Preclinical Efficacy of the Camptothecin-Polymer Conjugate IT-101 in Multiple Cancer Models

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Abstract Preclinical efficacy of i.v. IT-101, a nanoparticulate conjugate of 20(*S*)-camptothecin and a cyclodextrin-based polymer, was investigated in several mouse xenografts. The effects of different multiple dosing schedules on tumor growth of LS174T colon carcinoma xenografts are elucidated. All multiple dosing schedules administered over 15 to 19 days resulted in enhanced efficacy compared with untreated or single-dose groups. Further improvements in antitumor efficacy were not observed when the dosing frequency was increased from three weekly doses to five doses at 4-day intervals or 5 days of daily dosing followed by 2 days without dosing repeated in three cycles using similar cumulative doses. This observation was attributed to the extended release characteristics of camptothecin from the polymer. Antitumor efficacy was further evaluated in mice bearing six different s.c. xenografts (LS174T and HT29 colorectal cancer, H1299 non–small-cell lung cancer, H69 small-cell lung cancer, Panc-1 pancreatic cancer, and MDA-MB-231 breast cancer) and one disseminated xenograft (TC71-luc Ewing's sarcoma). In all cases, a single treatment cycle of three weekly doses of IT-101 resulted in a significant antitumor effect. Complete tumor regression was observed in all animals bearing H1299 tumors and in the majority of animals with disseminated Ewing's sarcoma tumors. Importantly, IT-101 is effective in a number of tumors that are resistant to treatment with irinotecan (MDA-MB-231, Panc-1, and HT29), consistent with the hypothesis that polymeric drug conjugates may be able to overcome certain kinds of multidrug resistance. Taken together, these results indicate that IT-101 has good tolerability and antitumor activity against a wide range of tumors.

20(*S*)-Camptothecin (CPT), a natural alkaloid first isolated from *Camptotheca acuminata*, is a potent antineoplastic agent with activity against a broad range of cancer cells (1). The reported mode of action is through inhibition of the topoisomerase I enzyme: formation of covalent and nonreversible topoisomerase I-DNA complexes during DNA replication results in strand breaks and subsequent induction of apoptosis (2). However, camptothecin itself was never commercialized as an anticancer agent due to poor solubility, lack of activity, and excessive toxicity observed in early clinical trials (3–5). Camptothecin undergoes a reversible, pH-dependent ring opening reaction between the active lactone (closed E-ring) and inactive carboxylate (open E-ring) form. At physiologic pH, this equilibrium lies towards the carboxylate form, which strongly binds to serum albumin, thus further driving the reaction towards the inactive form of CPT (6). To overcome some of these limitations, several small-molecule analogues of camptothecin have been synthesized (7). Two analogues, irinotecan (Camptosar; Pharmacia & Upjohn, Kalamazoo, MI) and topotecan (Hycamptin; GlaxoSmithKline, Research Triangle Park, NC), are currently approved for the treatment of certain cancers in humans (7).

An alternative approach to small-molecule topoisomerase I inhibitors is the attachment of camptothecin to polymeric carrier molecules (8–12). Attachment to hydrophilic polymers increases solubility and may result in extended release pharmacokinetics and improved biodistribution characteristics through the so-called enhanced permeability and retention effect. It has been shown that long-circulating macromolecules can extravasate through the abnormally leaky tumor vasculature and accumulate in tumor tissue due to a lack of effective lymphatic drainage (13). Additionally, a number of experiments have indicated that macromolecular drugs may overcome multidrug resistance mediated by drug efflux pumps because of their endocytic internalization (14, 15).

IT-101 is a conjugate of camptothecin with a cyclodextrin-based polymer (Fig. 1). This polymer was well tolerated at doses in excess of 240 mg/kg when injected i.v. in mice (12). When camptothecin is conjugated to this polymer, its solubility is increased by 3 orders of magnitude. CPT is attached to the polymer at the 20-OH position, which inhibits ring opening so that CPT remains in its active lactone form (11). Conjugates of different molecular weights (35-97 kDa) and linker chemistries were synthesized and tested *in vivo* (11, 12). From among these, IT-101, a conjugate with high molecular weight (82-97 kDa)

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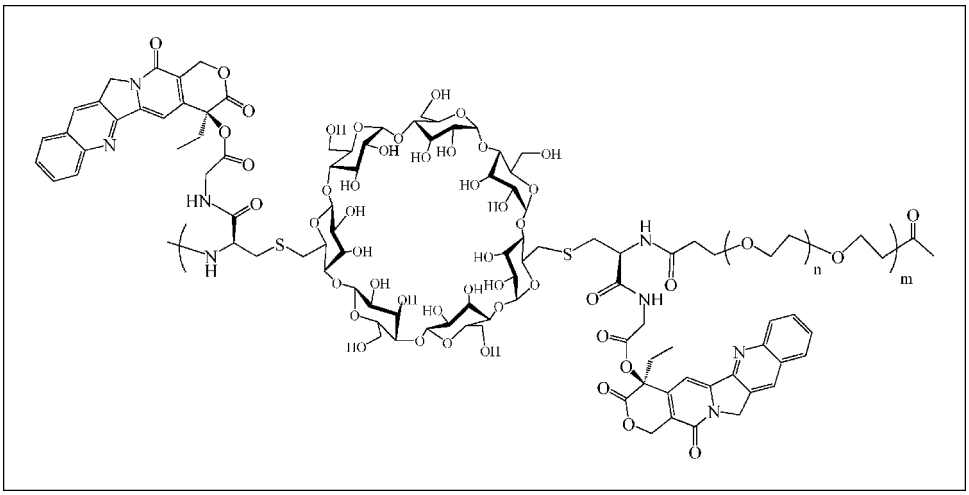

Fig. 1. Structure of IT-101, a conjugate between CPT and a linear cyclodextrin-based polymer. The components of the parent polymer are β-cyclodextrin and polyethylene glycol, both of which are widely used in pharmaceutical formulations, and the natural amino acid L-cysteine. Camptothecin is attached to the polymer via a single glycine amino acid linker. n, number of ethylene glycol repeating units (average $n = 77$ for polyethylene glycol with M_w 3400); m, number of repeating units of (cyclodextrin-based polymer-camptothecin) in the polymer-camptothecin conjugate (average $m = 15 \pm 4$ for parent polymer, M_w of 84.5 ± 22.5 kDa).

and a single glycine linker (G), was selected due to its higher efficacy and tolerability compared with low molecular weight conjugates and conjugates with different linker chemistries, respectively. The pharmacokinetics and biodistribution data for IT-101 in rats and tumor-bearing mice are available (16). The plasma half-life of IT-101 in rats ranges from 17 to 20 hours and is significantly greater than free CPT. At all times, the amount of free CPT in plasma was <1% of the amount bound to the polymer. At 24 hours postinjection in mice, the total CPT per gram of tissue is the highest in tumor when compared with liver, lung, spleen, and heart, and is ~2.5% of injected dose/g tissue.

