

High Efficacy of Intravenous Gimatecan on Human Tumor Xenografts

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Abstract. *Background/Aim:* The lipophilic, orally bioavailable camptothecin analogue gimatecan is characterized by improved efficacy over conventional camptothecins on human tumor xenografts. Gimatecan produced excellent outcomes orally with prolonged, low-dose, daily treatments. The aim of this study was to assess the antitumor efficacy of i.v. administered gimatecan. *Materials and Methods:* The antitumor activity of gimatecan delivered i.v. q4dx4 was evaluated in nude mice bearing human tumors and compared to clinically available anticancer drugs. *Results:* Intravenous administration of gimatecan showed superior efficacy with remarkable achievements at well tolerated doses. Moreover, prolonged treatments with i.v. administered gimatecan showed efficacy in models of cancer refractory to current therapeutic approaches, like an orthotopic brain tumor. *Conclusion:* Although the oral route is practical for gimatecan administration, the results support the interest in developing a suitable i.v. formulation in an attempt to further exploit the therapeutic potential of the compound.

Topotecan and irinotecan, water-soluble synthetic camptothecin analogues, are clinically important anti-neoplastic drugs with a singular mechanism of action and wide-spectrum activity (1). Nevertheless, the reversibility of drug-target interaction, opening of the lactone ring which results in loss of activity and preferential binding to human serum albumin of the open form represent limitations in exploiting drug efficacy. Gimatecan (ST1481), a new camptothecin substituted at position 7 with lipophilic chains, was selected for clinical development via the oral route up to Phase II studies due to the promising preclinical pharmacological profile (2). The agent showed a rapid

uptake and long persistence in cells with strong cytotoxic potency, likely related to potent inhibition of the target topoisomerase I and stabilization of the cleavable complex (DNA-enzyme-drug) (3). Gimatecan delivered orally, showed high effectiveness on a large panel of subcutaneous (*s.c.*) human tumor xenografts and orthotopic and metastatic tumor models (4, 5).

As an S phase-specific agent, the best *in vivo* efficacy of gimatecan was achieved by prolonged, daily, low-dose treatments (3-5). However, optimal antitumor effects in terms of complete responses and disease-free survivors were obtained by high doses following intermittent schedules (every 8-10 days). Thus, the drug seems less schedule-dependent than other camptothecins (3, 5). In a Phase I study the main toxicity of oral gimatecan, a dose-limiting thrombocytopenia, was related to the daily schedule employed. The authors stated that weekly, or twice-a-week schedules would be more appropriate for the drug (6). Moreover, in spite of the reported *in vitro* and *in vivo* ability of gimatecan to completely overcome resistance mediated by transporter proteins (3, 7), subsequent studies speculated that the high expression of drug-transporter proteins at intestinal level could complicate development of an oral analogue (8). These studies suggest the need to explore the antitumor efficacy of the compound administered via the *i.v.* route, given that the molecule is not histolesive (5). In *in vivo* experimental tumor systems more spaced and repeated treatment schedules are suitable to *i.v.* drug delivery allowing a more favorable recovery from local and systemic damages in treated animals.

The aim of this study was to assess the effects of gimatecan delivered *i.v.* following a short, intermittent administration schedule with respect to clinically available cytotoxic drugs in a panel of *s.c.* human tumor xenografts. The activity of gimatecan delivered *i.v.* with protracted schedules was, also, evaluated on experimental models, representative of cancers poorly responsive to current therapeutic approaches, like a *s.c.* lung carcinoma or an orthotopic brain tumor xenograft. The results demonstrated high efficacy of *i.v.* administration of the camptothecin

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independently of the employed schedule. Based on these favorable preclinical features, the therapeutic potential of *i.v.* gimatecan may be examined in a clinical setting.

Materials and Methods

Antitumor efficacy studies. All experiments were carried out using female athymic CD-1 nude mice, 7-10 weeks-old (Charles River, Calco, Italy). Mice were maintained in laminar flow rooms keeping temperature and humidity constant with free access to food and water. Experiments were approved by the Ethics Committee for Animal Experimentation of the Istituto Nazionale Tumori di Milan (years 2000-2005). Animal care and procedures were in compliance with the coeval national law D.L. 116/1992 and international policies.

