*Highlighted paper selected by Editor-in-chief***Promising Antitumor Activity of a Novel Quinoline Derivative, TAS-103, against Fresh Clinical Specimens of Eight Types of Tumors Measured by Flow Cytometric DNA Analysis**

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TAS-103, 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno-[2,1-*c*]quinolin-7-one dihydrochloride, is a dual topoisomerases I and II inhibitor. Antitumor activities of TAS-103 against fresh surgical specimens resected from 525 patients (32 types of tumors) were examined by flow cytometric (FCM) analysis of DNA integrity of tumor cells, and compared with those of five other investigational new drugs and 31 clinically available anticancer agents. Concentrations of clinically available anticancer agents were set at one-tenth of the peak plasma concentration (PPC) of the clinically recommended doses. On the other hand, since PPCs of investigational new drugs in humans were frequently unknown, these were estimated by a method that determines the theoretically achievable concentration in body fluid (TAC method). Correlations between TAC and PPC were examined for 16 clinically available anticancer agents, and it was found that TAC at 7*n* (the modified Fibonacci's dose-escalation scheme) of 14 drugs corresponded well with each one-tenth of PPC. By defining a 30% or more reduction in the integrated diploid peak as effective and a 60% or more reduction as definitely effective, TAS-103 at 5 µg/ml (7*n*) showed significantly higher effective rates and definitely effective rates than those of all other investigational new drugs, as well as almost all clinically available anticancer agents, against various malignancies, including non-small cell lung cancer, brain tumor and renal cancer. These results strongly suggest that TAS-103 will be expected to show excellent antitumor activities against a wide range of human tumors.

Key words TAS-103; new anticancer agent; clinical specimen; flow cytometry; antitumor activity *in vitro*

A novel quinoline derivative, TAS-103 (Fig. 1), has many attractive biological functions: i) it inhibits both topoisomerase (topo) I and topo II; ii) it stabilizes both topo I- and topo II-DNA cleavable complexes; iii) it shows potent cytotoxic effects *in vitro*; iv) it shows high antitumor activities against subcutaneously-implanted murine and human tumors *in vivo*, as well as various lung-metastatic murine tumors.¹⁾ However, it is unclear whether TAS-103 will show the similar level of activity against tumors in patients. In this setting, the chemosensitivity test is expected to show its potential for predicting activity of anticancer agents against human tumors.

Various *in vitro* drug sensitivity tests have been used to assay the responsiveness of tumors to anticancer agents.^{2–7)} Among various techniques, the colony-forming assay may be

the most widely applied *in vitro* assay for clinical use.⁸⁾ In a large study of prospective trials using colony-forming assay, it was shown that true-positive rate was 60% and true-negative rate was 85% based on clinical response.⁹⁾ Although this assay seems to be reliable for predicting the clinical response to chemotherapies, it is time- and cost-consuming, labor-intensive, and suffers from a low success rate.¹⁰⁾ In addition, this assay requires preparations of a single-cell suspension, which renders considerable damage to the tumor cells.

We have used direct *in vitro* measurements of apoptosis as a chemosensitivity test. Two different methods have been employed; morphological examinations of the nuclear damage, such as the chromatin condensation or degradation,⁵⁾ and flow cytometric (FCM) analysis of DNA integrity which shows apoptosis as the sub-G1 population.¹¹⁾ The FCM assay

Fig. 1. Structures of Investigational New Drugs

is a simple and reproducible method for detecting apoptosis, and has been widely used in basic laboratory studies.^{12,13)} One of the advantages of the FCM assay is that the single-cell suspension is theoretically unnecessary at the initial stage of the assay, which would contribute to a high success rate and high predictive accuracy.¹¹⁾ Predictability of clinical responses by the morphological method was shown to have the overall accuracy of 94% in ovarian cancer patients,¹⁴⁾ and that by the FCM assay was 82% in brain tumors.¹⁵⁾ In addition, favorable increase in survival time of patients with glioblastoma multiforme was observed by treatment with anticancer agents selected by the FCM assay.¹⁶⁾

Determination of the drug concentration employed in *in vitro* chemosensitivity test is another problem. One-tenth of the peak plasma concentration (PPC) has been commonly used as a standard concentration of anticancer drugs for *in vitro* chemosensitivity test of human tumors.^{17,18)} However, since the PPCs of the investigational new drugs in humans are frequently unknown, any method for predicting the PPCs of these drugs clearly needs to be developed. As a candidate, the theoretically achievable concentration (TAC) method¹⁹⁾ was examined and confirmed to be applicable to this purpose.

