recommended dose. At that dose level, hematological toxicity remained the most frequent treatment-related cause of discontinuation. The day 15 infusion was omitted in 10 of 18 cycles (55.5%) administrated to the six patients at the recommended dose (7 of 10 because of hematological toxicity, 3 of 10 for other reasons, such as patients refusal or early progression). In contrast, thrombocytopenia was infrequent and always ≤grade II, except for one patient treated at the 75 mg/m^2/week dose level,

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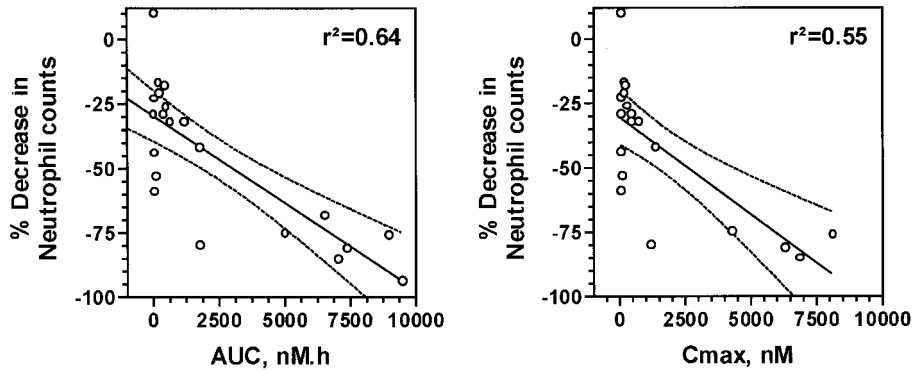

Fig. 4 Pharmacodynamic analysis. *Left panel*, percentage decrease in neutrophil counts according to *AUC* ($P < 0.0001$). *Right panel*, percentage decrease in neutrophil counts according to C_{max} ($P = 0.0003$).

who presented a grade III thrombocytopenia during the second cycle. Anemia was infrequent and moderate, except for three patients (grade III, two patients; grade IV, one patient), who had preexisting grade II anemia at study entry. Because of prior chemotherapy, most patients presented grade II–IV lymphopenia at the beginning of BBR 2778 administration and experienced slightly accentuated lymphopenia to grade III–IV. The hematological toxicity profile was similar between lightly and heavily pretreated patients (data not shown).

Nonhematological Toxicity. Grade I–II nausea and vomiting occurred in all patients receiving doses >75 mg/m^2/week but was never dose limiting. Such toxicity was consistently observed during the first infusion at the dose of 112.5 mg/m^2/week, was satisfactorily treated with ondansetron and prevented by the use of prophylactic antiemetic therapy with ondansetron before subsequent infusions. No mucositis was reported. Reversible alopecia was consistently observed at the dose of 112.5 mg/m^2/week and above. Because of the blue coloration of the BBR 2778 compound, a transient blue coloration of the skin and urine was observed at doses of 75 mg/m^2/week and above. From the dose of 112.5 mg/m^2/week and higher, the blue coloration of the skin was persistent between cycles. No clinical or functional cardiac side effect was reported. Six patients received a cumulative dose of >500 mg/m^2 (range, 560–1460 mg/m^2) of BBR 2778 without evidence of cardiac toxicity.

Pharmacokinetic and Pharmacodynamic Study. For doses of 25 mg/m^2 and higher, the individual plasma concentration-time profiles were completely described up to the last scheduled sampling time (24 h after the start of the infusion). Because of detection limits, BBR 2778 concentration measurements were feasible only over shorter intervals for doses of 5, 10, and 16.5 mg/m^2/week. For this reason, the apparent terminal rate constant and the derived parameters *AUC*, $t_{1/2,z}$, *CL*, V_z, and V_{ss} were estimated correctly only in subjects treated with 25 mg/m^2 or higher.

BBR 2778 concentrations decreased rapidly after the end of the infusion, following a typical biexponential kinetics. Fig. 2 shows the concentration × time profile for each dose level (mean values) and three individual patients treated at the recommended dose. As shown in Fig. 2, the AUC has been determined by extrapolation. The mean values of pharmacokinetic parameters are summarized in Table 4. Over the tested dose range, from 5 to 150 mg/m^2/week, a good correlation was observed between BBR 2778 dose, AUC_{tf}, and C_{max} values, according to linear regression analysis. From 25 to 150 mg/m^2/week, the values of $t_{1/2,z}$, *CL*, V_z, V_{ss}, *MRT*, *fe*, and CL_R remained relatively constant, whereas the *AUC* increased proportionally with the dose (Fig. 3), with only a moderate interpatient variability, consistent with a linear kinetics for BBR 2778 in the tested dose range. The drug displays a large volume of distribution, exceeding the body weight, suggesting a high penetration of BBR 2778 into intra- and extracellular body fluids associated with an accumulation in the tissue compartments. This is consistent with the prolonged blue coloration of the skin observed at doses ≥112.5 mg/m^2/week. BBR 2778 has a high plasma clearance and a long elimination half-life. Renal excretion does not represent the major drug elimination route; the mean percentage of the administrated dose recovered as unchanged drug in the 24 h urine sample ranged on between 5.5 and 9.2%.