Here, we report on a series of studies comparing different dosing schedules for the administration of IT-101 to mice bearing s.c. tumor xenografts. Results from these studies were then used to evaluate the efficacy of IT-101 in seven different mouse tumor xenografts. The models span a wide range of tumor types such as Ewing's sarcoma, colon carcinoma, non–small-cell lung cancer, small-cell lung cancer, breast cancer, and pancreatic cancer.

Materials and Methods

Reagents. Irinotecan (CPT-11, Camptosar) was from Pharmacia & Upjohn (Kalamazoo, MI). Camptothecin was from Boehringer Ingelheim (Ingelheim, Germany). IT-101 was synthesized as previously described (11). Properties of the polymer-camptothecin conjugates are described in Table 1. IT-101 is similar to compounds previously denoted as HG6, representing a conjugate with high M_w (H, 82-85 kDa), a single glycine linker (G), and ~6 wt % (5.0-7.4 wt %) drug loading (12).

Animal care. All animals were housed in a specific pathogen-free animal facility at Piedmont Research Center and at the California Institute of Technology in accordance with the Guide for Care and Use of Laboratory Animals and regulations of the Institutional Animal Care and Use Committee. The animal program at Piedmont Research Center is Association for Assessment and Accreditation of Laboratory Animal Care accredited.

Subcutaneous human tumor xenografts. The LS174T and HT29 colon, H1299 non–small-cell lung, and Panc-1 pancreatic cancer cell lines used for this study were maintained in athymic nude mice. H69 non–small-cell lung cancer and MDA-MB-231 breast adenocarcinoma cells were cultured in RPMI 1640 containing 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B, and 50 μg/mL gentamicin. The medium was supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L glutamine, 10 mmol/L HEPES, 0.075% sodium bicarbonate, and 1 mmol/L sodium pyruvate. The tumor cells were cultured in tissue culture flasks in a humidified incubator at 37°C in an atmosphere of 5% CO_2 and 95% air.

For tumors maintained in mice, a fragment (1 mm^3) was implanted s.c. into the right flank of Charles River female athymic nude mice. For tumors passaged in medium, cells were harvested during log-phase growth and resuspended in 50% matrigel. Subsequently, 1×10^7 cells (0.2-mL cell suspension) were injected s.c. in the right flank of female nude mice.

Tumors were measured in two dimensions with calipers and volume was calculated using the following formula: tumor volume = (length × width^2) / 2. Tumor volume was converted to tumor weight assuming 1 mm^3 is equal to 1 mg of tumor in weight. When the average tumor size was 80 to 120 mm^3, mice were sorted into groups of eight animals each and treatment was initiated (day 1).

Irinotecan and camptothecin were administered i.p. whereas all other treatments were given i.v. The tumor growth delay method was followed wherein each animal is sacrificed when its tumor reached a predetermined size. End-point tumor size was chosen to maximize the

Table 1. Properties of IT-101 preparations used

Batch no.	Studies*	M_w of the parent polymer (kDa)	M_w/M_n†	CPT loading (wt %)	%Free CPT	Particle size (nm)‡
1	SD	85	1.48	5.03	1.3	78
2	EF1-7	82	1.52	7.36	0.26	54

*SD, schedule and dose dependence of the antitumor activity in mice bearing LS174T xenografts; EF1-7, antitumor efficacy in seven different mouse xenograft models.
†Polymer polydispersity determined by light scattering techniques.
‡Mean particle size of the conjugate determined in water by dynamic light scattering using a ZetaPals instrument (Brookhaven Instruments, Holtsville, NY).

number of tumor doublings within the exponential growth phase in the control animals. End-point size varied for each cell line and was set at 1,500 mm^3 for LS174T and MDA-MB-231; 1,200 mm^3 for H1299, H69, and Panc-1; and 1,000 mm^3 for HT29.

Efficacy study in human TC71-luc Ewing's sarcoma xenografts. TC71 cells virally transduced to obtain stable luciferase expression were obtained from the laboratory of Timothy Triche at Children's Hospital-Los Angeles (see ref. 17 for characteristics of this cell line). TC71-luc cells were cultured in RPMI 1640 with 10% fetal bovine serum and antibiotics (penicillin/streptomycin). Female nonobese diabetic/severe-combined immunodeficient mice were injected with 5×10^6 TC71-luc cells suspended in 0.2 mL RPMI 1640 via the tail vein. This procedure yields mice with disseminated tumors that are located in areas where they are most commonly observed in Ewing's sarcoma patients (17). At 35 days post cell injection, mice were randomized into groups of six animals each and treatment was initiated (day 1).

Mice were imaged on days 1, 4, 8, 11, 15, 18, 23, 38, 46, 53, and 60 postadministration using an *in vivo* IVIS 100 bioluminescence/optical imaging system (Xenogen). D-Luciferin (Xenogen) dissolved in PBS was injected i.p. at a dose of 150 mg/kg (0.2 mL of a 15 mg/mL solution per 20-g mouse) 10 minutes before measurement of luminescence. General anesthesia was induced with 5% isoflurane and continued during the procedure with 2.5% isoflurane introduced via a nose cone.

After acquiring photographic images of each mouse, luminescent images were acquired with various (1-60 seconds) exposure times. The resulting grayscale photographic and pseudocolor luminescent images were automatically superimposed by the IVIS Living Image software to facilitate the matching of the observed luciferase signal with its location within the mouse. Regions of interest were drawn around the bodies of the mice to assess signal intensity emitted. Luminescent signal was integrated over these regions of interest and is expressed as photons emitted per second. Tumor bioluminescence in mice has been shown to be linearly correlated with the tumor volume (18, 19) and we have verified these findings (data not shown).