Gimatecan (7-t-butoxyiminomethylcamptothecin, Sigma-Tau, Pomezia, Rome, Italy) was dissolved in dimethylsulphoxide (DMSO), stored at -20°C , thawed and orally administered, suspended in sterile, distilled water (10% DMSO). For oral or *i.v.* treatment, gimatecan was dissolved in a mixture of ethanol and cremophor EL (50+50%), suspended in saline at several final concentrations, stored at -20°C , thawed and delivered under magnetic stirring. Irinotecan (CPT11, Sigma-Tau, Pomezia, Italy), topotecan (Smith-Kline Beecham Pharmaceuticals, King of Prussia, PA, USA) and paclitaxel (PTX, taxol, Indena, Milan, Italy) were dissolved and administered as previously described [(9-11)]. Cisplatin (DDP, Teva, ready to use) and doxorubicin (DX, Adriblastina, Pharmacia, Italy, dissolved in sterile, distilled water) were given every seventh day for three times (q7dx3). All drugs were delivered in a volume of 10 ml/kg of body weight, except PTX that was delivered in a volume of 15 ml/kg.

Human tumor xenografts from tumor lines were used in the study. The tumor lines derived from *s.c.* injection of cultured cells as previously described (9), except the CoBA colon carcinoma originating from patient biopsy specimen (12). Experimental groups of four-six mice were formed. On day 0 tumor pieces were implanted on both flanks and growing nodules were measured biweekly with Vernier caliper. Tumor volume (TV) was calculated according to the formula: $\text{TV (mm}^3\text{)} = d^2 \times D / 2$ where *d* and *D* are the shortest and the longest diameter, respectively. Treatments started when nodules were just palpable. Drug efficacy was assessed considering tumor volume inhibition percentage (TVI%) in treated *versus* control mice, Log_{10} cell kill (LCK) and complete regression (CR) rate, calculated as already described (9, 10). The highest body weight loss percentage induced by treatments is reported in Tables I-IV. Deaths occurring in treated mice before the death of the first control mouse were ascribed to toxicity. Two-tailed Student *t* and Fisher's exact tests were used for statistical comparison of tumor volumes and CR, respectively, in mice.

For the intracranial (*i.c.*) growing tumor model, cells of the SW1783 human astrocytoma (0.25×10^6 in 0.01 ml saline) were injected into the right frontal lobe of anesthetized mice as published (5). Experimental groups of 6-7 cell-injected mice were formed. For ethical reasons, the animals were monitored daily and sacrificed when body weight loss overcame 15% considering the day of sacrifice as day of death. Treatment started 3 days after cell injection. Drug activity was assessed as T/C%, *i.e.* the ratio of median survival time (MST) in treated over control mice (T/C) $\times 100$. Curves reporting the percentage of survivors over time were

estimated by the Kaplan–Meier product limit method and compared with the log-rank test (two-sided).

Results

The antitumor efficacy of *i.v.* administered gimatecan was assayed on two *s.c.* tumor xenografts considering the influence of solvents on drug activity in order to establish the best formulation. The compound was administered every fourth day for four times (q4dx4), a brief and intermittent schedule convenient in our experience at screening many compounds as new therapeutic agents.

The Panc-1 pancreatic tumor model highly responded to orally administered gimatecan at a dose of 2 mg/kg in 10% DMSO, which induced 5/10 CR and one toxic death (Table I). From previous studies doses of 2-3 mg/kg under the same treatment conditions were reported as maximum tolerated dose (MTD), *i.e.* 10% lethal dose (3, 4). Similar antitumor activity, but without toxic effects on the animals, could be attained only by orally delivering 4 mg/kg of the drug in 10% ethanol-cremophor (Figure 1). Marked antitumor efficacy was achieved by *i.v.* administration at all tested doses as evidenced by 100% of TVI, 26/26 CR and the fact that LCK values were not reached, reflecting lack of growth of the regressed tumors. Toxic effects were induced by the highest dose (2 mg/kg).

