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Clinical report

Phase I and pharmacologic study of i.p. 9-aminocamptothecin given as six fractions over 14 days

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We sought to define the tolerance of 9-amino-20(*S*)-camptothecin (9-AC) when given by the i.p. route to patients with cancer in the peritoneal cavity consisting of nodules that did not exceed 1 cm in maximum diameter. 9-AC was given in six fractions over 12 days, at doses ranging from 1.25 to 13.5 mg/m^2 in cycles repeated every 28 days. Dose escalations after the first two dose levels took place in cohorts of three patients, with expansion of the dose level once a dose-limiting toxicity (DLT) was encountered. All patients had blood and i.p. pharmacokinetic (PK) analysis during cycle 1 of each dose level. Topoisomerase (Topo) I signal was serially measured in peripheral blood mononuclear cells (PBMCs) in blood and cells collected in i.p. cytologic washings. Twelve patients received 31 cycles of 9-AC. Tolerance to repeated i.p. drug administration was generally excellent. The DLT was neutropenia encountered at the highest dose level in two patients, whereas the dose of 9 mg/m^2 was well tolerated. The DLTs were associated with peak plasma levels ranging from 47 to 81 ng/ml and also depletion of Topo I in PBMCs. The i.p.:plasma AUC ratio (±SD) was 11.5 (±3.8). Two patients had objective evidence of clinical benefit and only one of seven patients deemed evaluable for response had progressive disease. We conclude that i.p. 9-AC demonstrates excellent local tolerance at a dose and schedule associated with evidence of systemic effects. A dose of 9 mg/m^2/cycle administered in a schedule of six divided fractions is suitable for further evaluation against tumors involving primarily the peritoneal cavity. [© 2002 Lippincott Williams & Wilkins.]

Key words: 9-Aminocamptothecin, intraperitoneal, topoisomerase I.

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Introduction

Intraperitoneal (i.p.) therapy has been associated with prolongation in progression-free survival (PFS) of ovarian cancer patients, as compared to analogous regimens administered by the systemic route in randomized trials, except in one trial that was terminated early.[1–4] These differences in favor of i.p. therapy were statistically significant.[1–3] Moreover, the toxicity associated with i.p. cisplatin was less than with the same drug dose given i.v.[1] In an effort to improve on these results, we have been interested in studying drugs active in ovarian cancer with potential advantages when administered via the i.p. over the i.v. route for small volume disease throughout the peritoneal cavity. The topoisomerase (Topo) I inhibitors may be worth considering for such further development.[5]

Not only do they have activity against epithelial ovarian cancer, but initial studies indicated no dose-limiting local toxicity at doses leading to systemic myelosuppression.[6] Moreover, in an acid pH and low albumin environment, the active lactone form of camptothecins is favored. With these features, one would expect substantial pharmacologic advantage. Accordingly, we set out to study 9-amino-20(*S*)-camptothecin (9-AC) by the i.p. route utilizing a schedule that provides exposure of tumors to high doses and for prolonged periods.

9-AC is a semisynthetic analog of camptothecin and a specific inhibitor of Topo I with broad-spectrum, single-agent activity in animal tumor models including colorectal, prostate, bladder and ovarian cancers.

The duration of exposure above a threshold concentration of 10 mol/l (3.6 ng/ml) was associated with antitumor activity.[7] Because of poor water solubility, the initial phase I studies had problems in identifying a suitable formulation. Eventually, when formulated as a colloidal dispersion, continuous infusion schedules were generally well tolerated except for neutropenia that proved dose limiting. Clinical activity has been documented principally against platinum-resistant ovarian cancer in schedules of 120 h (Miller, GOG protocol #126I, unpublished) or longer, whereas initial studies with shorter schedules showed limited activity.[8,9] In our study design, therefore, we employed cycles consisting of six doses administered over a 2-week period. In the current phase I study, patient cohorts were entered to establish treatment tolerance, pharmacokinetics were determined in plasma and in the peritoneal cavity, and preliminary observations on any antitumor effects were made.