In the present study, with the FCM assay and the TAC method, the antitumor activity of TAS-103 was examined against various surgical specimens and compared with those of other investigational new drugs, as well as those of representative anticancer agents currently in clinical use. As a result, TAS-103 was shown to have the highest antitumor activities against a variety of human tumors among almost all drugs examined.

MATERIALS AND METHODS

Surgical Specimens The chemosensitivity tests were carried out with fresh surgical specimens resected from 525 patients (32 types of tumors) at Chiba Cancer Center Hospital and Chiba University Hospital from November 1994 to December 2005. Assay results for main eight types of tumors (which have large number of samples) are shown in the present study. These were non-small cell lung cancer (n=236), brain tumor (n=101), bone and soft tissue sarcomas (n=68), renal cancer (n=37), uterine tumor (n=21), breast cancer (n=15), small cell lung cancer (n=13) and ovarian cancer (n=10). The chemosensitivity tests were performed by request of physicians in order to get informations that assist them in considering the clinical protocol for the individual patients. Informed consent was obtained, by physicians, from all patients with respect to performing the chemosensitivity test by using their resected tumor specimens.

Drugs and Concentrations The tests have been done with 31 clinically available anticancer agents and six investigational new drugs, which had been successively provided (Table 1). Concentrations of clinically available anticancer agents were set at one-tenth of the PPC of the clinically recommended doses.^{20–35)} To evaluate the pro-drugs adequately, the *in vivo* activated forms, 4-hydroperoxycyclophosphamide, 4-hydroperoxyifosfamide and 7-ethyl-10-hydroxycamptothecin (SN-38), were used for cyclophosphamide, ifosfamide and irinotecan, respectively.

Since the PPC of the investigational new drugs, including

TAS-103, in humans was frequently unknown, the concentration of these agents was estimated by a method that determined the theoretically achievable concentration in body fluid (TAC method) advanced by Schabel *et al.*¹⁹⁾ For example, i) the highest nonlethal dose of TAS-103 in mice given intravenously (i.v.) by a single injection was 67 mg/kg,¹⁾ ii) converting this dose at mg/m² level by multiplying a K_m factor³⁶⁾ of 3 (for mice), 201 mg/m², iii) one-tenth of this dose has generally been employed as a starting dose (n) in the clinical phase I study,³⁷⁾ 20 mg/m², iv) converting this dose at mg/kg level by dividing with a K_m factor of 37 (for man), 0.54 mg/kg, v) the TAC could be estimated by multiplying a factor of 1.3, since the body fluid is approximately 80%¹⁹⁾; 0.71 μ g/ml, and vi) assuming the maximum tolerated dose (MTD) in man to be 7n to 9n at the modified Fibonacci's dose-escalation scheme,³⁷⁾ approximately 5 μ g/ml. In the present study, antitumor activity of TAS-103 was tested at both 5 μ g/ml (7n) and 0.5 μ g/ml (0.7n). Other investigational new drugs were tested for antitumor activity at 7n. These drugs were SUN-4599, P-1301, ADM-OXD (adriamycin-oxydextrane complex, adriamycin 28.3%), 69A and 4181 (Fig. 1). The highest nonlethal doses of these agents in mice (which were obtained from individual pharmaceutical companies) are shown in Table 1.

Determination of MTD in Mice Determination of the highest nonlethal dose in mice of 16 clinically available anticancer agents had been carried out at Cancer Chemotherapy Center, Japanese Foundation for Cancer Research. This study had been performed according to the guidelines of the Cancer Chemotherapy Center Experimental Animal Care and Use Committee, and indeed conducted in accordance with the guidelines. Adult C57BL/6 $\times$ DBA/2 F₁ (BDF₁) mice of either sex, 8–12 weeks old, were used in these studies. All the mice were supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. U.S.A. The animals were given food and water *ad libitum*. All drugs, except melphalan, were dissolved in physiological saline, diluted by a factor of 1.25 at several dose levels, and administered i.v. into a group of 10 mice/dose as a single injection or 3 injections at 4-d interval (q4dx3). Melphalan was suspended in 0.5% carboxymethylcellulose in physiological saline and given i.p. by a single injection. All animals were observed for 45 d after the last administration of drugs and the mortality was recorded.