The growth of the 501Mel melanoma model was moderately affected by oral gimatecan at a dose of 2 mg/kg in 10% DMSO (Table II). Like on Panc-1 xenograft, 4 mg/kg in 10% ethanol-cremophor achieved similar antitumor activity without lethal events, whereas antitumor activity (TVI: 73%, LCK: 1.1) and toxic effects (1/4 toxic deaths) were somewhat increased by the same dose formulated in 5% of ethanol-cremophor (Figure 2). Efficacy was not improved by delivering *i.v.* 1 mg/kg of the drug in 2.5% of solvent. However, the same dose of *i.v.* administered gimatecan suspended in 1.25% of ethanol-cremophor markedly inhibited the melanoma growth and safely induced 2/8 CR and a TVI% value significantly higher ($p < 0.01$) as compared to oral treatment (Figure 3). The highest antitumor activity obtained by the *i.v.* administration of 2 mg/kg was accompanied by toxic effects (*e.g.*, high weight loss or toxic deaths).

Overall, the ethanol-cremophor mixture seemed to reduce the potency of oral gimatecan relative to DMSO preparation. Moreover, in mice bearing the 501Mel melanoma oral administration of the drug at a dose of 4 mg/kg in 5% of ethanol-cremophor was more active and toxic than that prepared in 10%. Likewise, increasing concentrations of ethanol-cremophor in drug preparations seemed to affect potency of *i.v.* administered gimatecan resulting in decreased therapeutic activity against tumors and/or toxic effects in animals. Thus, the activity of gimatecan was reduced by the use of the ethanol-cremophor mixture as a solvent.

Table I. Effects of gimitecan delivered q4dx4 to athymic nude mice bearing s.c. the Panc-1 human pancreatic carcinoma. Several drug preparations were compared. On day 0 tumor fragments were implanted on both flanks. Treatment started when tumors were just palpable (day 3-4).

Route	Solvent (%)	Dose mg/kg	TVI% ¹	LCK ²	CR ³	BWL% ⁴	Tox ⁵
oral	DMSO ⁶ (10)	2	98 (25)	1.5	5/10	7	1/5
	et-cr ⁷ (10)	3	94	1	2/10	0	0/5
		4	99	1.5	5/10	5	0/5
<i>i.v.</i>	(2.5)	1	100	>>>3.7	10/10 ^f	1	0/5
	(5)	1.5	100	>>>5.5	8/8	7	0/4
	(5)	2	100	>>>5.5	8/8	20	1/4

¹Tumor volume inhibition % in treated over control tumors; in parentheses the day on which it was assessed; ²Gross log₁₀ cell kill to reach 500 mm³ of tumor volume; ³Complete response: *i.e.* disappearance of tumor lasting at least 10 days; ⁴Body weight loss % induced by drug treatment; the highest change is reported; ⁵Dead/treated mice; ⁶DMSO: dimethylsulphoxide suspended in sterile, distilled water; ⁷et-cr: ethanol and cremophor (50+50%) suspended in saline; ^f*p*<0.05 by Fisher's exact test vs. oral gimitecan 2 mg/kg, DMSO 10%.

Table II. Effects of gimitecan delivered q4dx4 to athymic nude mice bearing s.c. the Me501 human melanoma. Several drug preparations were compared. On day 0 tumor fragments were implanted on both flanks. Treatment started when tumors were just palpable (day 7).