Patients and methods

Patients with solid tumors were eligible for entry into this study if they had minimal (less than 1 cm in any one area) residual nodules in the peritoneal cavity. Ovarian cancer patients were required to have failed a platinum-based regimen. Disease outside the peritoneal cavity was permitted as long as it was asymptomatic and not anticipated to pose clinical problems requiring immediate treatment. Adequate performance status (ECOG scale of 2 or better), and adequate bone marrow, renal and hepatic function was required defined as an absolute neutrophil count $\geqslant 1500/\mu$l, thrombocytes $\geqslant 100\,000/\mu$l, total serum bilirubin $\leqslant 2$ mg/dl, AST $\leqslant 2 \times$ the upper limit of normal and creatinine concentration $\leqslant 2.0$ mg/dl. All patients were to have placement of an implantable port prior to entry and a baseline i.p. contrast computed tomography scan as previously described.[10]

9-AC was the colloidal dispersed (CD) formulation supplied by the NCI. The trial employed a dose-escalation design, but to maximize therapeutic intent, it initially entered one patient per level at the first two levels and allowed escalation in this first patient. At dose level 3, the trial reverted to a classic dose-escalation design with a minimum of three evaluable patients to be entered at each dose level and no dose escalation within cohorts, with expansion to enter up to three additional patients if one experienced a dose-limiting toxicity (DLT). A DLT consisted of any grade 4 hematologic toxicity, or any non-hematologic toxicities that were grade 3 or 4. Once two DLTs were recorded in any one level, dose escalation ceased and the next lower dose was to be similarly expanded. If one or no DLTs were observed at this lower level, it was defined as the recommended phase II dose.

The schedule selected consisted of treatment given over 2 weeks followed by a 2-week rest in a 4-week cycle. The treatment was administered in 6 fractions with a Monday–Wednesday–Friday $\times$ 2 week schedule. Dose levels are shown in Table 1 and with the volume being set a 1 l/m^2, a constant i.p. drug concentration per dose level was obtained. The patients were continued for 4 cycles or until disease progression; dose modifications were built in for hematologic toxicity and in the event of grade 3 or 4 non-hematologic toxicities, once they had resolved to grade 1 or less. Follow-up studies consisted of chemistries, tumor markers and cytologic assessment. Computed tomographic scans were repeated at 3-month intervals.

Pharmacokinetic (PK) studies

PK observations were performed in all patients during the first cycle. Aliquots of 2 ml of i.p. fluid and plasma in heparinized tubes were obtained, with blood being processed as previously described.[11] Samples were drawn at baseline, and at 5, 15 and 30 min, and 1, 2, 4, 6, 8, 12, 24 and 48 h after the first and fifth or sixth instillation. A solid-phase separation under neutral conditions or from acidified aliquots separated 9-AC lactone immediately after being drawn. Reversed-phase HPLC analysis was performed within 2 months of storage at -80°C. An acidic pH 2.55 isocratic HPLC mobile phase was used to enhance AC fluorescence 50-fold. Total 9-AC and the lactone form were determined separately. Pharmacodynamic assessment consisted of Topo I determinations by Western blot in peripheral blood

Table 1. Dose levels

Level	mg/m^2/ application	No. of 9-AC i.p. applications/cycle	mg/m^2/ per course
1	0.208	6	1.25
2	0.42	6	2.5
3	1.0	6	6.0
4	1.5	6	9.0
5	2.25	6	13.5

mononuclear cells (PBMCs), as previously described.[12]

Preparation of PBMCs for Topo I Western blotting

One Vacutainer cell preparation tube (CPT with sodium heparin; Becton Dickinson, Franklin Lakes, NJ) was obtained for each time point. The CPT tubes were centrifuged within 1 h at 1650 *g* for 20 min in a Beckman J-6B clinical centrifuge (Beckman Coulter, Brea, CA). The cellular layer above the dense solution and polyester gel (mononuclear cells and platelets) was removed and transferred to a 15 ml conical tube, adding 1 × Dulbecco's phosphate-buffered saline (DPBS) to 14 ml and centrifuged for 15 min at 300 *g*. After centrifugation, 7 ml of the supernatant was removed, the cell pellet was resuspended and brought to 14 ml with fresh DPBS, and then centrifuged for 10 min at 300 *g*. The supernatant was discarded and the cell pellet was resuspended in 300 μl DPBS.

