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Phase I and pharmacokinetic study of 502U83 (an arylmethylaminopropanediol) in cancer patients

Kit S Lam,[CA] David S Alberts, Yei-Mei Peng, Frank Brodar, Betty Matias, Manuel Modiano, R Tuttle, V Sol Lucas and W Wargin

KS Lam, DS Alberts, Y-M Peng and M Modiano are at both the Section of Hematology and Oncology, Department of Medicine, and the Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, USA. Tel: 602-626-7925. Fax: 602-626-2284. F Brodar and B Matias are at the Section of Hematology and Oncology, Department of Medicine, University of Arizona, Tucson, AZ 85724, USA. R Tuttle, V Sol Lucas and W Wargin are at the Burroughs Wellcome Company, Research Triangle Park, NC 27709, USA.

502U83, a novel arylmethylaminopropanediol, has proven active *in vivo* against a panel of murine leukemia and solid tumors as well as in a tumor clonogenic assay against a variety of fresh human cancers. A total of 35 previously treated cancer patients were enrolled in a phase I study of this compound. The maximally tolerated dose (MTD) appears to be 12 800 mg/m²/72 h by continuous intravenous infusion with severe granulocytopenia occurring in three of five patients. There were no objective clinical responses. Serum pharmacokinetic parameters were as follows: plasma terminal phase half-life ($t_{1/2}\beta$) = 3.84 h; total body clearance (CL_B) = 53.1 l/h/m²; volume of distribution at steady state (V_{dss}) = 127.9 l/m²; maximum plasma concentration (C_{max}) = 3.7 μg/ml (at 12 800 mg/m²/72 h dose).

Key words: Arylmethylaminopropanediol, chemotherapy, phase I

Introduction

502U83,[1] an arylemethylaminopropanediol (AMAP), is a DNA intercalating agent. The chemical structure of the drug is shown in Figure 1. It is one of four AMAPs that have undergone clinical trial. The other compounds are 770U82, 773U82 and 7U85. These compounds have a common methylaminopropanediol side chain linked to different carbocyclic or heterocyclic rings[1-3] with 502U83 having an anthracene ring. Although the AMAPs are DNA intercalators,[1] DNA intercalation alone does not account fully for their antitumor effects. It has been reported[4] that the ratio of DNA-protein crosslinks and DNA single strand breaks is 1:1 for 502U83 and the other AMAPs and the frequency of these DNA damage events correlates closely with the cytotoxicity patterns of these drugs. These observations suggest that the AMAPs may have inhibitory effects on the activity of DNA topoisomerase II.

502U83 has shown activity in an array of commonly used murine tumor screening systems, including the P338 mouse leukemia model. In addition, it is active against several tumors of the NCI panel, including L1210 leukemia, B16 melanoma, Lewis lung carcinoma, M5076 sarcoma and colon 38 carcinoma, although it proved inactive against the MX-1 mammary carcinoma.[2] Furthermore, it is active against a variety of fresh human cancers in the human tumor clonogenic assay. The drug is active via either intravenous, oral or intraperitoneal routes.

During preclinical studies, it was discovered that in addition to its antitumor properties, 502U83 has

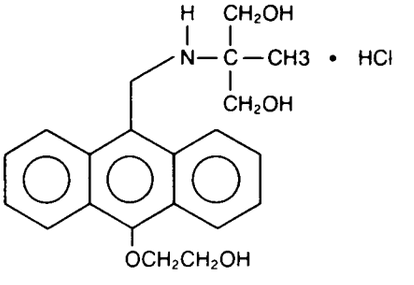

Figure 1. Chemical structure of 502U83.

[CA] Corresponding Author

local anesthetic-like activity in guinea pigs and mild negative chronotropic effects in isolated rat hearts or guinea pig atria.

The objectives of this phase I trial of 502U83 were to: (i) determine its maximally tolerated dose (MTD), (ii) determine its qualitative and quantitative toxicities, (iii) determine its pharmakokinetics and metabolism, and (iv) to evaluate clinical response.