Route	Solvent (%)	Dose mg/kg	TVI% ¹	LCK ²	CR ³	BWL% ⁴	Tox ⁵
Oral	DMSO ⁶ (10)	2	58 (41)	0.6	0/6	24	1/4
	et-cr ⁷ (10)	4	53	0.6	0/8	10	0/4
	(5)	4	73	1.1	0/6	7	1/4
<i>i.v.</i>	(2.5)	1	80	0.9	0/8	9	0/4
	(1.25)	1	92**	1.7	2/8	5	0/4
	(5)	2	97**	2.4	4/8	17	0/4
	(2.5)	2	97**	2.1	3/6	17	1/4

¹Tumor volume inhibition % in treated over control tumors; in parentheses the day on which it was assessed; ²Gross log₁₀ cell kill to reach 300 mm³ of tumor volume; ³Complete response: *i.e.* disappearance of tumor lasting at least 10 days; ⁴Body weight loss % induced by drug treatment; the highest change is reported; ⁵Dead/treated mice; ⁶DMSO: dimethylsulphoxide suspended in sterile, distilled water; ⁷et-cr: ethanol and cremophor (50+50%) suspended in saline. ***p*<0.01 by Student's *t*-test vs. oral gimitecan 2 mg/kg, DMSO 10%.

The pattern of antitumor efficacy of *i.v.* gimitecan was investigated in a panel of s.c. human tumor xenografts representative of cancer types intrinsically resistant to conventional therapy. The results are presented in Table III. Based on the data shown in Tables I and II, 1.5 mg/kg dissolved in 5% of ethanol-cremophor was the highest, active and non-toxic dose of *i.v.* gimitecan. Thus, under these treatment conditions, this dose level was supposed to be close to the MTD and employed against the tumors of the panel, except the melanomas. All model systems (9/9) highly responded to the antitumor effects of *i.v.* gimitecan in terms of TVI (88-100%), CR rates and LCK values. Specifically, on the Panc-1 pancreatic and MKN-28 gastric carcinomas, 501Mel and Me26414 melanomas the antitumor activity was impressive, as indicated by the rate of disappearance of tumors which did not grow after the end of the treatment with no evidence of disease until the end of the experiment, up to 170 days after tumor implant. Moreover, a comparison to clinically used anticancer drugs given *i.v.* at their optimal dose and schedule was carried out in eight xenografts. Some

data have been already reported (9-13). Gimitecan was more active in 6/8 tumors with similar or inferior activity only to cisplatin in the HT1376 bladder and paclitaxel in the GER pancreatic carcinoma, respectively. Interestingly, in five models the comparison included the clinical camptothecin irinotecan, which was less efficacious than gimitecan in all of them, mainly in the A549 lung and HT1376 bladder carcinomas and the melanomas. No better results were attained by topotecan on the CoBA colon and A549 lung carcinomas. Gimitecan delivered *i.v.* q4dx4 at a dose of 1.5 mg/kg was well tolerated with median body weight loss of 11.5% and no lethal toxicity (0/40) confirming to be close to the MTD, whereas 2 mg/kg were toxic (3/16).

The best antitumor efficacy of camptothecins was acquired by delivering the agents following protracted, daily treatments. Oral gimitecan administered every day for five days a week for six weeks (qdx5/wx6w) was highly effective in delaying the growth of the A549 lung carcinoma, which poorly responded to a q4dx4 schedule (4). On the same model a prolonged schedule was designed for *i.v.* gimitecan,

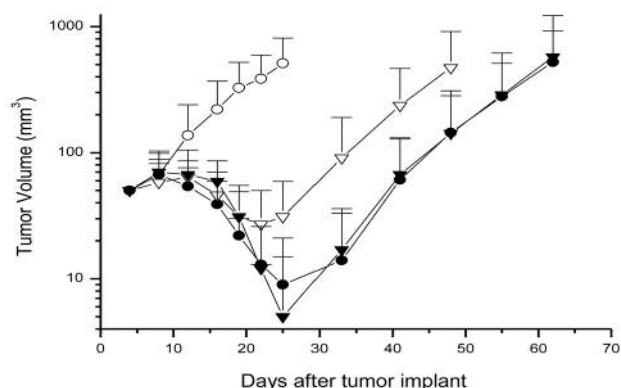


Figure 1. Curves of growth of the Panc-1 pancreatic carcinoma s.c. xenografted in nude mice receiving oral gimatecan at a dose of 2 mg/kg in 10% DMSO (filled circle), 3 mg/kg (open triangle) and 4 mg/kg (filled triangle) in 10% ethanol-cremophor or untreated (open circle). The drug was delivered on the day 4, 8, 12, 16 after tumor implant. Mean \pm s.d. of ten tumors/group is reported.

