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Evaluation of a novel bis-naphthalimide anticancer agent, DMP 840, against human xenografts derived from adult, juvenile, and pediatric cancers

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Abstract. The new bis-naphthalimide antitumor agent (R,R)2,2′-[1,2-ethanediylbis[imino(1-methyl-2.1- ethanediyl)]-bis{5-nitro-1H-benz[de]-isoquinoline-1,3- 2H) dione} dimethanesulfonate (DMP 840) was evaluated against parental and multidrug-resistant human KB cell lines in vitro and against these lines growing as xenografts in immune-deprived mice. In vitro, KB8-5 cells were 50-fold resistant to vincristine but only 16-fold resistant to DMP 840 as measured by clonogenic survival. For in vivo evaluation, DMP 840 was given by i. v. injection daily for 9 days or for 5 days/week for 2 consecutive weeks [(dx5)2]. In contrast to the cross-resistance of KB cell lines in vitro, both KB3-1 and KB8-5 tumors were highly and equally sensitive to DMP 840; only KB3-1 xenografts demonstrated sensitivity to vincristine, which was consistent with the in vitro results. DMP 840 was also evaluated against a panel of human tumors comprising colon adenocarcinoma and rhabdomyosarcoma xenografts. Against eight lines of colon adenocarcinoma, DMP 840 caused a high frequency of partial and complete regressions in two lines and significant inhibition of growth in two lines. DMP 840 caused complete regressions in five of six lines of advanced rhabomyosarcomas, demonstrating a broad range of effective dose levels. The pattern of activity against this tumor panel was similar but not identical to that of two inhibitors of topoisomerase I. There was no cross-resistance to DMP 840 in xenografts selected for resistance to vincristine or in a rhabdomyosarcoma selected for resistance to the topoisomerase I inhibitor topotecan. In contrast, a colon tumor selected for topotecan resistance was completely resistant to DMP 840. Slight cross-resistance to DMP 840 was demonstrated in a rhabdomyosarcoma xenograft that was selected for primary resistance to melphalan and was cross-resistant to topoisomerase I inhibitors. The pattern of activity and cross-resistance in these tumors was compared with that shown by two agents that inhibit topoisomerase I: topotecan and CPT-11.

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Introduction

Despite very significant advances in the treatment of childhood cancers, directly attributable to chemotherapy, there has not been similar success in the therapy of most adult carcinomas. Although this may in part relate to the greater tolerance of children to cytotoxic therapy, this difference is probably attributable to malignancies in adults having very different characteristics of growth and metabolism that lead to intrinsic drug resistance. Consequently, there has been a progressive development of preclinical models that more accurately represent specific human cancer types and may more readily identify new agents that have therapeutic utility against more frequently occurring chemorefractory tumors in adults.

Our studies have used childhood rhabdomyosarcoma as a model of a chemosensitive histiotype [10, 11] and colon adenocarcinoma as a model of a chemorefractory tumor [9, 13]. In a coordinated effort we have focused both on the biology, genetics, and metabolic characteristics of these tumors so as to identify more accurately particular targets against which therapeutic agents can be directed and on the development of more appropriate in vivo models that utilize tumor specimens heterografted into immune-compromised mice. The latter models have been used to identify therapeutically active agents that affect known loci [9–11] and also to identify agents with unknown mechanism(s) [18] that may prove valuable in identifying new targets for therapeutic intervention.

In the present study we evaluated a new bis-naphthalimide agent, (R,R)2,2′-[1,2-ethanediylbis[imino (1-methyl-2,1-ethanediyl)]-bis{5-nitro-1H-benz [de]-isoquinoline-1,3-(2H)dione} dimethanesulfonate (DMP 840),