Patients and methods

Eligibility

All patients had to have a confirmed diagnosis of metastatic cancer for which more effective regional or systemic therapy was not available. Additionally, they had to have a Karnofsky performance status of 60% or better and had to have been off previous anticancer therapy for at least 3 weeks. Patients with the following laboratory abnormalities were excluded from the study: WBC $< 3000/\mu l$, granulocytes $< 1500/\mu l$, platelets $< 100\,000/\mu l$, hemoglobin < 9 g/100 ml, bilirubin > 2.5 mg/dl, SGOT > 2 times upper limits of normal, alkaline phosphatase > 2 times upper limits of normal, creatinine > 2.0 mg/dl and blood glucose > 200 mg/dl. Additionally, patients with a significantly abnormal EKG (i.e. specifically heart block) or a history of significant heart disease were excluded from the study. The protocol was approved by the University of Arizona Human Subjects Committee. All patients enrolled in this study had informed consent.

Treatment Plan

502U83 was provided by Burroughs Wellcome (Research Triangle Park, NC). 502U83 was administered as a 72 h continuous infusion once every 3 weeks by infusion pump on an outpatient basis. The phase I dose escalation scheme was as follows: 400, 800, 1600, 3200, 4800, 6400, 9600 and 12800 mg/m^2/72 h. A dose level was considered safe if three patients were treated with less than moderate drug-related toxicities.

Criteria for toxicity and response

Toxicity was recorded according to World Health Organization criteria. In terms of clinical response, the following criteria were used: (i) complete response: total disappearance of all measurable tumor masses; (ii) partial response: at least a 50% reduction in the size of all measurable tumor masses; (iii) stable disease: neither progressive disease or objective response; and (iv) progressive disease: an increase of at least 50% in the size of all measurable tumor masses.

Pharmacokinetics

Blood samples (10 ml) were collected as follows: 0, 0.5, 1, 2, 4, 8, 24, 48 and 72 h of infusion, and at 10, 20, 40 and 60 min, 1.5, 2, 3, 4, 5, 6, 8 and 12 h post-infusion. The concentration of 502U83 in each plasma sample was measured by high performance liquid chromatography (HPLC). Prior to injection into the HPLC, each plasma sample was processed as follows: a 50 μl aliquot of methanol was added to a 0.5 ml aliquot of plasma sample. The sample was vortex-mixed and kept at 4°C for 10 min. The sample was then centrifuged at 2000 *g*, 4°C for 10 min to precipitate the plasma proteins. The resulting supernatant solution was further processed by a Vac-Elut^{TM} system with Bond-Elut^{TM} 1 ml C_2 mini-cartridges (Analytichem International, Harbor City, CA) which had been sequentially preconditioned by washing with 5 ml of methanol and 5 ml of water. After a 0.44 ml aliquot of the supernatant had passed through the C_2 cartridge, it was washed with 5 ml of water and the drug was eluted with a 200 μl aliquot of a cold 0.1 N methanolic HCl solution.

HPLC analysis was performed using a Perkin-Elmer LC series 4 solvent delivery system, a Perkin-Elmer ISS-100 autosampler, a Kratos Model 773 variable wavelength UV detector and a Nelson Analytical 3000 chromatography data station. A μBondapak C_{18} reverse-phase column (300 × 3.9 mm, particle size 10 μm, Waters Associates) preceded by a guard column (70 × 2.1 mm) packed with CO:Pell ODS (Whatman, Clifton, NJ) was used for all analyses.

502U83 was eluted isocratically at ambient temperature with acetonitrile and 0.2 M pH 2.3 phosphate buffer (16:84, v/v) as solvent at a flow rate of 2.5 ml/min; 502U83 was detected at 254 nm using a Kratos Model 773 variable wavelength detector.

Quantitation of 502U83 was done by the external standard method of analysis. 502U83 reference standard was obtained from Burroughs Wellcome (Research Triangle Park, NC). A plasma standard

calibration curve was obtained by plotting the resulting peak areas against the known concentration of standard added to a blank plasma sample. The standard curve was linear over the 25 to 5000 ng/ml concentration range tested. The average recovery of the assay was greater than 90% and the precision (coefficient of variation) was less than 7%.