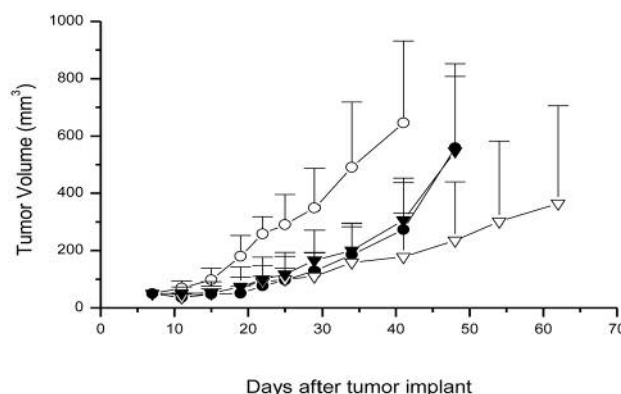


Figure 2. Curves of growth of the Me501 melanoma s.c. xenografted in nude mice receiving oral gimatecan at a dose of 2 mg/kg in 10% DMSO (filled circle), 4 mg/kg in 5% (open triangle) and in 10% (filled triangle) ethanol-cremophor or untreated (open circle). The drug was delivered on the day 7, 11, 15, 19 after tumor implant. Mean \pm s.d. of eight tumors/group is reported.

also, and significant antitumor effects were achieved (Table IV). The agent, at 2 mg/kg every seventh day for eight times (q7dx8), obtained high values of TVI (95%), CR rate (2/8) and LCK against this resistant model.

The effects of the *i.v.* camptothecin using a similar prolonged schedule (2 mg/kg q7-8dx4) were evaluated in mice bearing *i.c.* the SW1783 human astrocytoma (Table IV, Figure 4). This orthotopic model represents malignancies poorly affected by conventional therapies, because blood-brain barrier inhibits drug diffusion to central nervous system. Under these treatment conditions *i.v.* gimatecan succeeded to increase the life span of cell-injected mice (T/C: 174%, $p < 0.001$), in keeping with the already reported results achieved by the drug delivered orally, 0.25 mg/kg qdx5/wx4w (5).

Discussion

The lipophilic nature of gimatecan and its lactone stability make the drug suitable for oral administration with obvious pharmacological advantages (14-16). In a Phase II study, oral gimatecan was active against recurrent ovarian and peritoneal cancers with manageable safety profile in previously treated patients (15). However, this delivery route may present some limitations including, for instance, an erratic bioavailability. Moreover, toxic effects related to drug accumulation are expected following daily administrations. Specifically, pharmacokinetics of gimatecan in humans had a long terminal $t_{1/2}$ life leading to accumulation after daily dosing (6).

This preclinical study documents marked antitumor efficacy of the camptothecin analogue gimatecan administered *i.v.* The antitumor efficacy was superior compared to clinically

available anticancer drugs, using an intermittent treatment, *i.e.* the conventional q4dx4 schedule. The higher activity was clearly evidenced by CR rate in 4 out of 8 models (Table III). Relevant to this point was the impressive efficacy observed in models (*e.g.* MKN28 and 501Mel) poorly responsive to conventional drugs, including the clinical camptothecin analogue irinotecan. These achievements were attained by *i.v.* gimatecan at a well-tolerated dose without establishing a MTD. The remarkable tumor response to *i.v.* gimatecan likely reflects, compared to oral, a more favorable pharmacokinetic behavior related to the immediate increase in drug concentrations in plasma and tissue distribution of the active lactone form, which is known to be stable (16). The *i.v.* administration has the advantage of bypassing transporter proteins at intestinal level and retarding liver metabolism with respect to oral delivery. On the other hand, considering that the MTD of the drug in ethanol-cremophor is between 1.5 and 2 mg/kg *i.v.* and 4 mg/kg orally, the oral bioavailability of gimatecan might confirm the real ability of the drug to overcome resistance due to expression of transporter proteins in highly resistant cell lines. Gimatecan was, indeed, reported as a moderate substrate for the BCRP transporter protein, although to a lesser extent than topotecan (8).