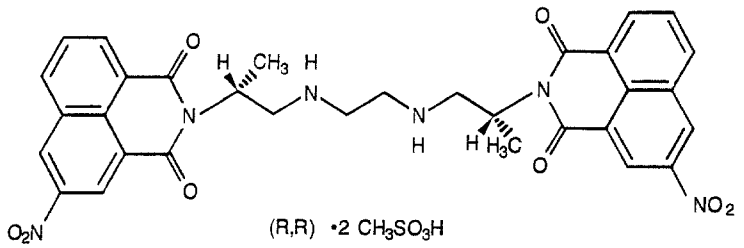

Fig. 1. Structure of DMP 840

against a panel of colon adenocarcinomas and rhabdomyosarcomas. DMP 840 is one of a series of bis-naphthalimide antitumor agents that bind with high affinity and G-C sequence specificity to DNA (Chen et al., manuscript in preparation). The lead compound, XB596, induced single- but not double-stranded DNA breaks in treated murine L1210 leukemia cells (Chen et al., manuscript in preparation). XB596 caused DNA unwinding, and the results were consistent with this agent being a DNA intercalator (Chen et al., manuscript in preparation). In nude mice, XB596 demonstrated good activity against two xenografted human tumors (DLD-2, colon; MX-1, mammary). Like XB596, DMP 840 (Fig. 1) binds to DNA, and vicosity studies suggest that this agent mono-intercalates (Kirshenbaum et al., manuscript in preparation). Unlike XB596, which had poor aqueous solubility (0.04 mg/ml), DMP 840 had an aqueous solubility (3 mg/ml) that allowed further clinical development (Chen et al., manuscript in preparation; Kirshenbaum et al., manuscript in preparation). In addition, the efficacy of DMP 840 in preclinical tumor models was far superior to that of XB596 (Chen et al., manuscript in preparation; McRipley et al., manuscript in preparation). In initial in vivo testing, DMP 840 demonstrated a broad spectrum of curative activity against several types of human solid-tumor xenografts in nude mice, but it also had the unusual property of producing up to 100% cures in mice transplanted with MX-1 mammary carcinoma. The present study extends the in vivo testing to colon tumors and childhood sarcoma, representing intrinsically resistant and chemosensitive histiotypes, respectively. The results obtained with DMP 840 are contrasted with those obtained using topoisomerase I inhibitors against the same panel of xenografts.

Materials and methods

In vitro studies. Cell lines KB3-1 and KB8-5 were obtained from Dr. I. Pastan and maintained in antibiotic-free medium containing 10% fetal calf serum (Gibco). KB8-5 was grown in the presence of 10 ng colchicine/ml [1]; this line overexpresses the *mdr1* gene 16-fold relative to the parenteral KB3-1 clone [8]. For drug sensitivity studies, cells were seeded at 3×10^3/35-mm culture dish and 24 h later were exposed to cytotoxic agents continuously for 7 days. Colony numbers were enumerated after 7 days [20].

Immune deprivation of mice. Female CBA/CaJ mice (Jackson Lab, Bar Harbor, Me.) aged 4 weeks were immune-deprived by thymectomy, followed 3 weeks later by whole-body irradiation (950 cGy) using a ^{137}Cs source. Mice received 3×10^6 nucleated bone marrow cells within 6–8 h of irradiation [18].

Table 1. Characterization of xenografts

Code	Histology	Patient's sex/age (years)	Reference
Colon adenocarcinomas (adult):			
HC_1	Moderately well differentiated	F 68	[16, 17]
GC_3	Poorly differentiated	M 61	[16, 17]
VRC_5	Poorly differentiated	M 72	[16, 17]
ELC_2	Poorly differentiated signet ring cell	F 83	[16, 17]
Colon adenocarcinomas (juvenile/young adult):			
SJC_2	Poor to moderately differentiated	F 14	[9]
SJC_3A	Poor to moderately differentiated	M 26	[9]
SJC_3B	Moderately well differentiated		
SJC_8	Well differentiated	M 11	Unpublished data
Childhood rhabdomyosarcoma: Histology/site			
Rh12	Embryonal/buttock	M 3	[15]
Rh18	Embryonal/perineum	F 1	[6, 15]
Rh28	Alveolar/lymph node	M 17	[6]
Rh30	Alveolar/bone marrow	M 16	[4]
IRS56	Embryonal/buttock	M 3	[9]
IRS68	Embryonal/shoulder	M 13	[18]

Tumor lines. Each of the six independently derived lines from previously untreated rhabdomyosarcoma (RMS) have been described previously [4, 6, 9, 15, 18]. The characteristics of each xenograft are summarized in Table 1. For chemotherapy studies, all tumors were used within 22 passages of their engraftment in mice. Each tumor grew routinely in over 90% of the recipient mice, and all were human as determined by karyotype and species-specific isoenzyme patterns. The chemosensitivity of these lines to conventional agents in the therapy of RMS [10] and to melphalan (L-PAM) and topotecan [9, 11] has been reported previously.

Sublines of Rh12, Rh18, and Rh28 selected in situ for resistance to vincristine (Rh12/VCR-3, Rh18/VCR-3) and melphalan (Rh28/L-PAM) have been described previously [7, 14]. Rh18/TOPO was selected in situ for resistance to topotecan. The colon adenocarcinomas used (HC_1, GC_3, VRC_5, and ELC_2) were derived from adult patients and have been characterized extensively [16–18]. SJC_2 was established from a moderately differentiated primary colon lesion in a 14-year-old girl. SJC_3A and SJC_3B tumors were independent primaries in a 26-year-old man, and SJC8 was derived from an 11-year-old boy. VRC_5/TOPO was selected for resistance to topotecan after 20 weeks of low-dose therapy in mice [9].