Chemosensitivity Testing Surgically resected tumor tissues were immediately minced and suspended in RPMI 1640. The micro-cell aggregate suspension was passed through a sterilized mesh several times to remove fibrous connective tissue, and centrifuged at 1000 rpm for 5 min to eliminate fatty tissues and necrotic portions. An aliquot of the cell suspension was incubated with each agent dissolved in RPMI 1640 supplemented with 10% fetal calf serum at 37 °C in 5% CO₂ for 8 h, and then cultured in fresh drug-free RPMI 1640 supplemented with 10% fetal calf serum and 100 μ g/ml of kanamycin for 72 h. To visualize the morphological changes of drug-induced cell death, cells were fixed with 100% methanol and stained with Giemsa on a slide glass.⁵⁾ Two hundred nuclei per slide were observed using the high-power field of a light microscope. Degenerative changes noted in the nuclei, including chromatin degradation or condensation, were judged as markers of apoptosis, and counted

Table 1. *In Vitro* Concentration and Highest Nonlethal Dose in Mice of Clinically Available Anticancer Agents and Investigational New Drugs

Agents	Abbreviation	0.1PPC ^{a)} (μ g/ml)	Ref.	TAC ^{b)} (μ g/ml)	HNLD ^{c)} (mg/kg/d)
Topoisomerase inhibitors					
Aclarubicin	ACR	0.6	34		
Actinomycin D	ACD	0.01	20		
Adriamycin	ADM	0.3	20	0.36	5.1 (q4dx3) ^{d)}
Daunomycin	DM	0.6	20	0.93	13.3 (q4dx3)
Epirubicin	EPI	0.4	33		
Etoposide	VP-16	3	20	1.8	25 (q4dx3)
Irinotecan	SN-38	0.02	35		
Mitoxantrone	MIT	0.06	24		
Pirarubicin	THP	0.3	32		
Antitumor antibiotics					
Bleomycin	BLM	1	20	4.5	64 (q4dx3)
Mitomycin C	MMC	0.2	20	0.23	3.3 (q4dx3)
Neocarzinostatin	NCS	0.15	31		
Peplomycin	PEP	0.5	22	0.70	10 (q4dx3)
Antimetabolites					
Cytosine arabinoside	Ara-C	4	20		
5-Fluorouracil	5-FU	10	20	11	164 (1 shot)
Methotrexate	MTX	3	20	2.4	34 (q4dx3)
Thioinosine	6-MPR	3	20		
Antimicrotubule agents					
Paclitaxel	TAX	0.6	30		
Vinblastine	VLB	0.1	20	0.62	8.9 (q4dx3)
Vincristine	VCR	0.1	20	0.13	1.9 (q4dx3)
Vindesine	VDS	0.1	20	0.19	2.7 (q4dx3)
Alkylating agents					
Buslphan	BUS	0.8	25		
Carboplatin	JM-8	4	29		
Carboquone	CQ	0.1	21	0.11	1.6 (1 shot)
Cisplatin	CDDP	0.5	20	0.34	4.8 (q4dx3)
Cyclophosphamide	CPM	1	26		
Ifosfamide	IFOS	1	27		
Melphalan	MPL	0.5	20	0.70	10 (1 shot)
Nimustine	ACNU	2	23	2.2	32 (1 shot)
Ranimustine	MCNU	2	42	2.2	32 (1 shot)
Thio-TEPA	TESPA	2	28		
Investigational new drugs					
4181	4181			1	50 (1 shot)
69A	69A			0.3	15 (1 shot)
ADM-oxydextran	ADM-OXD			3.2	152 (1 shot)
P-1301	P-13			0.5	8 (1 shot)
SUN-4599	SUN			5	18 (1 shot)
TAS-103	TAS			5, 0.5	67 (1 shot)

a) PPC: peak plasma concentration. b) TAC: theoretically achievable concentration in the body fluid and the concentration at 7n in the modified Fibonacci's dose-escalation scheme was shown. c) HNLD: highest nonlethal dose in BDF₁ mice and TAC was calculated based on this dose. d) q4dx3: 3 injections at 4-d interval.