Determination of treatment efficacy. Treatment efficacy is based on the determination of the time it took a specific tumor to reach the predetermined end point size. The time to end point (TTE) for each mouse was calculated from the equation TTE = $[\log(\text{end point}) - b] / m$, where b and m are the intercept and slope, respectively, of the line obtained by linear regression of a log-transformed tumor growth data set comprised of the first observation that exceeded the study end point volume and the three consecutive observations that immediately preceded the attainment of the end point volume. Animals that did not reach the end point were assigned a TTE value equal to the last day of the study. Animals classified as treatment-related deaths were assigned a TTE value equal to the day of death. Animals classified as non-treatment-related death were excluded from TTE calculations. Tumor growth delay (TGD) is calculated as the difference between the median TTE for a treatment group and the median TTE of the control group (TGD = $T - C$) and is expressed in days and as a percentage of the median TTE of the control group; %TGD = $[(T - C) / C] \times 100$, where T is equal to the median TTE for a treatment group and C is equal to the median TTE for the control.

Treatment may cause partial regression or complete regression of the tumor in an animal. In a partial regression response, the tumor volume is ≤50% of its day 1 volume for three consecutive measurements during the course of the study and ≥13.5 mm^3 for one or more of these three measurements. In a complete regression response, the tumor volume is <13.5 mm^3 for three consecutive measurements during the course of the study. An animal with a complete regression response at the end of the study was additionally classified as a tumor-free survivor.

Tolerability. Animals were weighed daily on days 1 to 5, then twice per week until the completion of the study. The mice were examined for overt signs of any adverse drug-related side effects. Acceptable toxicity for the maximum tolerated dose was defined as group mean body weight loss of <20% and not more than one toxic death among 10 treated animals.

Statistical and graphical analyses. Log-rank tests were employed to analyze differences in the TTE between treatment groups. The log-rank test was used to analyze the data for all animals except the non-treatment-related deaths. Two-tailed statistical analyses were conducted at $P = 0.05$. Mean tumor growth curves prepared for each group depicted the mean tumor size as a function of time. When an animal exited the study due to tumor size or treatment-related death, the final tumor size recorded for the animal was included with the data used to calculate the mean size at subsequent time points.

Results

Schedule dependence of IT-101 efficacy in LS174T xenografts. One of the primary indications for the topoisomerase I inhibitor irinotecan is colorectal cancer. We have previously shown that in the human LS174T colon carcinoma xenograft mouse model, IT-101 significantly delayed tumor growth compared with camptothecin alone and irinotecan (12). Here we explore how dosing frequency affects the efficacy of IT-101 in the same tumor model. For reference purposes, irinotecan was administered i.p. at 100 mg/kg once per week for 3 weeks (qwk × 3), which is a standard dosing schedule for irinotecan in the clinic. In non-tumor-bearing nude mice, the maximum tolerated dose for irinotecan i.p. on a qwk × 3 schedule was previously determined to be ~110 mg/kg (Piedmont Research Center internal data). This value is in good agreement with an i.v. mouse maximum tolerated dose of 100 mg/kg reported in the literature (20). In tumor-bearing mice, doses of 110 mg/kg qwk × 3 resulted in more than one toxicity related animal death per 10 animals, indicating that maximum tolerated dose was reached. Irinotecan was administered i.p. rather than i.v. because i.v. injection of irinotecan resulted in unacceptable gastrointestinal toxicity at 100 mg/kg whereas antitumor efficacy was equivalent for both routes of administration. A second control was camptothecin alone at 3 mg/kg, administered i.p. once daily on days 1, 5, 9, 13, and 17 (q4d × 5), which is the highest tolerated dose for camptothecin on this schedule. Tumor growth curves and Kaplan-Meier plots for the various treatments are shown in Fig. 2.

Camptothecin alone produced nonsignificant antitumor activity ($P > 0.05$) with 54% TGD whereas irinotecan administered at 100 mg/kg qwk × 3 resulted in a significant ($P < 0.05$) antitumor effect (101% TGD) compared with untreated control.

Treatment with IT-101 resulted in significant antitumor activity compared with untreated animals regardless of dosing schedule. A single dose (qd × 1) of IT-101 at 18.3 mg/kg (CPT equivalents) resulted in 79% TGD ($P < 0.05$). The multiple dose schedules of IT-101 investigated in this study all resulted in significant tumor growth delay compared with untreated animals (Fig. 2). Animals receiving 9.7 mg/kg (CPT equivalents) of IT-101 q4d × 5 showed 250% TGD ($P < 0.01$), those receiving 3.2 mg/kg (CPT equivalents) of IT-101 administered in 5 days of daily dosing followed by 2 days without dosing repeated in three cycles (5/2/5/2/5 schedule) showed 130% TGD ($P < 0.001$), and those receiving 18.3 mg/kg (CPT equivalents) of IT-101 qwk × 3 showed 221% TGD ($P < 0.001$), compared with untreated animals. To facilitate comparisons among the three different multiple dosing schedules, similar cumulative doses were administered over 15 to 19 days (see Fig. 2). Differences in antitumor effect between the different multiple dosing schedules were not significant ($P \geq 0.05$).