Growth-inhibition studies. Therapy was started in mice bearing bilateral s.c. tumors when the tumors had reached approximately 0.5–1 cm in diameter. The tumor response was determined at 7-day intervals using digital calipers (Maxcal) interfaced to an IBM PS/2 microcomputer. Two perpendicular diameters were used to compute volumes [18]. The growth delay was calculated from the difference in days required for treated

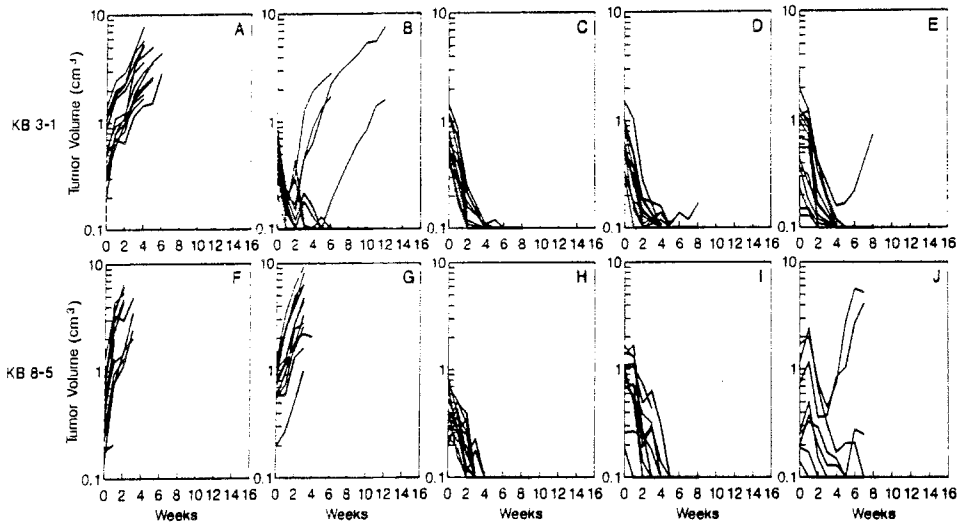

Fig. 2 A – J. Responses of KB3-1 and multidrug-resistant KB8-5 xenografts to vincristine and DMP 840. Data show growth curves for individual tumors in each treatment group. Panels **A – E** and **F – J** show the growth of individual KB3-1 and KB8-5 xenografts, respectively. Controls (**A, F**), vincristine given at 3 mg/kg i. p. (**B, G**), and DMP 840 given i. v. at 15 (**C, H**), 10 (**D, I**), and 5 mg/kg per dose (**E, J**) using the (dx5)2 schedule

tumors to grow to 4 times their volume after the start of treatment as compared with vehicle-treated controls. For each treatment group, six or seven tumor-bearing mice were used. Relative tumor volumes were calculated from the formula RTV = (V_x/V_o), where V_x is the volume on day X and V_o is the volume of the tumor at the time of initiating treatment. Treated/control (T/C) ratios for tumor volumes were calculated from the RTV values after each measurement. The minimal T/C ratio (i. e., smallest T/C ratio) and the day on which it was achieved are presented. To equate responses in tumor lines that demonstrated different rates of growth, the growth delay was normalized by expressing this as a function of tumor volume-doubling time. Grading of tumor responses is given in Table 2; the definition of $\geq$50% regression (PR) or complete regression (CR) required that each tumor within a group demonstrate such a reduction in volume at some time point after treatment.

Formulation and administration. DMP 840 was dissolved in dimethylsulfoxide (DMSO, 5% final volume) and immediately diluted in a 5% dextrose solution. The drug was stored at 4° C in foil-wrapped bottles. The drug was given by i. v. injection daily for 9 days (dx9) or for 5 days/week for 2 weeks (designated [dx5]2). Topotecan was dissolved in 0.9% saline or water for i. p. or p. o. administration and was given (dx5)3. CPT-11 was given i. v. (dx5)2 (0.1 ml/10 g body weight). Topotecan was generously provided by Dr. Randall K. Johnson, Smith Kline Beecham, and CPT-11 was a gift from Dr. Anne Mathieu-Boué, Laboratoire Roger Bellon, Paris.