to compare with non-treated controls. This morphological study showed that the average percentages of tumor cells in the preparations were consistently over 95. In addition to the morphological assay, the FCM analysis of propidium iodide-stained nuclei was performed, by which apoptotic cells were quantified as the proportion of the sub-G₁ population. For the FCM analysis, tumor specimens were prepared in the same way as for the morphological assay, and were mixed with phosphate buffered-saline (pH 7.2)/0.1% Triton X-100/0.1 mg/ml RNase (Sigma, St. Louis, MO, U.S.A.)/0.01% sodium azide for 15 min, and then with 100 μ g/ml propidium iodide (Sigma, St. Louis, MO, U.S.A.) for 15 min. Isolated nuclei were analyzed with a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA, U.S.A.). Apoptotic nuclei shifted to the hypodiploid (sub-G₁) area and the effectiveness of drugs was judged by the reciprocal reduction in the integrated diploid peak compared with that of untreated control

cells. A good correlation between increase in the sub-G₁ peak and decrease in the G₀/G₁ peak was observed in the treatment of the adenocarcinoma of the lung with 37 anticancer agents.³⁸⁾ More than 30% reduction in the integrated diploid peak (less than 70% in T/C) was judged to be positive.

Definition of Activity More than 30% reduction in the integrated diploid peak (70% cut-off) was defined as effective, and more than 60% reduction (40% cut-off) was defined as definitely effective. The 70% cut-off correlated with a more than 50% inhibition of P388 and L1210 cell growth *in vitro*, and the 40% cut-off correlated with a complete inhibition of the cell growth.³⁹⁾ In addition, when rats were inoculated intracranially with 9L rat brain tumors and were treated with drugs which were effective (70% cut-off) against these tumor cells *in vitro*, growth retardation of inoculated brain tumors and prolonged survival of inoculated rats were ob-

served.¹¹⁾ Furthermore, when the 70% cut-off was applied for the antitumor activity of eight anticancer drugs, which are active clinically,⁴⁰⁾ against 141 fresh specimens of non-small cell lung cancer, seven drugs showed a good correlation between the clinical activity and *in vitro* activity.³⁹⁾

Statistical Analysis Experimental results were analyzed for significance by Fisher's exact test for the morphological degenerative changes in the nuclei and FCM assay. A *p*-value of 0.05 or less was regarded as significant.

RESULTS

Correlation between Drug Concentrations Determined by PPC and TAC Methods Since the concentrations of clinically available anticancer agents were determined based on the PPC method and those of investigational new drugs on the TAC method, it became a matter of concern whether both concentrations corresponded to each other. The concentrations of 16 clinically available drugs determined by these two methods are shown in Table 1. As shown in Table 1, TACs at 7n of all drugs, except vinblastine and bleomycin, corresponded well with one-tenth of the PPC of each drug. In addition, TACs of vinblastine and bleomycin ranged, at maximum, within only 7-fold higher.

Association between Morphological Assay and FCM Assay Results The DNA integrities assessed by the FCM analysis were compared with the morphological changes of tumor cell nuclei. As shown in Table 2, good associations were observed between two methods; *i.e.*, the true-positive rate was 96% (27/28), the true-negative rate 88% (136/154), and the overall accuracy 90% (163/182).

Antitumor Activities of TAS-103 and Other Investigational New Drugs against Surgical Specimens As shown in Tables 3 and 4, TAS-103 at 5 $\mu\text{g/ml}$ (7n) showed significantly higher effective rates (ERs) and definitely effective rates (DERs) than those of other new drugs at 7n against all of the tumors examined. TAS-103 at 0.5 $\mu\text{g/ml}$ (0.7n) also showed the highest ERs among new drugs against the majority of tumors.