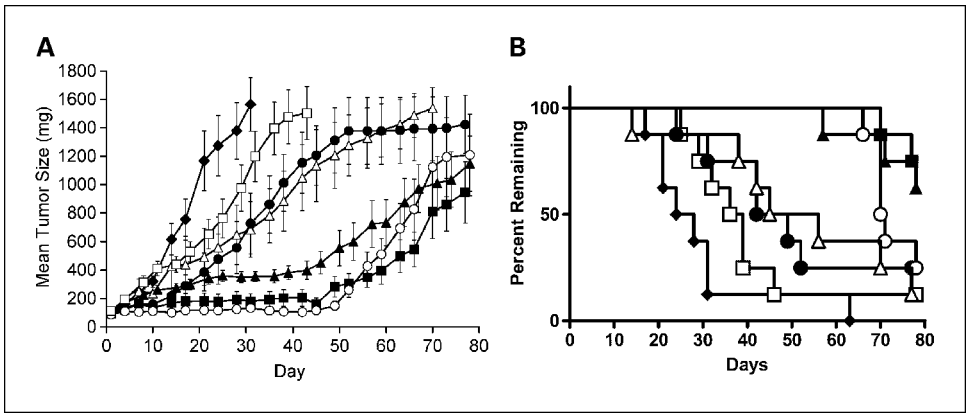

Fig. 2. Schedule dependence of IT-101 antitumor effect in a xenograft model of LS174T colon cancer s.c. implanted into nude mice. Animals were either untreated (♦) or treated with camptothecin q4d × 5 i.p. (3.0 mg/kg, □), irinotecan qwk × 3 i.p. (100 mg/kg, △), IT-101 qd × 1 i.v. (18.3 mg/kg, ●), IT-101 5/2/5/2/5 i.v. (3.2 mg/kg, ○), IT-101 q4d × 5 i.v. (9.7 mg/kg, ▲), and IT-101 qwk × 3 i.v. (18.3 mg/kg, ■). No significant difference in antitumor activity was seen between the various multiple dosing regimens of IT-101. Cumulative doses for the IT-101 treatment groups were as follows: qd × 1 = 18.3 mg/kg; q4d × 5 = 49 mg/kg; 5/2/5/2/5 = 48 mg/kg; qwk × 3 = 55 mg/kg. All IT-101 doses are in camptothecin equivalents.

All treatments were well tolerated with no animal deaths observed. Mean body weight loss was negligible for all treatment groups except the qd × 1 and 5/2/5/2/5 groups, which lost 8.9% on day 4 and 7.3% on day 17, respectively. However, weight loss was transient and not accompanied by any other overt signs of toxicity.

Efficacy in solid human tumor xenografts. The antitumor activity of IT-101 was further evaluated in several solid human tumor xenograft models in nude mice. Tumor types were chosen to reflect a broad range of tumors that have been shown to respond to treatment with either topotecan or irinotecan (21). Tumor lines selected included LS174T and HT29 (colorectal cancer), H1299 (non–small-cell lung cancer), H69 (small-cell lung cancer), Panc-1 (pancreatic cancer), and MDA-MB231 (breast cancer).

Irinotecan administered qwk × 3 at 100 mg/kg i.p. was included as an internal positive control as a means to judge the general sensitivity of the various tumor cell lines to an established small-molecule camptothecin derivative. Irinotecan has shown antitumor activity against a wide range of tumor types including colorectal, non–small-cell lung cancer, small-cell lung cancer, gastric carcinoma, cervical cancer, pancreatic cancer, and glioma (21). Preclinically, irinotecan has also been shown to be active against breast carcinoma (20) and was at least as active as topotecan against a number of different xenografts including colorectal, rhabdomyosarcoma, and brain tumors (22). Additionally, irinotecan, rather than topotecan, was selected because the parent compound and its active metabolite SN-38 have been reported to be have a more favorable pharmacokinetic behavior as well as increased cytotoxic activity compared with topotecan (21).

IT-101 was administered as a single high dose or multiple high and low doses, qwk × 3. Mean tumor growth curves as well as Kaplan-Meier curves for the s.c. xenograft models are shown in Fig. 3. A summary of results is also shown in Table 2. Data depicted in the Kaplan-Meier plots were used to determine statistical significance (see Materials and Methods for details). In the colorectal LS174T model, treatment with IT-101 resulted in a significant antitumor effect when administered as a single dose of 25.9 mg/kg (CPT equivalents) or as three weekly doses of 16.1 mg/kg (CPT equivalents). Three weekly doses of IT-101 at 25.9 mg/kg (CPT equivalents) also resulted in a reduction in tumor size but significance could not be evaluated due to two treatment-related deaths. Three weekly doses of irinotecan (100 mg/kg) resulted in a significant antitumor effect. This activity was, however, significantly lower than that of a single or multiple doses of IT-101.

HT29 colorectal carcinoma was chosen because it was reported to have a multidrug resistant phenotype (23). Consistent with these reports, three weekly doses of irinotecan (100 mg/kg) resulted in a nonsignificant tumor growth delay. Treatment with a single dose of IT-101 (20.7 mg/kg CPT equivalents) gave a tumor growth delay similar to three weekly doses of irinotecan but resulted in ~30% of treatment-related animal deaths. Three weekly doses of IT-101 at 12.9 mg/kg (CPT equivalents) resulted in a significant antitumor effect with minimal observed toxicity. This treatment also resulted in a partial regression response in two animals.

The H1299 non–small-cell lung cancer model was sensitive to treatment with the internal control irinotecan, resulting in a significant tumor growth delay (100%) and one tumor-free survivor. The mean tumor growth curve shows that irinotecan produced a transient decrease in tumor volume, which increased rapidly after day 30. A single dose of IT-101 at 25.8 mg/kg (CPT equivalents) resulted in a tumor growth delay similar to three weekly doses of irinotecan and produced four long-term tumor-free survivors. Multiple doses at this high dose level resulted in four treatment-related deaths after two doses and treatment was discontinued. IT-101 administered in three weekly doses at 16.1 mg/kg (CPT equivalents) resulted in complete tumor remission with no recurrence observed through the completion of the study (day 92) in 100% of animals treated. Log-rank analysis, which compares TTE values of two treatment groups, indicates significant activity relative to no treatment but nonsignificant activity relative to the irinotecan control. However, based on the number of tumor-free survivors in each group, efficacy was significant relative to irinotecan ($P < 0.001$, Fisher's exact test).

H69 tumors responded strongly to irinotecan at 100 mg/kg qwk × 3. This reference treatment produced significant antitumor activity with 216% tumor growth delay and one transient complete regression. IT-101 administered as a single dose of 16.1 mg/kg (CPT equivalents) produced a 178% TGD and yielded one tumor-free survivor. The lower dose regimen, 9.7 mg/kg (CPT equivalents) qwk × 3, produced a 183% TGD and yielded three partial regression responses. Each of these treatments was slightly less active than the irinotecan control ($P \geq 0.05$). IT-101 administered in three weekly doses of 16.1 mg/kg (CPT equivalents) produced 348% TGD and yielded two transient complete regression responses and four

partial regression responses. This regimen exhibited significantly greater activity than irinotecan reference therapy. All treatments were well tolerated and body weight loss was minimal in this model.