Statistical analysis. The results of individual tumor-inhibition studies were analyzed with one-way analysis of variance using the number of days required for tumors to reach 4 times their original volume as the dependent variable. Only tumors from mice that survived the entire study were included in the analyses, and any tumor that did not reach 4 times its original volume was assigned a default value of the maximal duration of the study. To compare the efficacy of various courses of treatment, data were collapsed across studies within a tumor line. The percentages of tumors showing a PR and/or CR and any regrowth were calculated for the individual tumor lines as described previously [18].

Results

Studies with KB cell lines in vitro and in vivo

Expression of P-glycoprotein has been detected in both colon adenocarcinomas and childhood RMS [2, 3, 5] and has been correlated with a poor outcome [2, 3]. One criterion that we have used to prioritize in vivo evaluation is that a new agent should be equally active against cells that exhibit a typical multidrug-resistant (MDR) phenotype conferred by the *mdr1* gene product. As compared with the parental KB3-1 cell line, KB8-5 was approximately 50-fold resistant to VCR [50% growth-inhibitory concentration (IC_{50}), 0.56 vs 28 n*M*] but only 16-fold resistant to DMP 840 (IC_{50}, 0.31 vs 5.1 n*M*). Consequently, we were interested in determining whether this level of resistance in vitro had a significant impact upon the sensitivity of these lines grown as xenografts. DMP 840 was given i. v. for two consecutive 5-day courses [(dx5)2], or tumor-bearing mice received a single administration of vincristine [3 mg/kg i. p., the dose lethal to 10% of the animals (LD_{10})]. The maximal dose of DMP 840 tolerated on either the (dx5)2 or the (dx9) schedule was 15 mg/kg per administration ($<LD_5$). As shown in Fig. 2, KB3-1 xenografts were sensitive to vincristine, with most tumors (71%) regressing completely subsequent to a single administration of the drug. In contrast, the growth of KB8-5 xenografts was not significantly inhibited by treatment. Thus, KB8-5 tumors retained resistance to vincristine in situ. Also shown in Fig. 2 are the dose responses to DMP 840. Both KB3-1 and KB8-5 xenografts were highly responsive to DMP 840, which caused CRs at 15 and 10 mg/kg. No significant resistance to DMP 840 was noted.

Colon adenocarcinomas

The responses of four colon adenocarcinoma xenografts derived from patients of advanced age (Table 1) were analyzed for growth inhibition, and the objective responses are shown in Table 2. DMP 840 given i. v. (dx9) caused significant growth inhibition in HC_1 and ELC_2 tumors and caused a high frequency of PRs in GC_3 xenografts. It should be noted that although DMP 840 did not cause regressions in ELC_2 tumors, it caused a protracted delay in growth even at 10 mg/kg per dose (Fig. 3).

The activity of DMP 840 was next examined against four colon adenocarcinomas derived from young patients

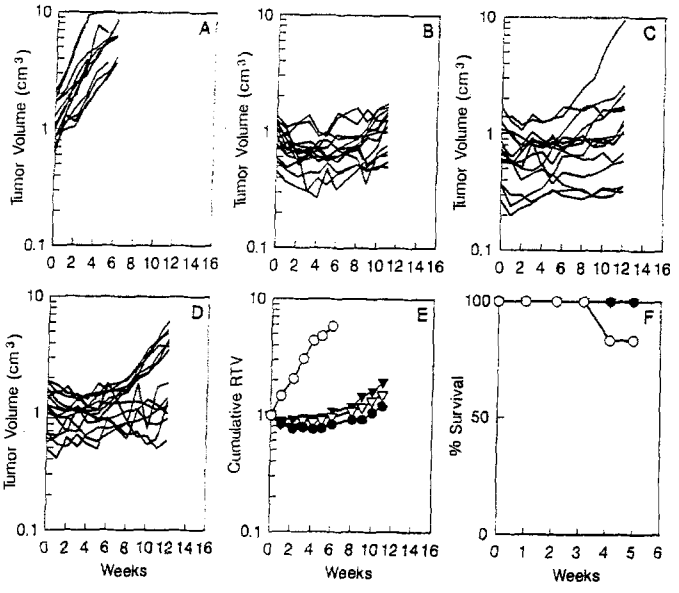

Fig. 3 A–F. Responses of ELC_2 colon adenocarcinomas to DMP 840. Mice received vehicle (**A**, control) or DMP 840 (dx9) i.v. at **B** 15, **C** 12.5, and **D** 10 mg/kg per dose (each curve shows the growth of an individual tumor). **E** Relative tumor volume for all treatment groups: controls (○) and mice receiving 15 (●), 12.5 (▽), and 10 mg/kg per dose (▼). **F** Survival during the study (symbols as defined in **E**)