Antitumor Activities of Clinically Available Anticancer Agents and TAS-103 against Surgical Specimens As shown in Tables 3 and 4, aclarubicin showed the highest ERs and DERs against all of the tumors among topoisomerase inhibitors, and these activities are comparable to those of TAS-103 at 5 $\mu\text{g/ml}$. Mitomycin C, cytosine arabinoside and paclitaxel showed the highest ERs against the majority of tumors among antitumor antibiotics, antimetabolites and antimicrotubule agents, respectively. Among alkylating agents, several drugs (melphalan, cyclophosphamide, cisplatin and carboplatin) showed the highest ERs against, at least, one type of tumor. However, the ERs and DERs of all these drugs were significantly lower than those of TAS-103 at 5 $\mu\text{g/ml}$. More-

over, ERs of TAS-103 at 0.5 $\mu\text{g/ml}$ against non-small cell lung cancer, bone and soft tissue sarcomas, breast cancer and small cell lung cancer were higher than those of the majority of clinically available anticancer agents.

DISCUSSION

We have developed the FCM assay as a drug sensitivity test on the basis of morphological changes of apoptotic nuclei. The assays based on morphological changes of the whole tumor cells were employed in the 1970s and the predictive accuracy was reported to be 50%.⁴¹⁾ However, a unique morphological method employed in the present study was shown to have the overall accuracy of 94% in ovarian cancer patients.¹⁴⁾ Supporting this, good associations were observed between results of the morphological assay and the FCM assay (Table 2).

Correlations between TAC and PPC were examined for 16 clinically available anticancer agents, and it was found that TACs at 7n of 14 drugs corresponded well with one-tenth of the PPC of each drug (Table 1). Moreover, a good correlation between the concentration of ranimustine (MCNU) estimated by the TAC method and the actual concentration in human brain tumors has been shown,⁴²⁾ in which 6 patients with malignant gliomas or metastatic brain tumors had been given MCNU by one-shot intravenous injection at 150 mg/body (2.34–3.75 mg/kg; TAC, 3.04–4.88 $\mu\text{g/ml}$), while the maximum concentration of MCNU in the tumor tissues has been found to be 2.38–3.31 $\mu\text{g/g}$.

In the present study, with the FCM assay and the TAC method, the antitumor activity of TAS-103 was examined against fresh surgical specimens resected from 501 patients (8 types of tumors). As a result, TAS-103 at 5 $\mu\text{g/ml}$ showed significantly higher ERs and DERs than those of all other investigational new drugs and all clinically available anticancer agents (except aclarubicin) against all the tumors examined (Tables 3, 4). Furthermore, TAS-103 at 0.5 $\mu\text{g/ml}$ also showed excellent antitumor activities (Tables 3, 4).

TAS-103 was shown to have unique biological actions and high antitumor activities against murine and human tumors *in vitro* and *in vivo*.¹⁾ In addition, it was also shown in the toxicological studies in cynomolgus monkeys that the dose-limiting toxicity of TAS-103 given by single or intermittent (once per 3 weeks, 2 doses) administration was leukopenia and other toxicity to major organs was not observed.⁴³⁾

Aclarubicin showed the highest ERs and DERs against all of the tumors examined among topoisomerase inhibitors, and these activities are comparable to those of TAS-103 at 5 $\mu\text{g/ml}$ (Tables 3, 4). It is interesting to note that aclarubicin is seemed to be a topo I and topo II inhibitor,⁴⁴⁾ like TAS-103.¹⁾

A clinical phase I study of TAS-103 has been carried out against 32 patients; including 16 colorectal, 7 lung, and 5 head and neck.⁴⁵⁾ TAS-103 was administered to patients as a continuous intravenous infusion over 1 h. One dose of TAS-103 (50 to 200 mg/m²) was given weekly for 3 weeks, repeated every 35 d. The MTD was approximately 160 mg/m² and the dose-limiting toxicity was neutropenia. The authors recommended a dose of 130 to 160 mg/m², administered using the weekly schedule, for phase II studies. In the present study, based on the TAC method, a starting dose (n) of TAS-

Table 2. Association between Morphological Assay and Flow Cytometric Assay Results

No. of drugs ^{a)}	+/+ ^{b)}	+/-	-/+	-/-
182	27	1	18	136

a) Six tests (5 glioblastoma multiforme and 1 adenocarcinoma of the lung) were done with a total of 182 (56, 34, 26, 12, 12 and 42, respectively) anticancer drugs. b) Flow cytometric assay/morphological assay; +, positive; -, negative.