Western blot determinations of PBMC and i.p. fluid cell Topo I

The monitoring of the PBMC Topo I signal by Western blot analysis was carried out essentially as described by Liebes *et al.* with electrophoresis on 6% acrylamide gels and transferred onto New England Nuclear (Boston, MA) PolyScreen PVDF transfer membranes.[12] HeLa cells were used as positive controls to normalize for the amount of Topo I. The development of the Topo I signal employed a preselected and qualified SCL-70 antibody preparation.[12] Horseradish peroxidase-conjugated Protein A (1:1000) was used along with the visualization of the primary and secondary antibody complex with ECL detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ) along with exposure to Kodak BioMax MS scientific imaging film (Rochester, NY).

Assay for determination of the steady-state levels of 9-AC

Plasma for the 9-AC analysis was collected in one heparinized tube and centrifuged at 1000 *g* for 10 min within 0.5 h. after collection. The plasma was removed and stabilized for the preservation of the lactone form of 9-AC by the transfer to a 15-ml polypropylene tube where the volume present was estimated to the nearest 0.5 ml using the markings on the tube and recorded on a log sheet. An equal volume of stabilizing buffer (250 mM $KHPO_4$, pH 6.0) was added followed by mixing. The stabilized plasma was stored at 4°C until processed further for HPLC analysis within 4–24 h. The plasma specimens were processed according to a modification of a previously described study by Takimoto *et al.* using solid-phase extraction.[13] Briefly, Bond-Elut, 200 mg, C_{18} solid-phase extraction cartridges (Varian, Harbor City, CA) were conditioned by passing 1 ml of methanol, followed by 1 ml of water through the cartridge packing with the aid of a vacuum manifold. Camptothecin was added at 10 ng/ml plasma as an internal standard to the patient and reference samples. The samples were vortexed and loaded onto the activated solid-phase extraction cartridges. After the sample had passed through, the cartridge was washed twice with 1 ml water followed by 1 ml of 25% (v/v) methanol. The samples were eluted with 0.75 ml of 75% (v/v) methanol/25 mM $KHPO_4$, pH 2.55 and 0.25 ml of 25mM $KHPO_4$, pH 2.55. The HPLC separation was achieved using a C_{18} reversed-phase, 150 × 4.6 mm ID, 3 μm, Phenosphere (ODS2) column (Phenomenex, Torrance, CA). Mobile phase was isocratic 25% acetonitrile/25 mM $KHPO_4$ at 1 ml/min. Detection, using post-column acidification by 25% acetonitrile/1% triflouroacetic acid at 0.2 ml/min, was by fluorescence at an excitation wavelength of 365 nm and emission wavelength of 470 nm as recommended by Supko and Malspeis.[11]

Assessment of PK parameters

Topotecan plasma levels were fit to non-linear models available in WINONLIN version 3.0 (Pharsight, Mountain View, CA) with the short-infusion, and oral absorption one- and two-compartment models.

Results

Patients and toxicities

Five dose levels were explored (Table 1) and the characteristics of the patients entered are shown in Table 2. DLTs were confined to dose level 5. At this dose level, all three patients entered experienced DLTs and in one instance the drug-induced myelosuppression may have contributed to the patient's death. This 68-year-old man with colon cancer,

Table 2. Patient characteristics

	No. of patients
No. entered	12
Age (years)	
median	56
range	29–74
Sex	
female	6
male	6
ECOG PS	
0	6
1	6
Tumor type	
ovarian/peritoneal	3
gastric	2
cholangiocarcinoma	1
colon/appendiceal	5
endometrial	1
Previous treatment	
chemotherapy	
none	3
1 regime	5
> 1 regimen	4

ascites and liver metastases, had been treated with five prior regimens, including 5-fluorouracil, irinotecan and oxaliplatin. He sustained grade 4 hematologic toxicity, in the setting of increasing deterioration from disease, and died 23 days after completing his first cycle. The second patient experiencing DLT had a carcinoma of the endometrium/peritoneum and diabetes mellitus. Following her first cycle, she experienced aggravation of liver function studies, from an abnormal baseline of grade 2–3. A third patient with gastric cancer had hematemesis with aseptic peritonitis which required transfusions. Table 3 details all major toxicities observed with respect to the dose level and the plasma AUC. Two patients were dose escalated without toxicity. No grade 3 toxicities were observed at plasma AUCs <300 ng/ml · h. An account of all toxicities appears in Tables 4 and 5. At 9 mg/m^2, the treatment tolerance was excellent, and lends clinical and pharmacological support for establishing this level as the recommended phase II dose. Figure 1 shows decay plots that reflect the trend found across all five dose levels