The MDA-MB-231 xenograft model was included in the panel as a representative of an estrogen-independent breast adenocarcinoma. IT-101 administered as a single dose or three weekly doses of 25.8 mg/kg (CPT equivalents) resulted in four and five treatment-related deaths, respectively. These doses were therefore not statistically evaluable. When IT-101 was administered on the qwk × 3 schedule at 16.1 mg/kg (CPT equivalents), treatment was well tolerated, producing a tumor growth delay of 221% and one long-term tumor-free survivor. This schedule showed significant antitumor activity ($P < 0.01$). The internal positive control irinotecan administered qwk × 3 at 100 mg/kg produced nonsignificant activity in this model.

In the Panc-1 model, a single dose of IT-101 at 20.7 mg/kg (CPT equivalents) resulted in nonsignificant antitumor activity and 31% TGD. One treatment-related death was also observed at that dose level. When administered on a qwk × 3 schedule,

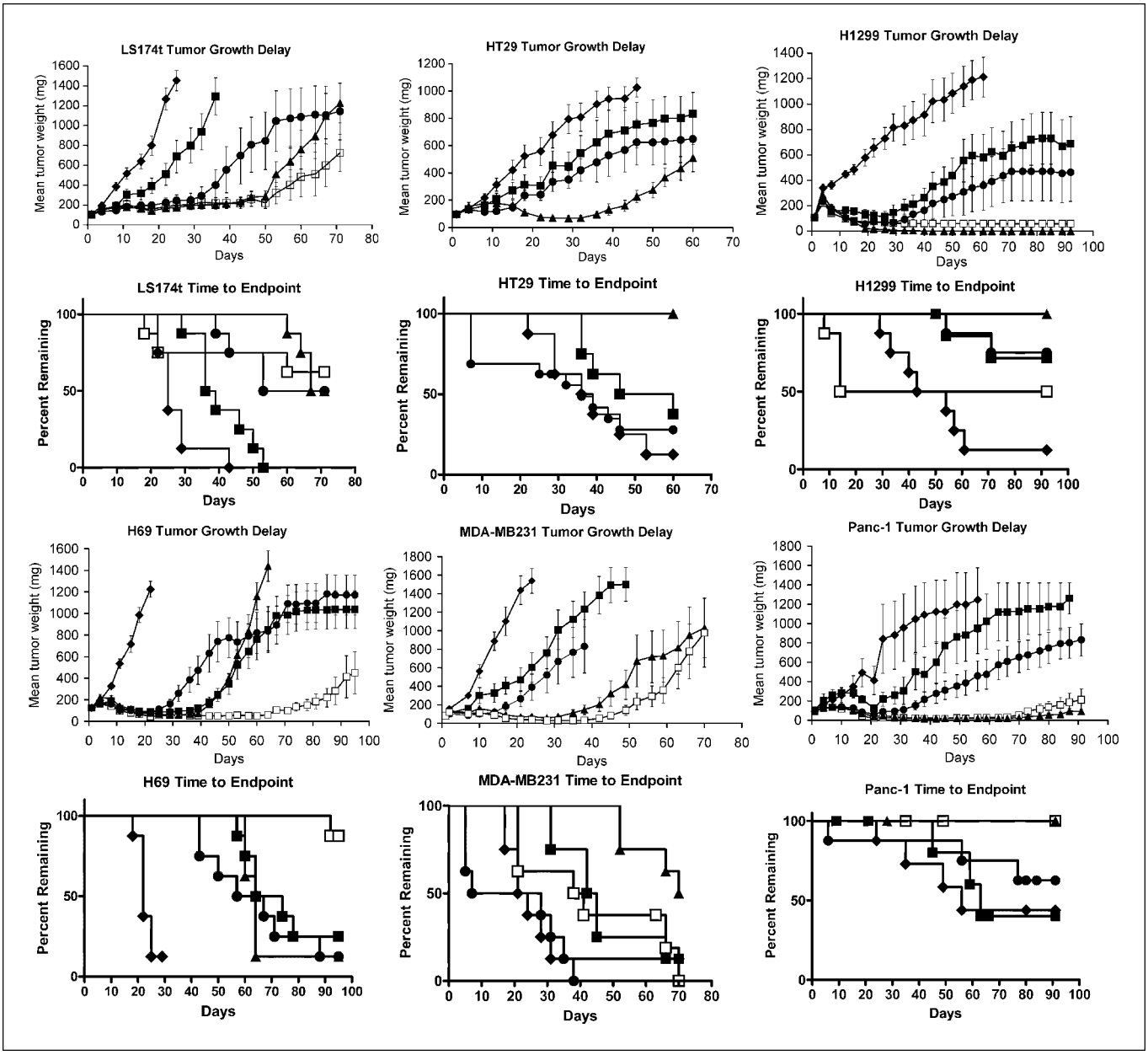

Fig. 3. Inhibition of six different human xenografts by IT-101. Mice were either treated with vehicle (♦) or the internal positive control irinotecan administered i.p. on days 0, 7, and 14 at 100 mg/kg (■). IT-101 was administered i.v. as a single high dose (●) or as three weekly doses on days 0, 7, and 14 at a high (□) or low (▲) dose level. Dose levels were adjusted based on differences in dose limiting toxicity observed in the different models: High dose = 25.8 mg/kg (LS174T, H1299, and MDA-MB-231), 20.7 mg/kg (HT29 and Panc-1), or 16.1 mg/kg (H69). Low dose = 16.9 mg/kg (LS174T, H1299, and MDA-MB-231), 12.9 mg/kg (HT29 and Panc-1), or 9.7 mg/kg (H69). Points, mean tumor growth; bars, SE. Survival is indicated as the percentage of animals remaining on study because mice bearing tumors that reached a predetermined cutoff size were removed from the experiment. End-point tumor size was chosen to maximize the number of tumor doublings within the exponential growth phase in the control animals. End-point size varied for each cell line and was set at 1,500 mm^3 for LS174T and MDA-MB-231; 1,200 mm^3 for H1299, H69, and Panc-1; and 1,000 mm^3 for HT29. All IT-101 doses are in camptothecin equivalents.