Table 3. Antitumor Activities of Investigational New Drugs and Clinically Available Anticancer Agents against Tumors with More than 30 Cases

Drug	Conc. ($\mu\text{g/ml}$)	Category	Non-small cell lung cancer			Bone and soft tissue sarcomas			Brain tumor			Renal cancer		
			Total No.	No. of DE ^{a)} (%)	No. of E ^{b)} (%)	Total No.	No. of DE (%)	No. of E (%)	Total No.	No. of DE (%)	No. of E (%)	Total No.	No. of DE (%)	No. of E (%)
TAS-103	5	New ^{c)}	236	91 (39)	183 (78)	68	30 (44)	55 (81)	101	45 (45)	83 (82)	37	5 (14)	24 (65)
TAS-103	0.5	New	236	15 (6)***	89 (38)***	64	9 (14)***	27 (42)***	100	0***	16 (16)***	36	2 (6)	8 (22)***
ADM-OXD	3.2	New	114	6 (5)***	38 (33)***	54	2 (4)***	19 (35)***	45	1 (2)***	11 (24)***	13	0	3 (23)*
P1301	0.5	New	113	5 (4)***	26 (23)***	47	1 (2)***	4 (9)***	42	0***	2 (5)***	10	0	0***
SUN4599	5	New	211	2 (1)***	30 (14)***	81	0***	14 (17)***	175	1 (0)***	9 (5)***	36	0	3 (8)***
69A	0.3	New	99	2 (2)***	13 (13)***	45	0***	3 (7)***	41	0***	1 (2)***	9	0	0***
4181	1	New	99	0***	12 (12)***	45	0***	3 (7)***	41	0***	4 (10)***	9	0	0***
ACR	0.6	Topol. II ^{d)}	238	69 (29)*	181 (76)	70	27 (39)	55 (79)	109	11 (10)***	47 (43)***	37	10 (27)	28 (76)
DM	0.6	Topol. II ^{d)}	236	31 (13)***	131 (56)***	68	11 (16)***	34 (50)***	100	1 (1)***	19 (19)***	36	3 (8)	18 (50)
THP	0.3	Topol. II	238	14 (6)***	99 (42)***	70	5 (7)***	32 (46)***	106	3 (3)***	20 (19)***	37	4 (11)	15 (41)
EPI	0.4	Topol. II	238	5 (2)***	59 (25)***	68	1 (1)***	22 (32)***	106	1 (1)***	15 (14)***	37	1 (3)	8 (22)***
ACD	0.01	Topol. II	238	5 (2)***	58 (24)***	70	2 (3)***	24 (34)***	105	3 (3)***	13 (12)***	36	0	9 (25)***
MIT	0.06	Topol. II	238	4 (2)***	51 (21)***	68	2 (3)***	8 (12)***	106	0***	9 (8)***	36	1 (3)	9 (25)***
SN-38	0.02	Topol. I ^{f)}	239	5 (2)***	40 (17)***	69	2 (3)***	11 (16)***	105	1 (1)***	6 (6)***	36	1 (3)	5 (14)***
ADM	0.3	Topol. II	238	5 (2)***	37 (16)***	70	1 (1)***	16 (23)***	106	1 (1)***	10 (9)***	37	0	6 (16)***
VP-16	3	Topol. II	239	4 (2)***	32 (13)***	70	0***	4 (6)***	107	1 (1)***	3 (3)***	37	0	3 (8)***