Table 3. Toxicity and plasma AUC correlation

Dose level	Patient no.	Toxicities (grade 3 and 4)	9-AC plasma AUC (ng/ml · h)
1	N01	none	29
2	N01	none	35
2	N02	infection sutures grade 3 not related to drug	108
3	N02	none	549
3	N03	abdominal pain grade 3 from catheter blockage; probably mechanical—not related to drug	ND
3	N04	none	457
4	N05	neutropenia grade 4 after third cycle	309
4	N06	syncope grade 3	309
4	N07	none	256
4	N12	leukopenia grade 3	1134
5	N08	hemorrhage from i.p. catheter, hematemesis, aseptic peritonitis grade 3 from progression of disease	995
5	N09	abnormal LFT grade 3 from baseline grade 2	430
5	N10	ANC, febrile neutropenia grade 4, expired after 1 cycle	436
5	N11	none	350

Table 4. Hematologic toxicities [grade] (worst per patient)

Dose level	No. of patient/cycles	Granulocytes				Febrile neutropenia				Platelets				Anemia			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	1/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2/4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	3/4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	4/15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	4/6	0	0	0	1[a]	0	0	0	1[a]	0	0	1	0	0	2	0	0

[a]Same patient.

Table 5. Non-hematologic toxicities [grade] (worst per patient)

Dose level	No. of patients/cycles	Nausea and vomiting				Diarrhea				Abdominal pain				Headache				Fatigue			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	1/2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
2	1/4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2/4	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
4	4/15	4	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
5[a]	4/6	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0

[a]One patient in dose level 5 had grade 3 elevated LFT, but had an abnormal baseline of grade 2; another in dose level 5 had a grade 3 hematemesis with aseptic peritonitis.

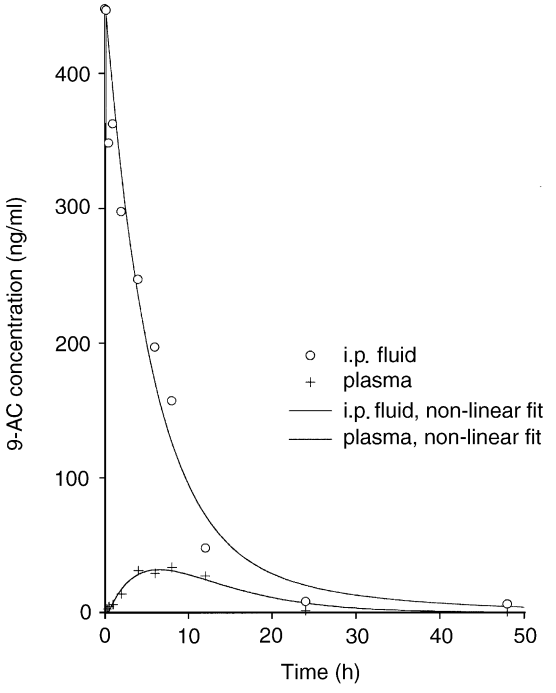

Figure 1. Decay of plasma and i.p. fluid 9-AC levels from a patient on dose level 2. The plotted data are fit with the non-linear fit modeling of the individual data points. A 13-fold ratio in the AUC i.p. fluid:plasma results from these data.