The role of the pharmacokinetic behavior is also supported by the influence of the solvent on the drug antitumor potency, since ethanol-cremophor concentration reduced the drug potency and increased the MTDs of gimatecan. A plausible explanation of this effect is the alteration of tissue distribution produced by the solvent. Cremophor EL was already reported to increase doxorubicin efficacy with reduced mortality in mice bearing the Ehrlich ascites carcinoma (17). In fact, this

Table III. Efficacy of gimatecan vs. clinically used drugs *i.v.* on *s.c.* human tumor xenografts. Optimal doses and schedules were employed, *i.e.* gimatecan (GIM), paclitaxel (PTX, 54 mg/kg), irinotecan (CPT11, 50 mg/kg), topotecan (TPT, 15 mg/kg) q4dx4, cisplatin (DDP, 6 mg/kg) and doxorubicin (DX, 7mg/kg) q7dx3. Treatment started when tumors were just palpable (day 3-7).

Tumor type	Drug	Dose mg/kg	TVI% ¹	CR ²	NED ³	LCK ⁴	BWL% ⁵	Tox ⁶
Panc-1 pancreas	GIM	1.5	100, 100	18/18	14/18 (125,170)	3.7, >5.5	3, 7	0/9
	PTX		97	2/8	-	0.9	5	1/5
GER pancreas	GIM	1.5	96	0/9	-	1.8	11	0/5
	PTX		100	8/8	5/8 (90)	5.8	5	0/4
	DDP		54	0/9	-	0.8	8	0/5
MKN-28 stomach	GIM	1.5	97	3/10	2/8 (70)	1.9	16	0/5
	DDP		51	0/10	-	0.4	14	0/5
	DX		71	0/8	-	0.8	4	0/4
MKN-45 stomach	GIM	1.5	96	1/12	-	3.1	10	0/6
CoBA colon	GIM	1.5	97	0/10	-	1.8	13	0/5
	CPT11		86	0/8	-	1.1	2	0/4
	TPT		85	0/10	-	1.0	10	0/5
	DDP		58	0/10	-	0.3	5	0/5
HT1376 bladder	GIM	1.5	88	1/12	-	1.1	12	0/6
	CPT11		46	0/10	-	0.2	9	0/5
	DDP		80	1/10	-	1.5	6	0/5
	DX		64	0/10	-	1.2	26	1/5
A549 lung	GIM	1.5	94	0/8	-	1.8	14	0/4
	CPT11		42	0/8	-	0.5	4	0/4
	TPT		42	0/8	-	0.5	8	1/6
	PTX		86	0/12	-	1.3	13	0/6
	DX		50	-	-	0.5	-	-
Me26414 melanoma	GIM	2	99	5/6	4/6 (78)	4.1	24	1/4
	CPT11		56	1/8	-	0.7	9	0/4
	PTX		92	2/8	-	1.7	3	0/4
	DDP		67	0/8	-	1.0	8	0/4
501Mel melanoma	GIM	2	97, 97	7/14	2/14 (112)	2.1, 2.4	17, 17	1/8
	CPT11		70	0/10	-	1.3	18	0/5
	DDP		68	0/10	-	1.0	7	0/5

¹Tumor volume inhibition % in treated over control tumors; ²Complete response: *i.e.* disappearance of tumor lasting at least 10 days; ³No evidence of disease at the end of the experiment (in parentheses the day); ⁴Gross log₁₀ cell kill to reach an established tumor volume; ⁵Body weight loss % induced by drug treatment; the highest change is reported; ⁶Dead/treated mice.