MMC	0.2	Antibio ^{g)}	239	6 (3)***	72 (30)***	70	1 (1)***	16 (23)***	107	0***	9 (8)***	38	0*	13 (34)*
PEP	0.5	Antibio	238	5 (2)***	63 (26)***	69	1 (1)***	23 (33)***	105	0***	8 (8)***	36	0	10 (28)***
BLM	1	Antibio	239	5 (2)***	56 (23)***	70	1 (1)***	15 (21)***	107	1 (1)***	6 (6)***	38	0*	10 (26)***
NCS	0.15	Antibio	237	4 (2)***	30 (13)***	67	1 (1)***	7 (10)***	104	1 (1)***	4 (4)***	36	0	4 (11)***
Ara-C	4	Antimet ^{h)}	236	8 (3)***	79 (33)***	68	2 (3)***	25 (37)***	103	2 (2)***	9 (9)***	37	1 (3)	11 (30)***
6-MPR	3	Antimet	235	3 (1)***	48 (20)***	67	1 (1)***	9 (13)***	100	2 (2)***	6 (6)***	36	1 (3)	3 (8)***
5-FU	10	Antimet	239	2 (1)***	48 (20)***	69	2 (3)***	13 (19)***	106	1 (1)***	3 (3)***	36	0	3 (8)***
MTX	3	Antimet	237	0***	26 (11)***	70	0***	5 (7)***	104	0***	4 (4)***	36	0	1 (3)***
PTX	0.6	Mitotic ⁱ⁾	235	5 (2)***	66 (28)***	65	0***	13 (20)***	101	3 (3)***	25 (25)***	36	2 (6)	10 (28)***
VDS	0.1	Mitotic	239	7 (3)***	38 (16)***	68	2 (3)***	10 (15)***	105	2 (2)***	10 (10)***	36	1 (3)	5 (14)***
VLB	0.1	Mitotic	238	2 (1)***	26 (11)***	68	1 (1)***	7 (10)***	105	0***	5 (5)***	36	0	3 (8)***
VCR	0.1	Mitotic	239	1 (0)***	22 (9)***	70	0***	7 (10)***	109	0***	8 (7)***	36	0	3 (8)***
MPL	0.5	Alkylat ^{j)}	239	3 (1)***	55 (23)***	70	2 (3)***	11 (16)***	105	0***	5 (5)***	36	0	6 (17)***
TESPA	2	Alkylat	179	2 (1)***	34 (19)***	28	1 (4)***	4 (14)***	81	0***	2 (2)***	26	0	4 (15)***
CDDP	0.5	Alkylat	202	1 (0)***	38 (19)***	40	0***	4 (10)***	92	1 (1)***	6 (7)***	31	0	2 (6)***
CPM	1	Alkylat	229	3 (1)***	39 (17)***	62	0***	9 (15)***	102	0***	7 (7)***	36	0	7 (19)***
CQ	0.1	Alkylat	239	8 (3)***	39 (16)***	67	1 (1)***	7 (10)***	104	0***	2 (2)***	36	2 (6)	6 (17)***
IFOS	1	Alkylat	238	5 (2)***	37 (16)***	62	1 (2)***	3 (5)***	102	0***	4 (4)***	36	0	1 (3)***
JM-8	4	Alkylat	201	6 (3)***	28 (14)***	40	0***	4 (10)***	89	0***	3 (3)***	31	0	2 (6)***
BUS	0.8	Alkylat	179	0***	21 (12)***	28	0***	0***	80	0***	1 (1)***	26	0	1 (4)***
MCNU	2	Alkylat	238	1 (0)***	25 (11)***	67	1 (1)***	6 (9)***	105	0***	5 (5)***	36	0	2 (6)***
ACNU	2	Alkylat	239	0***	24 (10)***	66	1 (2)***	4 (6)***	108	0***	6 (6)***	36	1 (3)	5 (14)***