Pharmacokinetic analysis

The 502U83 plasma concentration–time data were analyzed using a two-compartment model with zero-order infusion. Non-linear least-squares regression analysis was performed using NONLIN with a weighting function of 1/concentration. The estimates of the intercompartment rate constants and V_c were used to calculate the terminal phase half-life ($t_{1/2}\beta$), volume of distribution at steady state (V_{dss}), area under the plasma concentration–time curve (AUC) and plasma clearance (CL). The C_{max} was the highest 502U83 plasma concentration observed during the infusion.

Results

Patient characteristics

A total of 35 patients were enrolled in the study over a period of 15 months. Of these 35, 22 were male and 13 were female. The various tumor diagnoses are shown in Table 1. The dose and number of courses administered to each patient are summarized in Table 2.

Toxicity

502U83 was well tolerated, the major side effect, myelosuppression, became dose limiting in patients given 12 800 mg/m^2/72 h. The results are summarized in Table 3. Five of six patients treated with 502U83 experienced grade 3 or 4 granulocytopenia. Although grade 4 granulocytopenia (below 500/μl) was observed in one patient at 9600 mg/m^2/72 h, and two patients at 12 800 mg/m^2/72 h, none of these patients developed an infection requiring hospitalization. Nadir blood counts occurred between 10 and 14 days in most cases. The minor toxicities observed in some patients are summarized in Table 4. None of the patients required termination of therapy because of these toxicities. Splinter hemorrhages in the nail bed were observed in 11 of the 35 patients and did not appear to be dose related (see Figure 2). The MTD of 502U83 was considered 12 800 mg/m^2/72 h over 3 weeks, because it produced nearly consistent grade 3 granulocytopenia.

Table 1. Patients entered

Diagnosis	No. of patients
Colon carcinoma	18
Breast carcinoma	4
Ovarian carcinoma	3
Rectal carcinoma	3
Renal carcinoma	2
Pancreatic carcinoma	1
Esophagus carcinoma	1
Endometrial carcinoma	1
Adenocarcinoma of unknown primary	2
Total	35

Clinical response

Of the 35 patients, only two had evidence of a clinical response and these were mixed responses. Patient no. 11 had a 60% decrease in her large left supraclavicular mass that lasted for 2 months, but she had slight progression of pulmonary and abdominal metastases. Patient no. 13 had a 70% regression of her right lobe liver metastases that lasted for 2.5 months, but she had progression of her left lobe liver lesion (see Figure 3). There were no complete or partial responses in any of the 35 patients treated.

Pharmacokinetics

The pharmacokinetic results associated with doses of 400–12 800 mg/m^2 administered over 72 h are summarized in Table 5. Plasma concentration–time data from 21 patients was available for analysis. Plasma concentrations decayed in a biexponential manner after cessation of infusion. A plasma concentration-time profile for a typical patient (patient no. 32: 12 800 mg/m^2) is shown in Figure 4. The average $t_{1/2}\beta$ was 3.84 h, CL was 53.1 l/h/m^2 and V_{dss} averaged 127.0 l/m^2. At the highest dose tested the mean plasma C_{max} was 3.7 ug/ml and the mean AUC was 218 h μg/ml h.

Table 2. Summary of patients entered at dose level

Patient no.	No. of courses of 502U83 (mg/m²/72 h) given at								Total no. of courses
	400	800	1600	3200	4800	6400	9600	12800	
1	4	4							8
2	1								1
3	3	2							5
4	1								1
5		1							1
6		1							1
7		1							1
8			2						2
9			1						1
10			1						1
11				2					2
12				1					1
13				3					3
14						1			1
15					1	1[a]			2
16						3			3
17					2	2			4
18					2	1			3
19					1	1			2
20						2			2
21					2				2
22					1				1
23						1			1
24						2			2
25						4			4
26						3			3
27							1		1
28							1		1
29							1		1
30								1	1
31							3	1[b]	4
32								2	2
33								1	1
34								1	1
35								1	1
Total									71

[a] Because patient no. 15 developed facial and periorbital swelling, his second dose was cut back to 4800 mg/m²/72 h.
[b] Patient no. 31 developed grade II neutropenia and grade IV granulocytopenia, therefore his subsequent doses were cut back to 9600 mg/m²/72 h.