Table 2. Antitumor activity of IT-101 in various s.c. human xenograft models in nude mice

Model	*n*	Agent	Schedule	Dose (mg/kg)	Median TTE	TGD (%)	Log-rank significance		CR	TFS	TR	BWLmax (%)
							vs UT	vs CPT-11				
LS174T (colon)	8	UT	—	—	24.6	—	—	—	0	0	0	—
	8	CPT-11	qwk × 3	100	37.1	51	*	—	0	0	0	—
	8	IT-101	qd × 1	25.8	62.0	152	†	*	0	0	0	12.1 (5)
	8	IT-101	qwk × 3	25.8	71.0	189	ne	ne	0	2	2	15.9 (18)
	8	IT-101	qwk × 3	16.1	69.0	180	†	†	0	0	0	4.7 (11)
HT29 (colon)	8	UT	—	—	36.6	—	—	—	0	0	0	—
	8	CPT-11	qwk × 3	100	52.0	42	ns‡	—	0	0	0	—
	16	IT-101	qd × 1	20.7	32.0	—	ne§	ne	0	0	5	20.9 (5)
	8	IT-101	qwk × 3	12.9	60.0	64	†	*	0	0	0	2.5 (11)
H1299 (NSCLC)	8	UT	—	—	45.6	—	—	—	0	0	0	—
	8	CPT-11	qwk × 3	100	91.0	100	*	—	1	1	0	—
	8	IT-101	qd × 1	25.8	91.5	101	*	ns	4	4	0	4.2 (4)
	8	IT-101	qwk × 2	25.8	14.0	—	ne	ne	4	3	4	23.4 (12)
	8	IT-101	qwk × 3	16.1	92.0	102	†	ns‖	8	8‖	0	8.0 (19)
MDA-MB-231 (breast)	8	UT	—	—	21.4	—	—	—	0	0	0	—
	8	CPT-11	qwk × 3	100	41.1	92	ns	—	0	0	0	—
	8	IT-101	qd × 1	25.8	17.2	—	ne	ne	0	0	4	14.2 (5)
	8	IT-101	qwk × 3	25.8	38.0	78	ne	ne	0	0	5	9.1 (5)
	8	IT-101	qwk × 3	16.1	68.6	221	*	¶	1	1	0	—
H69 (SCLC)	8	UT	—	—	21.2	—	—	—	0	0	0	—
	8	CPT-11	qwk × 3	100	67.0	216	†	—	1	0	0	—
	8	IT-101	qd × 1	16.1	59.0	178	†	ns	1	1	0	—
	8	IT-101	qwk × 3	16.1	95.0	348	†	*	2	0	0	—
	8	IT-101	qwk × 3	9.7	38.9	183	†	ns	0	0	0	—
Panc-1 (pancreatic)	8	UT	—	—	48.8	—	—	—	0	0	0	—
	8	CPT-11	qwk × 3	100	58.6	20	ns	—	0	0	0	—
	8	IT-101	qd × 1	20.7	64.1	31	ns	ns	0	0	1	12.7 (5)
	8	IT-101	qwk × 3	20.7	91.0	86	¶	¶	4	1	0	13.5 (5)
	8	IT-101	qwk × 3	12.9	91.0	86	¶	*	3	1	0	—

NOTE: See Materials and Methods for statistical methods. Irinotecan (CPT-11) was included as a positive internal control.
Irinotecan (CPT-11) was administered i.p.; IT-101 was administered i.v.; IT-101 dose is in camptothecin equivalents.
UT, untreated control group. TTE, time to end point (days); 1,500 mm^3 (LS174T and MDA-MB-231); 1,200 mm^3 (H1299, H69, and Panc-1); 1,000 mm^3 (HT29). TGD, percent tumor growth delay versus control group: $[(T - C) / C] \times 100$. CR, number of animals classified as complete responders. TFS, number of animals classified as tumor-free survivors. TR, number of treatment-related deaths. BWLmax, percent maximum mean body weight loss (numbers in parentheses indicate days after drug dosing when BWLmax was observed). NSCLC, non – small-cell lung cancer. SCLC, small-cell lung cancer.
*Log-rank test is equivalent to Mantel-Haenszel test; $P < 0.01$.
†Log-rank test is equivalent to Mantel-Haenszel test; $P < 0.001$.
‡Log-rank test is equivalent to Mantel-Haenszel test; ns, not significant ($P \geq 0.05$).
§Log-rank test is equivalent to Mantel-Haenszel test; ne, nonevaluable.
‖Fisher's exact test comparing complete versus non – complete responders showed IT-101 to be significantly more effective than irinotecan ($P < 0.001$).
¶Log-rank test is equivalent to Mantel-Haenszel test; $P < 0.05$.

both the 20.7 and 12.0 mg/kg dose levels produced significant activity and 86% TGD. At the higher dose level, IT-101 yielded one tumor-free survivor, three transient complete regression responses, and two partial regression responses. At the lower dose level, IT-101 yielded one tumor-free survivor, two transient complete regression responses, and four partial regression responses. Both multiple dose schedules of IT-101 produced significantly greater activity than irinotecan, which showed nonsignificant antitumor effect relative to no treatment. Whereas the single and multiple high doses caused 12.7% and 13.4% mean body weight loss, the low dose qwk × 3 schedule resulted in negligible body weight loss.

Efficacy in disseminated tumor xenograft. Recently, there has been an increased interest in the use of camptothecin derivatives in Ewing's sarcoma (24–27). We were therefore interested in seeing if IT-101 would have antitumor activity in the disseminated TC-71 luc model of Ewing's sarcoma. In this model, tumor burden was monitored by the amount of light emitted from the tumor cells that constitutively express luciferase. Figure 4 shows the mean tumor signal of all treated groups. Treatment with a single dose of IT-101 at 12.5 mg/kg (CPT equivalents), irinotecan qwk × 3 at 100 mg/kg, and IT-101 qwk × 3 at 12.5 mg/kg (CPT equivalents) caused increasing degrees of tumor growth delay. Whereas TGD