Table 2. Xenografts derived from adult colon adenocarcinomas

Tumor	Dose (mg/kg)	Time to 4× (days)	Growth delay (days)	T/C (day)	%			Response[a]
					PR	CR	C	
HC_1	0	28±6						
	10	53±10	25	0.34 (35)	0	0	0	++
	12.5	64±19	36	0.52 (35)	0	0	0	++
	15	63±12	35	0.43 (35)	0	0	0	++
GC_3	0	35±16						
	10	71±17	36	0.18 (35)	64	36	36	++
	12.5	70±12	35	0.14 (35)	64	29	21	++
	15	80±8	45	0.10 (56)	79	29	21	++
VRC_5	0	17±4						
	10	39±4	22	0.31 (28)	0	0	0	++
	12.5	39±10	22	0.32 (28)	7	0	0	++
	15	41±7	24	0.34 (21)	0	0	0	++
ELC_2	0	31±8						
	10	>83	>52	0.19 (42)	0	0	0	+++
	12.5	>83	>52	0.17 (42)	0	0	0	+++
	15	>83	>52	0.14 (72)	21	0	0	+++

[a] Tumor response criteria: –, no growth inhibition; +, growth inhibition $\geq Td_2$; ++, growth inhibition $\geq 2 \times Td_2$; +++, growth inhibition $\geq 3 \times Td_2$; ++++, growth inhibition $>3 \times Td_2$ plus volume regression ≥50%; +++++, complete regression with subsequent regrowth; ++++++, complete regression with no growth during the period of observation (≥84 days). Td_2, Mean time required for tumor volume to double

(ages 11–26, Table 1), and the results are presented in Table 3. Tumor SJC_3B was quite sensitive to DMP 840 treatment, with a high frequency of CRs being noted in replicate experiments (100% and 78%) at the highest dose level tolerated (Fig. 4). In contrast, SJC_3A tumors derived from an independent primary in the same patient were less responsive to treatment (Table 3).

Rhabdomyosarcomas

Xenografts derived from childhood RMS were highly sensitive to DMP 840 therapy (Table 4). At the highest dose level (15 mg/kg per administration [dx5]2), DMP 840 caused CRs without subsequent regrowth in 5/6 tumor lines during the 12-week period of observation. Similar activity was observed at 10 and 5 mg/kg per dose, demonstrating a relatively broad range of activity against these tumor lines. The least responsive tumor was Rh12, against which DMP 840 exerted significant inhibitory activity but did not cause objective regressions.

Cross-resistance to DMP 840

The efficacy of DMP 840 against RMS selected in situ for resistance to vincristine (Rh12/VCR, Rh18/VCR), the topoisomerase I inhibitor topotecan (Rh18/TOPO), and mel-

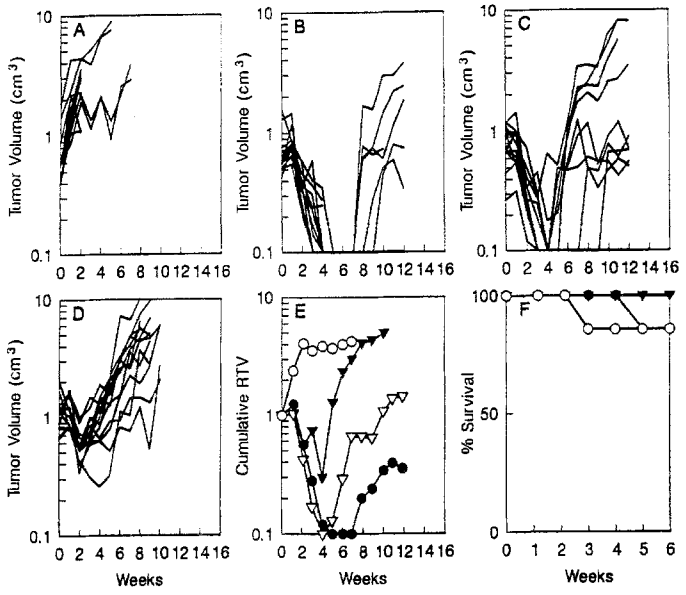

Fig. 4 A – F. Responses of SJC_3B colon adenocarcinomas to DMP 840. Mice received vehicle (**A**, control) or DMP 840 (dx9) i.v. at **B** 15, **C** 10, and **D** 5 mg/kg DMP 840 per dose (each curve shows the growth of an individual tumor). **E** Relative tumor volume for all treatment groups: controls (○) and mice receiving 15 (●), 12.5 (▽), and 10 mg/kg DMP 840 per dose (▼). **F** Survival during the study symbols as defined in **E**)