Table 3. Myelotoxicity resulting from 502U83 at the final two dose levels of the phase I study

	No. of courses with significant myelotoxicity at							
	9600 mg/m²/72 h				12800 mg/m²/72 h			
	grade 1	2	3	4	grade 1	2	3	4
Neutropenia	0	2	1	0	0	5	1	0
Granulocytopenia[a]	0	1	1	1	1	2	2	2
Thrombocytopenia	1	0	0	0	1	0	0	0
Anemia	1	0	1	0	1	0	0	0
Total no. of courses given		6[b]				7[c]		

[a] Nadir counts occurred between 10 and 14 days in most cases.
[b] Of the four patients tested at this dose level, all the myelotoxicities occurred in one patient, except for the grade 1 anemia which occurred in another patient.
[c] Five of the six patients tested at this dose level experienced some degree of myelotoxicities.

Figure 2. Splinter hemorrhage was observed in 11 of the 35 patients.

Discussion

We demonstrated in this study that 502U83 has a MTD or 12 800 mg/m^2/72 h by continuous infusion. The dose limiting toxicity is severe granulocytopenia. However, none of the patients developed an infection requiring hospitalization.

Two other phase I 502U83 trials at a different dose schedule were reported,[6,7] using a dose of up to 2000 mg/m^2 over 1 h infusion, the dose limiting toxicity was reversible PR, QRS, QT and QT (corrected) interval prolongation.[6] At 800 mg/m^2 when these EKG changes began to occur, the plasma C_{max} of these patients was about 10 μg/ml. However, we did not observe such consistent EKG changes in our phase I trial patients even at the highest dose level which was 12 800 mg/m^2/72 h, at which time the mean plasma C_{max} was 3–7 μg/ml. Similarly at a dose schedule of 8000 μg/m^2/24 h,[7] no EKG interval prolongation has been observed although the dose limiting toxicity at this dose schedule was reported to be respiratory insufficiency. Similar to the other phase I studies of this agent, we did not observe any significant clinical response in our present study.

Table 4. Non-myelosuppressive toxicities[a]

	No. of patients
Mouth sores	2
Alopecia (Grade 2)[b]	3
Facial swelling	5
Hand/arm swelling	9
Splinter hemorrhage in nailbed	11

[a] The remaining toxicities besides alopecia were observed mostly at dose levels of 4800–6400 mg/m^2/72 h.
[b] Alopecia was observed only at the dose level of 12 800 mg/m^2/72 h.