The DLT of BBR 2778 was shown to be hematological toxicity, and although the number of points was limited, a significant correlation was observed between the percentage decrease of neutrophils and both *AUC* and C_{max} (Fig. 4).

Antitumor Activity. Three patients experienced objective responses. A 57-year-old female with breast cancer whose metastatic disease involving the bone had progressed during prior chemotherapy with paclitaxel, 5-fluorouracil, and folinic acid showed complete clinical disappearance of pain, biological normalization of alkaline phosphatases, and CA 15.3 levels lasting 7 months after treatment with BBR 2778 at the 112.5 mg/m^2/week dose level. This patient, who had received a cumulative epirubicin dose of 400 mg/m^2 as therapy for local relapse prior to metastatic dissemination, showed no evidence of cardiac toxicity subsequent to treatment with BBR 2778. A 49-year-old female with facial cylindroma, previously untreated by chemotherapy, showed a partial response of the local site of relapse (cavum), as measured by magnetic resonance imaging, and a stabilization of lung and liver metastatic sites after two cycles of 112.5 mg/m^2/week of BBR 2778. This response lasted for 6 months. A 57-year-old patient with a small cell lung cancer with hepatic metastasis presented a partial response after two cycles of 75 mg/m^2/week BBR 2778, sustained after the third cycle. However, this patient was withdrawn from the study after the third cycle for the occurrence of brain metastasis.

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DISCUSSION

The identification of new compounds structurally related to anthracyclines represents a critical challenge in the improvement of acute and long-term cardiac tolerance of active therapeutic approaches, particularly in patients with breast cancer and lymphoma. With this aim, the anthracenedione family was developed >10 years ago and was found to be associated with a lower degree of cardiac and gastrointestinal side effects. However, preclinical models and careful follow-up of mitoxantrone-treated patients have shown that this therapeutic agent was not totally devoid of cardiac side effects (17, 18). More recently, BBR 2778, the lead compound of the aza-anthracenediones, was found to not cause any histopathological alteration of cardiac tissues when repeatedly administrated in rodent models. The reason for this absence of toxicity remains uncertain, possibly involving the differential lipophilic characteristics of the compounds (19).

We report here the results of a Phase I study of BBR 2778 administrated as a 1-h i.v. infusion, 3 consecutive weeks of 4. The MTD of this schedule was 150 mg/m^2/week, because two of three patients experienced a DLT consisting of febrile and/or severe grade III–IV reversible neutropenia. The nadir neutrophil count occurred at day 14, and recovery occurred by day 28. This toxicity did not appear to be cumulative. Two other Phase I studies exploring different schedules were simultaneously performed. Neutropenia was the limiting toxicity in these studies, where BBR 2778 was administrated weekly in patients with hematological malignancies or given as a 1-h infusion every 3 weeks in patients with solid tumors (20). In our study, as well as in the studies performed with alternate schedules, other hematological or nonhematological toxicities were mild to moderate. It is notable that no mucositis occurred in this study. Importantly, no clinical cardiac side effects were reported, even in patients receiving a cumulative dose of >500 mg/m^2 of BBR 2778, who included two patients pretreated with anthracyclines. These results suggest an improvement in the tolerance profile in comparison to classical anthracycline compounds, but this requires further confirmation in the frame of phase II/III trials including careful cardiac monitoring.

In this study, the recommended dose for Phase II studies was 112.5 mg/m^2/week. Neutropenia was the main limiting factor for treatment dose density because three of six patients experienced treatment delay for neutropenia at day 15 (44.5% of day 15 infusions). Thus, although the recommended dose of 112.5 mg/m^2/week for 3 consecutive weeks was suitable for half of the patients, the injection might be cancelled in patients that experienced an absolute neutrophil count <1500/μl at day 15.