Table 3. Xenografts derived from juvenile colon adenocarcinomas

Tumor	Dose (mg/kg)	Time to 4× (days)	Growth delay (days)	T/C (day)	%			Response
					PR	CR	C	
SJC2	0	23±13						
	12.5	44±31	21	0.22 (28)	28	14	14	+
	15	54±24	31	0.23 (28)	78	36	29	++
SJC_3A	0	24±16						
	10	29±2	5	0.73 (14)	0	0	0	–
	12.5	31±20	7	0.23 (14)	64	0	0	–
	15	38±26	14	0.20 (21)	71	21	21	+
SJC_3B	0	24±6						
	5	45±12	21	0.14 (21)	14	0	0	(+)
	12.5	>84	>60	0.04 (28)	100	79	57	++++
	15	>84	>60	0.03 (35)	100	78	71	++++
SJC8	0	17±8						
	10	35±21	18	0.36 (21)	0	0	0	+
	15	42±10	25	0.26 (21)	0	0	0	+

phalan (Rh28/L-PAM) was next examined. As shown in Fig. 5 and Table 5, there was no cross-resistance to DMP 840 in RMS xenografts selected for vincristine or topotecan resistance. However, whereas DMP 840, even at 5 mg/kg per dose, caused CR with regrowth in only 1 of 14 Rh28 tumors, Rh28/L-PAM tumors were less responsive, with tumor regrowth occurring at each dose level (Fig. 5 G, H). In contrast to Rh18/TOPO, the topotecan-resistant variant of colon tumor VRC_5 was completely resistant to DMP 840 (Table 5).

Discussion

Our initial studies in vitro determined that in an MDR cell line, KB8-5, that overexpresses *mdr1* transcripts, there was approximately 50-fold resistance to vincristine and 16-fold resistance to DMP 840 relative to the parental KB3-1 cells. To examine whether this level of resistance in vitro was biologically significant, we evaluated DMP 840 against xenografts established from parental and MDR cell lines. The sensitivity to vincristine was assessed simultaneously with that to DMP 840 in these experiments. As shown in Fig. 2, vincristine given at the maximal tolerated dose (MTD, 3 mg/kg i.p.) caused CRs in most KB3-1 xenografts but had no effect on the growth of KB8-5 tumors. In contrast, DMP 840 had very similar activity against both lines at dose levels as low as 5 mg/kg. Consequently, the significance of resistance in vitro as a predictor of in vivo chemosensitivity, at least for DMP 840, is not known.

The spectrum of therapeutic activity of this bis-naphthalimide agent was examined subsequently in a comprehensive model of colon adenocarcinoma, representative of a chemoresistant histiotype, and in childhood RMS xenografts representing chemosensitive tumors. DMP 840 was