where a dramatic differential effect in 9-AC drug levels is observed with respect to the i.p. fluid levels compared to the plasma. Table 6, which summarizes the key pharmacokinetic calculated parameters, shows the pharmacologic advantage as detailed in the ratio of AUC i.p.:plasma values which range from 7.6 to 16.5. This trend is also apparent in the peak levels detected in plasma (range 4–41 ng/ml) versus i.p. fluid (range 112–2827 ng/ml). It is also worth noting that starting at the third dose level, a progressive increase in the plasma elimination half-lives is apparent compared to the i.p. fluid elimination mean half-life of 6.7 h, which was also seen for the first two dose levels for the calculated plasma elimination half lives. In addition, Figure 2 shows a plot of the respective plasma and i.p. fluid 9-AC with respect to dose levels. These PK data show linearity with dose level for both i.p. and i.v. AUC. The difference in the slope of the AUC plots for i.p. fluid and plasma is 10.4 comparable to the mean $\pm$ SD of 11.5 $\pm$ 3.8 observed across all five dose levels for the AUC i.p. fluid:plasma ratio. Figure 3 shows the typical pharmacodynamic effects observed with respect to the Topo I signal as monitored in PBMCs obtained from the i.p. fluid washes. A range of 83–99% depletion of the Topo I signal was observed in the PBMCs, while the available i.p. fluid cells examined showed a reduction that increased with the number of cycles of treatments, where the data shown in Figure 3 show a 66% reduction on cycle 1 and 90% reduction for cycle 2. Table 7 summarizes the degree of Topo I signal depletion for cycle 1 between the baseline and the 2-h sample for both PBMCs and i.p. fluid cells. The data were only descriptive due to the variable yield of i.p. fluid cells from the samples analyzed, but in general, i.p. fluid cells appear to show a greater degree of Topo 1 depletion.

Discussion

9-AC is a Topo I inhibitor derivative of camptothecin that possesses some advantages over others in this drug family. It has greater lipophilicity and it is likely to be less of a substrate than either topotecan or irinotecan for the breast cancer resistance protein (BCRP) that may account for the emergence of resistance to these agents. In addition, the BCRP in the gastrointestinal and biliary tracts may be responsible for the poor oral bioavailability of these drugs, as well as for toxicities such as diarrhea. Moreover,

Table 6. Pharmacokinetic parameter summary for plasma and i.p. fluid

No. of patients (dose level)	Dose (mg/m^2)	AUC i.p. (ng/ml · h)	Elimination half-life i.p. fluid (h)	Cmax i.p. fluid (ng/ml)	AUC plasma (ng/ml · h)	Elimination half-life plasma (h)	Cmax plasma (ng/ml)	AUC i.p./ plasma ratio
1 (1)	0.208	473	5.2	112	28.7 ± 27.1	4.5 ± 1.8	4 ± 2.1	16.5
2 (2)	0.42	933 ± 126	3.2 ± 1.3	243 ± 163	71.2 ± 51.6	8.9 ± 4.0	7 ± 4.1	13.1
3 (3)	1	3846 ± 105	7.9 ± 3.6	486 ± 104	504 ± 77	16.5 ± 11.2	25.4 ± 8.6	7.6
3 (4)	1.5	4983 ± 1329	10.2 ± 6.1	373 ± 162	462 ± 313	21.3 ± 12.6	20.9 ± 3.4	10.8
3 (5)	2.25	6160 ± 1056	6.5 ± 0.6	2827 ± 3086	640 ± 430	15.7 ± 11.6	40.8 ± 14.5	9.6

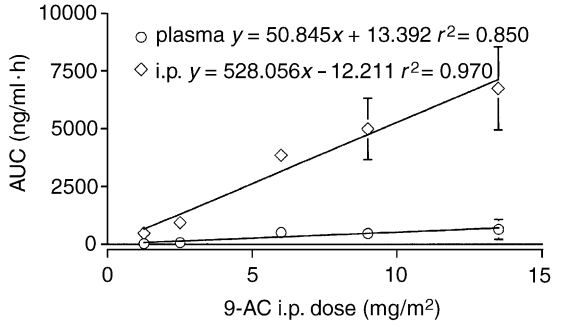

Figure 2. The correlation of the dose proportionality of the AUC for 9-AC for plasma and i.p. fluid. The difference in the slope of the AUC plots for i.p. fluid and plasma is 10.4, comparable to the mean ± SD of 11.5 ± 3.8 observed across all five dose levels for the AUC i.p. fluid:-plasma ratio.