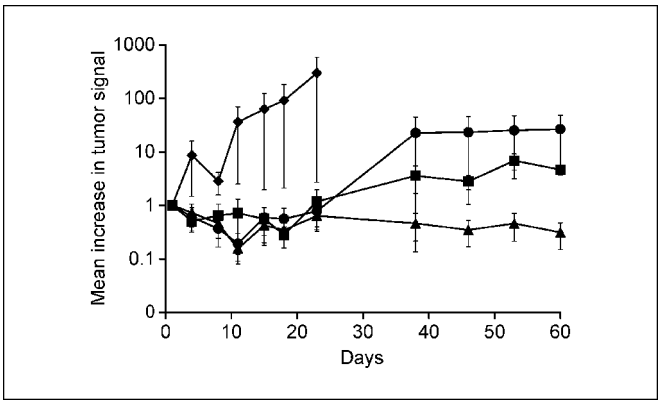

Fig. 4. Inhibition of tumor growth in the TC71-luc disseminated tumor model. Mice were treated with vehicle (*diamonds*), the internal positive control irinotecan at 100 mg/kg administered i.p. once per week for 3 weeks (*squares*), a single dose of IT-101 (12.5 mg/kg CPT equivalents, *circles*), or three weekly doses of IT-101 (12.5 mg/kg CPT equivalents, *triangles*). Points, tumor growth expressed as the mean ratio of light signal at each time point to the light signal at time 0; bars, SE.

differences were not statistically evaluated due to the high variability in tumor signal in the untreated group, treatment with IT-101 qwk × 3 resulted in complete tumor regression in this model and five of six animals remained tumor-free until the end of the study (day 60). Comparing the number of tumor-free survivors in each group, this result was significant compared with all other groups ($P < 0.05$, Fisher's exact test). Figure 5 shows typical examples of mice bearing TC71-luc tumors treated with irinotecan (*top*) or IT-101 (*bottom*). Treatment with irinotecan resulted in a decrease in tumor burden within the first 2 weeks of treatment. However, tumors recurred after the end of the treatment. In the animal shown in Fig. 5, a secondary tumor not detected at the study start was detected 2 days after the last irinotecan administration, with the primary tumor reappearing several weeks later. Treatment with IT-101 (*bottom*) resulted in complete tumor regression.

Tolerability. Treatments with irinotecan were well tolerated. Mean body weight loss for animals treated with three weekly doses of 100 mg/kg of irinotecan i.p. was negligible. Whereas the maximum tolerated dose of a single dose of IT-101 in non-tumor-bearing mice was previously found to be ~25 mg/kg (CPT equivalents), maximum tolerated dose in tumor-bearing mice was >16.1 mg/kg but <25 mg/kg in a model-dependent fashion. Toxicity was characterized by animal deaths preceded by body weight loss, with maximum body weight loss occurring between days 4 and 5 after a single dose. The maximum tolerated dose for animals on a qwk × 3 schedule was similar to the maximum tolerated dose for a

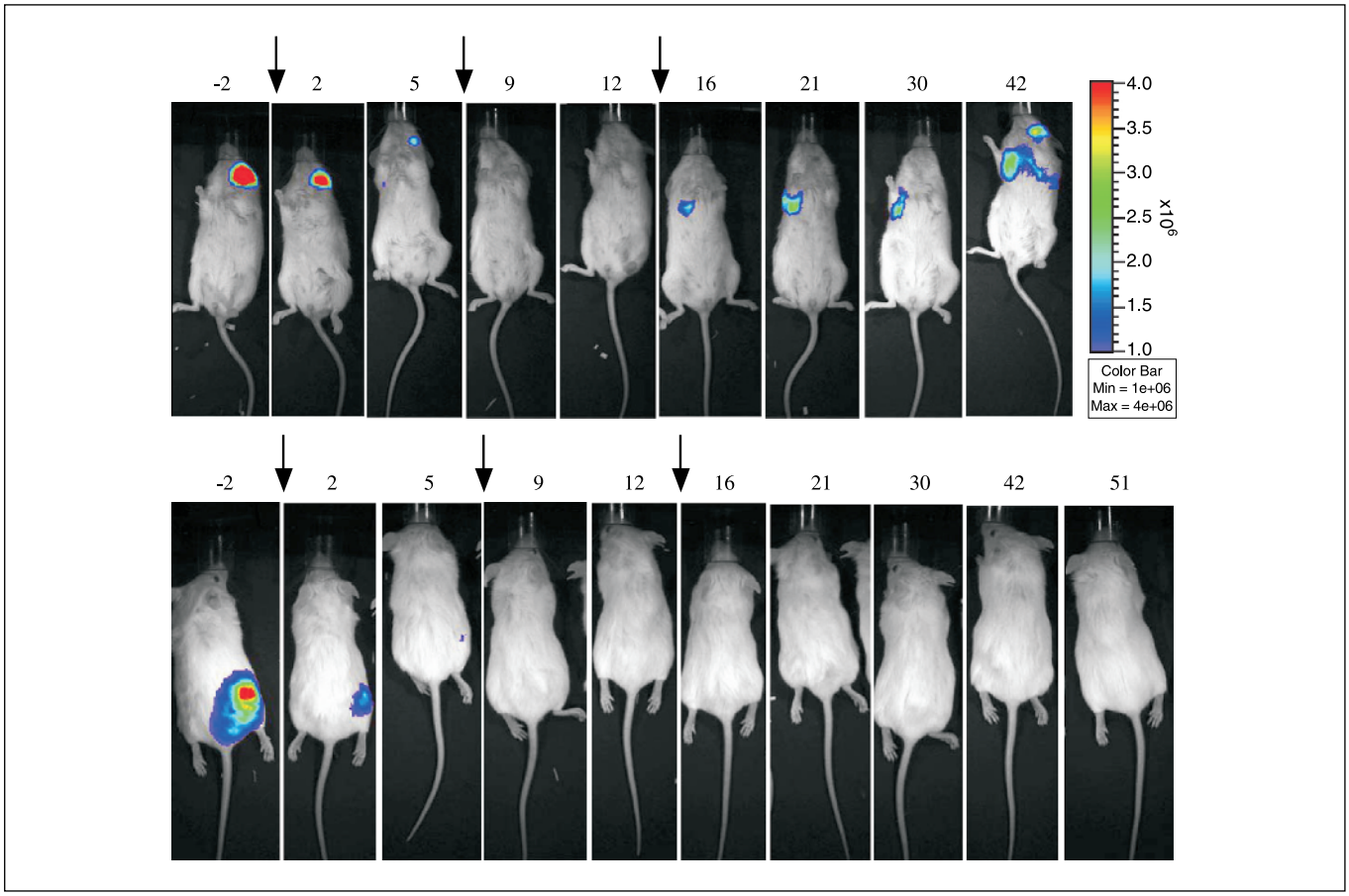

Fig. 5. Antitumor effect of irinotecan at 100 mg/kg (*top*) and IT-101 at 12.5 mg/kg CPT equivalents (*bottom*) in a disseminated TC71-luc tumor model (Ewing's sarcoma) in nonobese diabetic/severe-combined immunodeficient mice. Tumor burden as a function of time (in days) was monitored using a Xenogen imaging system. Arrows, treatment on days 0, 7, and 14. Color bar, light intensity emitted in photons per second.