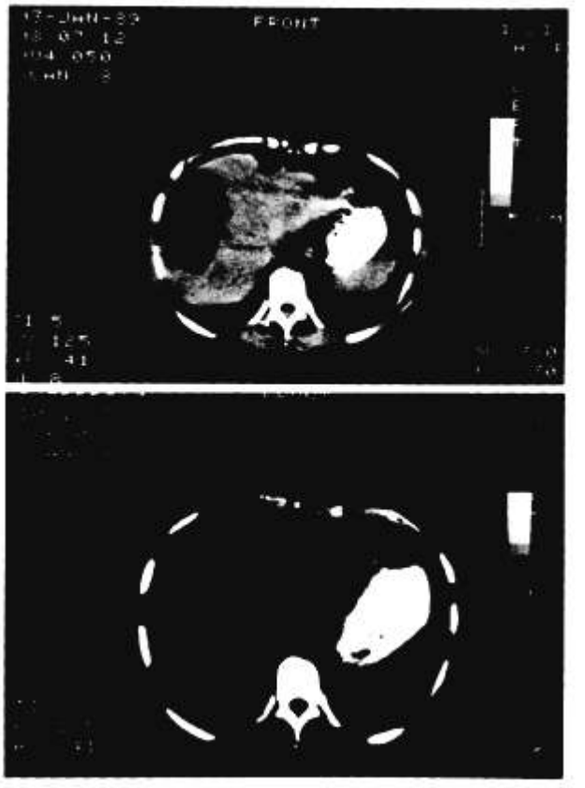

Figure 3. CT scan of the abdomen. Patient no. 13 with 70% regression of her right lobe liver mestastases. The top scan was taken on January 13, 1989, and the bottom scan was taken on February 27, 1989.

Table 5. Plasma pharmacokinetic parameters in 21 study patients treated with 502U83 by continuous 72 h intravenous infusion

Patient no.	Dose over 72 h (mg/m^2)	Total dose (mg)	Infusion rate (mg/h)	$t_{1/2}(\beta)$ (h)	CL (l/h/m^2)	C_{max} (μg/ml)	V_c (l/m^2)	V_{dss} (l/m^2)	AUC (μg/ml h)
1	400	960	13.28	7.46	12.0	0.40	23.8	42.6	33
2	400	732	10.10	3.93	69.2	0.09	65.6	372.6	6
5	800	1496	20.61	8.36	10.0	0.93	2.6	7.2	80
6	800	1240	17.09	3.73	5.7	1.33	0.5	4.0	140
7	800	1272	17.53	4.32	10.4	1.07	0.9	7.4	77
8	1600	3792	52.43	2.35	43.5	0.57	8.7	64.8	37
9	1600	2784	38.39	8.50	12.9	1.72	14.7	30.0	124
11	3200	4768	66.07	2.32	21.8	1.27	6.5	34.8	147
12	3200	6592	90.82	3.16	73.1	0.97	58.3	181.7	44
14	6400	12352	169.39	1.76	71.6	1.23	9.5	101.1	89
17	4800	10176	141.27	3.81	104.1	1.25	17.0	237.6	46
18	4800	9504	129.22	3.11	46.8	1.40	6.6	70.6	103
19	4800	9312	128.65	3.24	62.9	1.03	30.6	123.3	76
20	6400	8688	120.20	2.49	97.7	1.05	66.3	247.4	49
24	6400	12160	168.61	3.73	40.7	2.22	8.8	91.4	157
26	6400	14592	201.49	3.42	78.2	1.42	26.6	186.0	82
27	9600	19008	264.00	2.42	95.3	1.90	57.8	246.6	101
29	9600	18912	261.14	2.92	67.7	2.17	24.5	157.5	142
30	12800	20864	285.73	2.47	78.5	2.80	14.7	178.2	163
31	12800	23808	327.17	4.14	40.8	5.23	17.2	130.8	314
32	12800	21376	292.14	3.01	71.9	3.07	9.6	170.2	178
Mean				3.84	53.1		22.4	127.9	
SD				1.91	31.6		21.4	97.7	

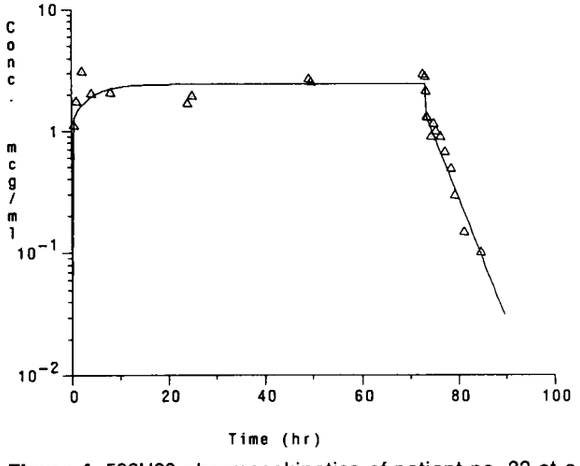

Figure 4. 502U83 pharmacokinetics of patient no. 32 at a dose level of 12 800 mg/m^2/72 h.

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