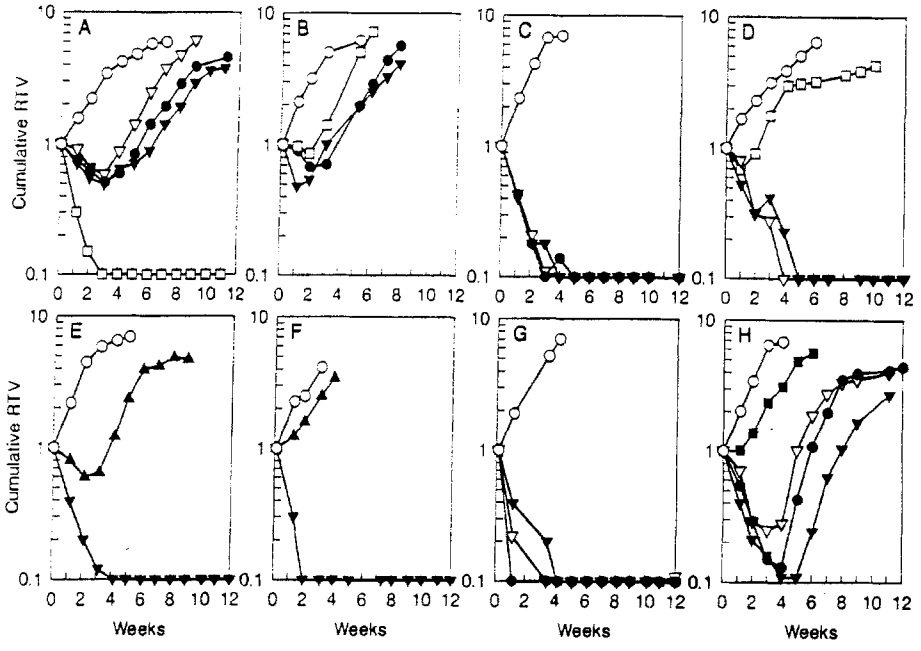

Fig. 5 A – H. Efficacy of i. v. DMP 840 against parental and vincristine; topotecan-, or melphalan-resistant sublines of rhabdomyosarcomas. **A** Rh12. **B** Rh12/VCR. **C** Rh18. **D** Rh18/VCR. **E** Rh18. **F** Rh18/TOPO. **G** Rh28. **H** Rh28/LPAM. DMP 840 was given i. v. (dx5)2, and topotecan was given i. p. (dx5)3. ○, Control. Treatment groups: ▼, 15; ●, 10; and ▽, 5 mg/kg DMP 840 per dose. Both vincristine at 3 mg/kg i. p. (□) and melphalan at 13 mg/kg i. p. (■) were given as a single administration. Topotecan was injected i. p. at 1.75 mg/kg per dose (▲) using the (dx5)3 schedule. Each curve shows the relative tumor growth for 12 or 14 tumors/group

Table 4. Responses of rhabdomyosarcoma xenografts

Tumor	Dose (mg/kg)	Time to 4× (days)	Growth delay (days)	T/C (days)	%			Response
					PR	CR	C	
Rh12	0	23±13						
	5	43±21	20	0.17 (21)	50	8	8	+
	10	46±26	23	0.15 (28)	79	14	14	++
	15	59±13	36	0.14 (42)	79	43	43	+++
Rh18	0	14±8						
	5	>84	>70	0.01 (21)	100	100	100	++++++
	10	>84	>70	0.01 (21)	100	100	100	++++++
	15	>84	>70	0.01 (28)	100	100	100	++++++
Rh28	0	15±10						
	5	>84	>69	0.01 (28)	100	100	8	+++++
	10	>84	>69	0.01 (28)	100	100	100	++++++
	15	>84	>69	0.01 (28)	100	100	100	++++++
Rh30	0	25±16						
	5	>84	>69	0.02 (35)	100	100	100	++++++
	10	>84	>69	0.02 (35)	100	100	100	++++++
	15	>84	>69	0.02 (35)	100	100	100	++++++
IRS56	0	31±8						
	5	>84	>53	0.02 (42)	100	100	100	++++++
	10	>84	>53	0.03 (35)	100	100	100	++++++
	15	>84	>53	0.01 (14)	100	100	100	++++++
IRS68	0	18±13						
	10	>84	>66	0.02 (35)	100	100	86	+++++
	15	>84	>66	0.02 (35)	100	100	100	++++++

given i. v. either as a bolus on 9 consecutive days (colon tumors) or as two 5-day courses on consecutive weeks (RMS). The MTD was 15 mg/kg per administration, causing weight loss, but lethality occurred in <5% of mice treated at this level. Against colon tumors derived from "typical patients" (see Table 1 for patient data), DMP 840 demonstrated only modest activity. Treatment caused a high frequency of PRs in GC_3 tumors, but these rapidly regrew. In contrast, the growth of ELC_2 xenografts was significantly inhibited for the duration of observation (84 days), but treatment did not cause volume regressions. Against colon tumors established from younger patients, DMP 840 caused CRs in SJC_3B xenografts but had less activity against SJC_3A tumors derived from an indepen-

Table 5. Cross-resistance in xenografts

Tumor	Dose (mg/kg)	Time to 4× (days)	Growth delay (days)	T/C (days)	%			Response
					PR	CR	C	
Rh12/VCR	0	19±7						
	10	43±11	24	0.14 (21)	79	7	7	+++
	15	44±20	25	0.17 (14)	100	14	14	+++(+)
Rh18/VCR	0	29±10						
	5	>84	>55	0.02 (42)	100	100	100	++++++
	15	>84	>55	0.02 (42)	100	100	100	++++++
Rh18/TOPO	0	21±4						
	15	>84	>63	0.03 (35)	100	100	100	++++++
Rh28/LPAM	0	13.1±8						
	5	25±26	12	0.04 (28)	100	25	25	+++
	10	59±27	46	0.02 (28)	100	71	21	++++
	15	64±32	51	0.02 (28)	100	71	29	++++
VRC_5/TOPO	0	9±4			0	0	0	–
	10	12±3	3	0.95 (7)	0	0	0	–
	15	12±4	3	0.42 (15)	0	0	0	–