solvent is not an inert vehicle, but exerts several biological effects after *i.v.* delivery in drug formulations. Importantly, cremophor EL is reported to alter pharmacokinetics of many drugs and, in particular, paclitaxel. Since taxol affinity to the solvent is higher than that to plasma, the encapsulation of the drug within micelles of cremophor EL has been proposed for explaining the non-linear drug disposition (18). Likewise, the highly lipophilic gimatecan could be entrapped by micelles of cremophor increasing persistence of the camptothecin in plasma after *i.v.* injection or in bowel after “gavage”. As a consequence, a slower diffusion to tissues could induce a lower, but prolonged cytotoxic activity. It would be expected that higher rates of cells will be targeted in the S phase with higher antitumor efficacy and reduced toxic effects, in particular in the bone marrow. The role of pharmacokinetics in antitumor efficacy of *i.v.* gimatecan could be, also,

supported by a previous study performed with the hydrophilic analogue topotecan (10). At same dose and schedule (q4dx4), topotecan oral treatment was characterized by better tolerability and increased antitumor efficacy over the *i.v.* treatment. This improvement is likely related to the persistent plasma levels of oral topotecan which appear more critical than the higher, but transient plasma concentrations produced by the *i.v.* administration. In addition, in *i.v.* treated mice the peak of topotecan in plasma was associated with the high rate of lethal events, probably because of myelotoxicity. In contrast, after *i.v.* administration the lipophilic nature of gimatecan and the different pharmacokinetic behavior, possibly modulated by the solvent cremophor EL, might favor a slow and persistent tissue accumulation and account for the high antitumor efficacy and tolerability of *i.v.* gimatecan. Interestingly, in experimental and clinical studies therapeutic

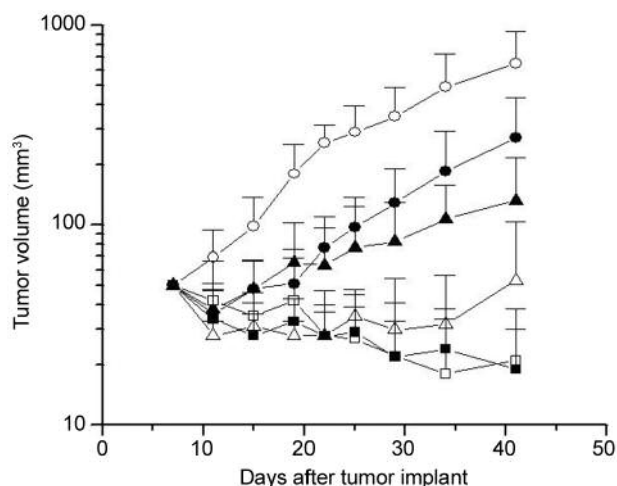


Figure 3. Curves of growth of the Me501 melanoma s.c. xenografted in nude mice receiving oral gimitecan at a dose of 2 mg/kg in 10% DMSO (filled circle), or i.v. 1 mg/kg in 1.25% (open triangle) and in 2.5% (filled triangle), 2 mg/kg in 2.5% (open square) and in 5% (filled square) ethanol-cremophor or untreated (open circle). The drug was delivered on the day 7, 11, 15, 19 after tumor implant. Mean \pm s.d. of eight tumors/group is reported.

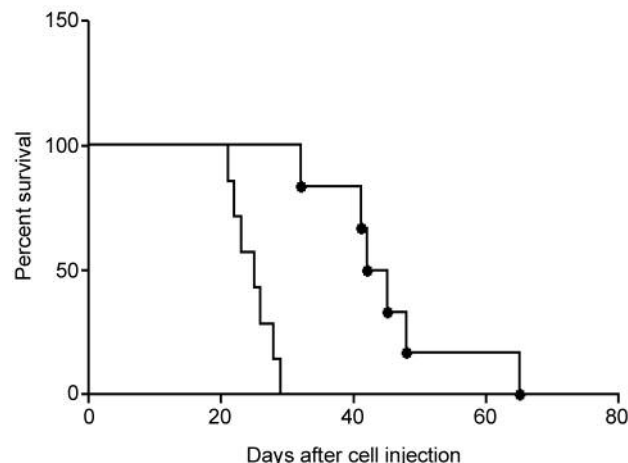


Figure 4. Kaplan-Meier plot of percentage of survivors over time among mice i.c. injected with SW1783 astrocytoma cells, receiving i.v. gimitecan at a dose of 2 mg/kg every eighth-seventh day for four times (filled circle) or untreated (no symbol). Mean of six-seven animals/group is reported.