single dose, but maximum body weight loss tended to appear at later time points. At doses of ≤16.1 mg/kg (CPT equivalents), IT-101 was well tolerated with negligible (<5%) mean body weight losses in the LS174T, HT29, H69, Panc-1, MDA-MB-231, and TC71-luc models, which received three weekly doses of 16.1 mg/kg (LS174T, H69, and MDA-MB-231), 12.9 mg/kg (HT29 and Panc-1), or 12.5 mg/kg (TC71-luc) polymer-bound CPT. Maximum mean body weight loss in the H1299 non–small-cell lung cancer model at 16.1 mg/kg qwk × 3 was 8.0% on day 19. Observed body weight losses were transient and no concomitant overt signs of toxicity were noted.

Discussion

We have previously shown that IT-101, a conjugate of camptothecin and a cyclodextrin-based polymer, shows increased antitumor activity compared with camptothecin alone or irinotecan in a colorectal cancer model (12). IT-101 is a high molecular weight conjugate (62-107 kDa) with a single glycine linker between the polymer and camptothecin and was shown to be more effective than a low molecular weight conjugate (35 kDa) and have fewer side effects than a conjugate with a triglycine linker (12).

Here we first investigated if variations in dosing frequency would affect the efficacy and tolerability of IT-101 in the same LS-174T colorectal xenograft model. As previously observed, conjugation of camptothecin to a cyclodextrin-based polymer allowed for administration of higher doses (in CPT equivalents) compared with CPT alone: CPT alone was found to be toxic at 9 mg/kg on a q4d schedule (12) whereas the maximum tolerated dose of IT-101 is >16 mg/kg CPT equivalents on a q4d × 3 or qwk × 3 schedule. Compared with camptothecin alone, IT-101 also showed increased antitumor effect: one single dose of IT-101 resulted in greater antitumor effect than five doses of camptothecin (q4d × 5). Additionally, one single dose of IT-101 showed approximately the same antitumor effect as three weekly doses of the internal positive control irinotecan. This prolonged antitumor effect is consistent with the enhanced permeability and retention effect first proposed by Matsumura and Maeda (13). Long-circulating macromolecules such as IT-101 can accumulate in the tumor tissue by extravasation from the abnormally leaky tumor vasculature and collect within the tumor due to a lack of effective lymphatic drainage. Recently completed pharmacokinetics and biodistribution studies in rats and tumor-bearing mice further confirm the enhanced permeability and retention hypothesis: we observed both a long plasma half-life of ~19 hours and tumor tissue concentrations at 24 hours postadministration that were greater than any other tissue analyzed (16). In comparison, the plasma half-life for CPT was found to be <2 hours and tumor tissue concentrations were negligible at 24 hours postadministration.

All multiple dosing schedules administered over 15 to 19 days resulted in significantly increased efficacy compared with a single dose. Additional increases in antitumor efficacy were not seen when dosing frequency was increased from qwk × 3 to q4d × 5 or a 5/2/5/2/5 schedule. We attribute this observation to the extended half-life of IT-101 which may be additionally extended in tissues. We have previously reported that IT-101 is readily taken up by cancer cells into the endocytic compartment (12). Acidification in the endocytic compartment slows the esterolytic cleavage of camptothecin from the polymer, resulting in extended release kinetics. All treatments were well tolerated. Comparing all multiple dosing schedules, maximum body weight loss was observed with the 5/2/5/2/5 schedule (−7.3%) whereas a strategy of less frequent dosing resulted in negligible body weight loss. Based on these results, we used a qwk × 3 dosing schedule for our further studies.

Efficacy of IT-101 was further evaluated in six s.c. and one disseminated xenograft model. One cycle of three weekly doses of IT-101 showed significant antitumor activity in all of these models. Tumor growth was significantly delayed in all models. Complete tumor regression was observed in all animals bearing H1299 non–small-cell lung cancer tumors and in the majority of animals with disseminated Ewing's sarcoma tumors. These animals remained tumor-free until the end of the study. The excellent antitumor effect in non–small-cell lung cancer is interesting because camptothecin derivatives are not currently used in front line therapy for this indication. More recently, however, there has been an increased interest to use camptothecin derivatives as combination therapies in non–small-cell lung cancer and a number of phase II trials with promising results have been reported (28–31). The observation of tumor eradication in the disseminated Ewing's sarcoma model is significant because it shows that even very small (micro-) tumors may effectively be treated with IT-101. Such small tumors are often present in the TC71-luc model but will only become visible at a much later time compared with the primary tumor or will appear after the primary tumor undergoes transient regression (i.e., after treatment with irinotecan; see Fig. 4). IT-101 may therefore have potential for the treatment of metastatic disease wherein the size and age variation in metastases, their dispersed anatomic locations, and their heterogeneous composition over time hinder complete surgical removal of disease and can limit the effectiveness of many systemic anticancer drugs (32).

Interestingly, IT-101 was effective in a number of tumors that were resistant to treatment with irinotecan (e.g., MDA-MB-231, Panc-1, and HT29). This result is consistent with literature reports that polymeric drug conjugates may be able to overcome multidrug resistance through avoidance of P-glycoprotein or multidrug resistance protein–associated drug efflux. One hypothesis is that this is achieved by endocytotic uptake and localization in lysosomes, followed by the release of drug from the polymer chains and ultimately diffusion via the cytoplasm into the cell nuclei (14, 15, 33). Panc-1 overexpresses MRP (34, 35) whereas HT29 overexpresses P-glycoprotein (23), conferring the multidrug resistant phenotype.

Taken together, these results indicate that IT 101 has potent antitumor activity against a wide range of solid tumors when administered alone. Additional studies are under way to test IT-101 in combination with other chemotherapeutic agents.

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