Table 6. Comparison of efficacy and cross-resistance to topoisomerase I inhibitors

Tumor	CPT-11[a, b]	Topotecan[c]	DMP 840
HC_1	++++[d]	+++	++
GC_3	+++	+	++
VRC_5	++++	+++	++
ELC_2	+++	+	+++
SJC2	+++	+++	++
SJC3A	++++++	+++	+
SJC3B	++++	+++	+++++
SJC8	+++	+++	+
Rh12	++	+++	+++
Rh18	+++++	+++	++++++
Rh28	++++++	+++++	++++++
Rh30	++++++	+++++	++++++
IRS56	++++++	+++++	++++++
IRS68	++++++	++++++	++++++
VRC_5/TOPO	+++	+	–
Rh12/VCR	++++	+++	++++
Rh18/VCR	++++++	+++++	++++++
Rh18/TOPO	++++++	±	++++++
Rh28/LPAM	+++++	±	+++++

[a] CPT-11, (dx5)2; topotecan, (dx5)3
[b] Data from Houghton et al. [21]
[c] From Houghton et al. [9] with additional data
[d] For response criteria see Table 2

dent primary in the same patient. Only the response of SJC_3B tumors would be regarded as an objective response by clinical criteria (13%). However, DMP 840 exhibited curative action against colon xenografts DLD-2 and CX-1 in nude mice (McRipley et al., manuscript in preparation).

DMP 840 was highly active against five of six RMS lines derived from previously untreated patients. When given i.v. (dx5)2 at 15 mg/kg per dose, DMP 840 caused CRs without regrowth during the period of observation (84 days) in five tumor lines, and it maintained this level of activity at 5 mg/kg per dose in three of five lines. Thus, this agent demonstrated very significant activity over a 3-fold range of doses.

We next examined the efficacy of this bis-naphthalimide antitumor agent against a panel of RMS selected in situ for resistance to vincristine, topotecan, or melphalan. There was no cross-resistance in either of the vincristine-resistant RMS lines (Rh12/VCR and Rh18/VCR). In the variant of Rh18 selected for topotecan resistance, no cross-resistance to DMP 840 was measured, but the colon adenocarcinoma VRC_5/TOPO was completely resistant to DMP 840. There was slight cross-resistance also in Rh28/L-PAM xenografts selected for resistance to melphalan.

Although the mechanism of the tumoricidal action of DMP 840 is not yet known, its spectrum of activity against these xenografts is similar but not identical to that of two topoisomerase I inhibitors that were evaluated in these models. The activity of topotecan and CPT-11 is contrasted with that of DMP 840 in Table 6. Thus, against colon adenocarcinomas the spectrum of activity of DMP 840 was similar to that of topotecan, but it was less active than CPT-11, an agent reported to cause objective responses in patients with colon adenocarcinoma [19]. All three agents have very significant activity against RMS xenografts, with Rh12 tumors being least responsive to each agent. However, the pattern of cross-resistance is particularly interesting. Vincristine-resistant RMS retained complete sensitivity to DMP 840; thus, this agent is similar to topotecan [9] and CPT-11 [21]. VRC_5 colon tumors selected for resistance to topotecan were completely resistant to DMP 840, whereas this line retained full sensitivity to CPT-11. On the other hand, Rh18/TOPO retained complete sensitivity to DMP 840 (and CPT-11), perhaps indicating different mechanisms of resistance to topotecan and DMP 840 in this line. The sensitivity of Rh28/L-PAM is also of interest. This line was selected for melphalan resistance but is cross-resistant to vincristine [7], etoposide (VP-16) [12], and topotecan [9] and is partially resistant to CPT-11. Both

the activity of DMP 840 against Rh28 cells and the level of cross-resistance in Rh28/L-PAM cells are almost identical to those reported for CPT-11 [21].

In summary, DMP 840 represents a novel chemotherapeutic agent with good activity in vivo. Although the mechanism(s) of activity remain to be elucidated, its spectrum of activity and cross-resistance profile may indicate activity at the level of topoisomerase I or II. Preliminary data show that in vitro, DMP 840 reduces the level of topoisomerase I and II covalently bound to DNA in the presence of topotecan and VP-16, respectively (Danks and Brown, manuscript in preparation). The activity of DMP 840 together with the relatively broad range of effective doses suggests that this agent should be evaluated in childhood and other solid malignancies.

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