For doses ranging from 25 to 150 mg/m^2/week, BBR 2778 pharmacokinetics were linear. The distribution and elimination patterns do not appear to be affected by the dose increase from 25 to 150 mg/m^2. BBR 2778 is a high clearance drug. Preclinical studies showed that fecal excretion is the principal elimination route for [^{14}C]BBR 2778. Eight days after dosing, cumulative radioactivity excreted in urine and feces reached, respectively, 15.2 and 39.5% of the administered dose in male mice and 5.0 and 35.5% in rats (Novuspharma; data on file). Cumulative urinary excretion of unchanged BBR 2778 in male mice and rats accounted for 10.9 and 1.6% of the administered dose, respectively (Novuspharma; data on file). Data in humans on the excretion of unchanged BBR 2778 in urine are consistent with animal data; the renal excretion of the unchanged drug appears to be a minor elimination route for BBR 2778. Most likely, excretion in the bile and metabolism accounts for most of elimination of the administered dose. A clearance of 0.90 l/h/kg was observed after administration of the MTD dose of 150 mg/m^2, approaching the sum of kidney (Q_R, 0.47 l/h/kg) and liver (Q_H, 0.71 l/h/kg) plasma flows reported for humans (21). Considering that the renal clearance of BBR 2778 (CL_R) approaches 0.1 l/h/kg, we may calculate a renal extraction ratio (E_R) of 0.2 from the relationship $E_R = CL_R/Q_R$. This means that BBR 2778 is extracted from the kidney with a modest efficiency, as confirmed by urinary excretion data. The nonrenal clearance, CL_{NR}, which is the difference between the systemic clearance and the renal clearance, is therefore much higher than the renal clearance (CL_{NR}, 0.8 L/h/kg) and is similar to the hepatic plasma flow, suggesting hepatic extraction as described previously for mitoxantrone (22).

In vitro studies have shown that BBR 2778 is in fact only weakly bound to plasma proteins. The unbound fraction in humans is 0.43 (Novuspharma; data on file). Therefore, binding is not expected to affect the rate and extent of BBR 2778 elimination by the liver and kidneys. The values of V_z and V_{ss} are high and suggest a high penetration of BBR 2778 into intra- and extracellular body fluids and accumulation in the body, a property shared by many anticancer drugs and by mitoxantrone (22). The estimates of Vss are lower than those of Vz. This means that a significant part of the drug elimination occurs during the distribution process. The pharmacodynamic study shows a significant correlation between the percentage of decrease of neutrophils (nadir) and both C_{max} and *AUC, i.e.,* the administrated dose of BBR-2778.

Although clinical activity was not the primary end point of this study, BBR 2778 achieved antitumor activity in the 75–150 mg/m^2/week dose range. Activity was reported in a patient with anthracycline-pretreated breast cancer, who experienced a complete relief of clinical symptoms and a normalization of biological parameters during 7 months of treatment, with no cardiac toxicity despite a cumulative dose of 1460 mg/m^2 of BBR 2778. Of note, this patient obtained no benefit from subsequent treatment with mitoxantrone. In preclinical models, <0.05% of the administrated dose was found in the brain of mice and rats, suggesting that BBR 2778 does not cross the blood-brain barrier (14). This was supported by the clinical development of brain metastasis in a patient with small cell lung cancer who concomitantly experienced a partial response on target tumor sites outside the central nervous system.

In summary, this trial demonstrates that BBR 2778 has a manageable toxicity profile on a weekly schedule, 3 consecutive weeks of 4, and recommends the dose of 112.5 mg/m^2/week for Phase II trials, with optional administration at day 15 determined on an individual basis. This compound shows promising antitumor activity and should be investigated in patients at high risk of cumulative cardiotoxicity, such as anthracycline-pretreated breast cancer and lymphoma patients.

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REFERENCES

1. Tormey, D. C. Adriamycin (NSC-123 127) in breast cancer: an overview of studies. Cancer Chemother. Rep., *6:* 319–327, 1975.

2. Von Hoff, D. D., Layard, M. W., Basa, P., Davis, H. L., Jr., Von Hoff, A. L., Rozencweig, M., and Muggia, F. M. Risk factors for doxorubicin-induced congestive heart failure. Ann. Intern. Med., *91:* 710–717, 1979.

3. Ryberg, M., Nielsen, D., Skovsgaard, T., Hansen, J., Vittrup Jensen, B., and Dombernowsky, P. Epirubicin cardiotoxicity: an analysis of the 469 patients with metastatic breast cancer. J. Clin. Oncol., *16:* 3502–3508, 1998.

4. Lopez, M., and Vici, P. European trials with dexrazoxane in amelioration of doxorubicin and epirubicin-induced cardiotoxicity. Semin. Oncol., *25:* 55–60, 1998.