Table IV. Effects of i.v. gimitecan at a dose of 2 mg/kg, in nude mice bearing human tumors. Tumor fragments or cells were inoculated on day 0.

Solvent et-cr (%) ⁹	Tumor type	Site	Days of treatment	T/C% ¹	TVI% ²	P vs. ³ controls	CR ⁴	NED ⁵	LCK ⁶	BWL% ⁷	Tox ⁸
(5)	A549 lung carcinoma	s.c.	5, 12, 19, 26, 33, 40, 47, 54	-	95 (57)	0.0097	2/8	2/8 (125)	2.2	10	1/5
(2.5)	SW1783 astrocytoma	i.c.	3, 11, 18, 25	174	-	0.0005	-	-	-	-	-

¹Ratio of median survival time (MST) in treated over control mice (T/C) \times 100; ²Tumor volume inhibition % in treated over control mice; in parentheses the day on which it was assessed; ³By two-tailed Student's *t*-test (A549) or log-rank test (SW1783); ⁴Complete responses, i.e. disappearance of tumor lasting at least 10 days; ⁵No evidences of disease at the end of experiment (in parentheses the day); ⁶Gross log₁₀ cell kill to reach 600 mm³ of tumor volume; ⁷Body weight loss % induced by drug treatment, the highest change is reported; ⁸Dead/treated mice; ⁹Ethanol and cremophor (50+50%) concentration suspended in saline.

index of cisplatin was seen improved after formulation in cremophor EL. A markedly reduced myelosuppression was found on the basis of these observations (19).

Given the high cytotoxic potency of gimitecan, prolonged administration schedules may produce potential benefits in the treatment of slowly-growing tumors. Thus, the efficacy of oral gimitecan against the slowly growing lung carcinoma A549, which poorly responded to the intermittent and brief q4dx4 schedule at the MTD, was increased by employing protracted daily administrations of a low dose level (4). Similarly, intermittent and prolonged administrations of a high dose *via* the i.v. route resulted into a valid antitumor

outcome, at least in terms of growth inhibition and complete responses (Table IV).

Similar results were obtained by the i.v., prolonged and high-dose treatment against the orthotopic brain tumor xenograft SW1783 (Figure 4) confirming the ability of the drug to cross blood-brain barrier and reach brain tissues (3). Recent studies employing i.v. gimitecan in mice knock out for ABC transporter proteins suggest the drug, given the high cell membrane permeability, as a good candidate for clinical evaluation against intracranial malignancies (20). Unfortunately, a Phase II trial showed minimal efficacy of oral gimitecan in adults with recurrent glioblastoma. The failure of the treatment might

be ascribed to the daily dosing used or lack of selectivity in the choice of the patients with respect to topoisomerase I levels (21).

The range of malignancy models responsive to antitumor effects of gimatecan has been recently enlarged including gastro-esophageal cancers and hepatocarcinomas (22-24). Liver is a common site of primary and metastatic involvement for several cancer types. Thus, marked therapeutic activity of oral gimatecan against liver metastases of ovarian carcinoma in nude mice was reported employing prolonged, daily, low-dose schedule. The efficacy was related to relevant drug accumulation in the organ after oral administration (3). However, considering the high lipophilicity and cytotoxic potency of the molecule, the achievement of an appropriate *i.v.* formulation of gimatecan might be a valid implement for intra-arterial or -portal treatments in locoregional therapies of liver cancers.

In conclusion, despite known drawbacks of the solvent required for *i.v.* formulation, the study emphasizes that *i.v.* gimatecan is characterized by an optimal therapeutic index, as indicated by marked efficacy at well tolerated doses. Based on this preclinical evidence, the development of suitable formulations for *i.v.* delivery could result into a more versatile exploitation of gimatecan with optimization of drug efficacy over the currently employed therapies.

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