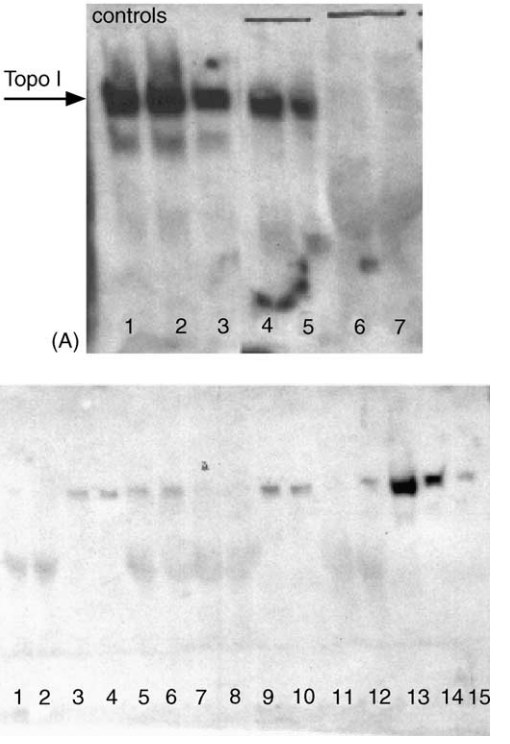

Figure 3. (A) Western blot results of the Topo I signal in PBMCs from a patient receiving i.p. 9-AC treatment. The first three lanes are the Topo I signal from HeLa cells used to normalize for the Topo I copy number, while lanes 4 and 5 are the baseline PBMC Topo I levels, and lanes 6 and 7 are the results from 2 h post i.p. administration where a limited amount of Topo I signal is evident. (B) Western blot results of the Topo I signal in i.p. cells following treatment with 9-AC for two consecutive cycles of drug treatment. A 66% reduction of the Topo I signal was observed on cycle 1 (lanes 5 and 6) and a 90% depletion was detected on the second cycle (lanes 11 and 12).

9-AC studies in prolonged infusion schedules indicate that it is active against ovarian cancer, at response rates not unlike those of topotecan.

The PK data demonstrate the consistent pharmacologic advantage of i.p. administration with an average 11-fold differential effect for 9-AC drug exposure in the peritoneal cavity as opposed to the systemic levels. This trend is also reflected in the i.p. fluid:plasma C_{max} values. What also seems apparent starting at the third dose level is that the high drug levels in the i.p. cavity serve as an additional compartment for prolonging the systemic elimination of the 9-AC from the plasma. This effect combined with the levels less than 1 μg/ml detected in the i.p. fluid at dose level 5 likely accounted for the consistent toxicities observed at this dose level.

This phase I study establishes a recommended phase II dose in a novel schedule over 6 fractions that was generally well tolerated below level 5. In view of the known schedule dependency of these compounds, this schedule would appear suitable for further study. Although the treatment of epithelial ovarian cancer does not currently include i.p. consolidation, such a strategy is worth testing. Currently, the relapse rate after initial carboplatin + paclitaxel approaches 50% at 2 years and 12 cycles of paclitaxel maintenance or drugs such as hexamethylmelamine have been tried to prolong this

Table 7. Topo I depletion following 9-AC i.p. dosing

No. of patients data points	Dose (mg/m^2)	Plasma PMBCs % depletion (baseline: 2 h)	i.p. fluid cells % depletion (baseline: 2 h)
1	0.208	ND	–
0	0.42	–	–
1	1	62.8	–
3	1.5	92.9 ± 8.5	99
3	2.25	62 ± 35	93.7 ± 9.1

remission. The i.p. administration of a Topo I inhibitor could also be considered a suitable consolidation treatment for testing in an effort to achieve prolongation of survival without progression after the initial induction. However, such a strategy does require reassessment, a maneuver that continues to be controversial, to be followed by insertion of an i.p. catheter. Nevertheless, a number of randomized trials continue to support some advantage for the i.p. over the i.v. route in ovarian cancer and encourage such new approaches. In this respect, it is noteworthy that at the recommended phase II dose, blood levels of 9-AC are associated with depletion of Topo message in PBMCs and provide some systemic effects without major toxicities. In addition, i.p. therapy has never been adequately treated in gastrointestinal malignancies that routinely metastasize to the peritoneal cavity, such as gastric and appendiceal carcinomas. The activity of i.p. 9-AC in one patient with appendiceal cancer was encouraging and suggests that further trials utilizing this route of administration should be performed in this disease.

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