5. Krapcho, A. P., Petry, M. E., Getahun, Z., Landi, J. J., Stallman, J., Polsenberg, J. F., Gallagher, C. E., Maresch, M. J., Hacker, M. P., Giuliani, F. C., Beggiolin, G., Pezzoni, G., Menta, E., Manzotti, C., Oliva, A., Spinelli, S., and Tognella, S. 6,9-Bis[(aminoalkyl)amino]benzo[*g*]isoquinoline-5,10-diones: a novel class of chromophore-modified antitumor anthracenediones. Synthesis and antitumor evaluations. J. Med. Chem., *37:* 828–837, 1994.

6. Hazlehurst, L. A., Krapcho, A. P., and Hacker, M. P. Comparison of aza-anthracenedione-induced DNA damage and cytotoxicity in experimental tumor cells. Biochem. Pharmacol., *50:* 1087–1094, 1995.

7. De Isabella, P., Palumbo, M., Sissi, C., Capranico, G., Carenini, N., Menta, E., Oliva, A., Spinelli, S., Krapcho, A. P., Giuliani, F. C., and Zunino, F. Topoisomerase II DNA cleavage stimulation, DNA binding activity, cytotoxicity and physico-chemical properties of 2-aza and 2-aza-oxide-anthracenedione derivative. Mol. Pharmacol., *30:* 30–38, 1995.

8. National Cancer Institute. Report of National Cancer Institute Development Therapeutics Program In-Vitro Screening Data Review Checklist, 1995.

9. De Isabella, P., Capranico, G., Beggiolin, G., Pezzoni, G., Giuliani, F. C., and Zunino, F. Ridotto accumulo intracellulare del mitoxantrone in cellule resistenti HT29, che non exprimono la GP170. Atti del XII Congresso della Societa Italiana di Cancerologia, Stresa-Baveno, Italy, abstract 66, 1994.

10. Von Hoff, D. D., and Degen, D. Investigation of the effects of BBR 2778 on human tumor colony forming units. Final Report of Institute for Drug Development, 1995.

11. Beggiolin, G., Pezzoni, G., and Giuliani, F. G. BBR 2778 (6,9-bis{[(2-amino)ethyl]amino}benzo[*g*]isoquinoline-5,10-dione dimaleate): *in vitro* cytotoxic activity. BM Italy Report, 02/ONC, 1994.

12. Manzotti, C., Pezzoni, G., and Giuliani, F. G. BBR 2778 (6,9-bis{[(2-amino)ethyl]amino}benzo[*g*]isoquinoline-5,10-dione dimaleate): *in vivo* antitumor activity. BM Italy Report, 03/ONC, 1994.

13. Crippa, L. Pivotal intravenous single dose toxicity study in the male mouse. BM Italy Internal Report, 1994.

14. Crippa, L. BBR 2778 and mitoxantrone: comparative delayed cardiotoxic effect after repeated treatment in mice. BM Italy Internal Report, 1993.

15. Guidelines for Reporting of Adverse Drug Reactions. Bethesda, Maryland, National Cancer Institute, Division of Cancer Treatment, 1988.

16. WHO. *In:* WHO Handbook for Reporting Results of Cancer Treatment, No 40. Geneva: WHO, 1979.

17. Aviles, A., Arevila, N., Diaz Maqueo, J. C., Gomez, T., Garcia, R., and Nambo, M. J. Late cardiac toxicity of doxorubicin, epirubicin, and mitoxantrone therapy for Hodgkin disease in adults. Leuk. Lymphoma, *11:* 275–279, 1993.

18. Viglione, P. N., Praprotnick, A., and Pinto, J. E. *In vitro* evaluation of acute effects of mitoxantrone (Novantrone) in rat and guinea pig atria. Pharmacol. Toxicol., *72:* 208–212, 1993.

19. Andersson, B. S., Eksborg, S., Vidal, R. F., Sunberg, M., and Carlberg, M. Anthraquinone-induced cell injury: acute toxicity of carminomycin, epirubicin, idarubicin and mitoxantrone in isolated cardiomyocytes. Toxicology, *135:* 11–20, 1999.

20. Faivre, S., Borchmann, P., Jodrell, D., Camboni, G., Bernareggi, A., Raymond, E., Imadalou, K., Engert, A., and Diehl, V. BBR2778 a new anthracenedione analogue with no preclinical cardiac toxicity: results from three Phase I studies. Proc. Am. Assoc. Cancer Res., *40:* 67, 1999.

21. Guyton, A. C. Textbook of Medical Physiology, p. 279. Philadelphia: W. B. Saunders, 1996.

22. Ehninger, G., Schuler, U., Proksch, B., Zeller, K. P., and Blanz, J. Pharmacokinetics and metabolism of mitoxantrone: a review. Clin. Pharmacokinet., *18:* 365–380, 1990.

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