a) DE: Definitely effective. b) E: Effective. c) New: Investigational new drug. d) Topo I, II: Topoisomerase I and II inhibitor. e) Topo II: Topoisomerase II inhibitor. f) Topo I: Topoisomerase I inhibitor. g) Antibio: Antitumor antibiotic. h) Antimet: Antimetabolite. i) Mitotic: Antimicrotubule agent. j) Alkylat: Alkylating agent. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with TAS-103 (5 $\mu\text{g/ml}$) group.

Table 4. Antitumor Activities of Investigational New Drugs and Clinically Available Anticancer Agents against Tumors with Less than 30 Cases

Drug	Conc. ($\mu\text{g/ml}$)	Category	Breast cancer			Ovarian cancer			Uterine tumor			Small cell lung cancer		
			Total No.	No. of DE (%)	No. of E (%)	Total No.	No. of DE (%)	No. of E (%)	Total No.	No. of DE (%)	No. of E (%)	Total No.	No. of DE (%)	No. of E (%)
TAS-103	5	New	15	7 (47)	11 (73)	10	3 (30)	7 (70)	21	4 (19)	13 (62)	13	5 (38)	9 (69)
TAS-103	0.5	New	14	1 (7)*	7 (50)	10	0	2 (20)	21	0	6 (29)	13	2 (15)	4 (31)
ADM-OXD	3.2	New	17	1 (6)*	6 (35)*	N.D. ^{a)}			3	0	1 (33)	6	1 (17)	2 (33)
69A	0.3	New	12	1 (8)*	4 (33)	N.D.			3	0	0	5	0	0*
SUN4599	5	New	25	0*	5 (20)**	10	0	1 (10)*	22	0	5 (23)	16	2 (13)	2 (13)**
P1301	0.5	New	13	1 (8)*	2 (15)**	N.D.			2	0	0*	5	0	0*
4181	1	New	12	0**	1 (8)**	N.D.			3	0	0	5	0	0*
ACR	0.6	Topol, II	15	5 (33)	7 (47)	10	3 (30)	6 (60)	21	4 (19)	13 (62)	14	4 (29)	10 (71)
DM	0.6	TopolII	15	1 (7)*	7 (47)	10	0	3 (30)	21	1 (5)	8 (38)	14	2 (14)	6 (43)
THP	0.3	TopolII	15	1 (7)*	5 (33)	10	0	3 (30)	21	1 (5)	7 (33)	14	2 (14)	5 (36)
ACD	0.01	TopolII	15	1 (7)*	4 (27)*	10	0	0**	21	0	3 (14)**	13	0*	3 (23)*
MIT	0.06	TopolII	15	1 (7)*	4 (27)*	10	0	0**	22	0	5 (23)*	14	0*	3 (21)*
SN-38	0.02	Topol	14	1 (7)*	3 (21)**	10	0	0**	22	1 (5)	5 (23)*	14	1 (7)	1 (7)**
VP-16	3	TopolII	15	0**	3 (20)**	10	0	1 (10)*	22	0	3 (14)**	14	0*	3 (21)*
ADM	0.3	TopolII	15	0**	2 (13)**	10	0	0**	21	0	2 (10)**	14	0*	3 (21)*
EPI	0.4	TopolII	15	0**	1 (7)**	10	0	0**	21	0	5 (24)*	14	0*	3 (21)*
PEP	0.5	Antibio	15	0**	4 (27)*	10	0	1 (10)*	21	0	4 (19)*	14	1 (7)	2 (14)**
BLM	1	Antibio	15	1 (7)*	4 (27)*	10	0	0**	21	0	3 (14)**	14	1 (7)	2 (14)**
NCS	0.15	Antibio	14	0**	2 (14)**	10	0	0**	21	0	2 (10)**	14	1 (7)	2 (14)**
MMC	0.2	Antibio	15	0**	2 (13)**	10	0	1 (10)*	22	0	7 (32)	14	1 (7)	6 (43)
Ara-C	4	Antimet	15	2 (13)	4 (27)*	10	0	0**	20	1 (5)	5 (25)*	14	1 (7)	3 (21)*
MTX	3	Antimet	15	0	2 (13)**	10	0	0**	20	0	2 (10)**	14	1 (7)	1 (7)**
6-MPR	3	Antimet	15	1 (7)	1 (7)**	10	0	1 (10)*	21	0	3 (14)**	14	1 (7)	1 (7)**
5-FU	10	Antimet	15	0	1 (7)**	10	0	0**	21	0	3 (14)**	14	0*	1 (7)**
VLB	0.1	Mitotic	15	0	4 (27)*	10	0	1 (10)*	22	0	5 (23)*	14	0*	2 (14)**
PTX	0.6	Mitotic	15	0	3 (20)**	10	0	1 (10)*	20	1 (5)	7 (35)	13	1 (8)	5 (38)
VDS	0.1	Mitotic	15	0	3 (20)**	10	0	1 (10)*	22	0	5 (23)*	14	1 (7)	2 (14)**
VCR	0.1	Mitotic	15	0	1 (7)**	10	0	0**	22	0	2 (9)**	14	0*	2 (14)**
JM-8	4	Alkylat	10	0	3 (30)*	10	0	0**	20	0	2 (10)**	11	1 (9)	2 (18)*
TESPA	2	Alkylat	5	0	1 (20)	10	0	0**	20	0	4 (20)*	8	1 (13)	2 (25)
MPL	0.5	Alkylat	15	1 (7)	2 (13)**	10	0	0**	22	0	5 (23)*	14	0*	2 (14)**
CQ	0.1	Alkylat	15	1 (7)	2 (13)**	10	0	0**	22	0	0***	14	1 (7)	1 (7)**
ACNU	2	Alkylat	15	0	2 (13)**	10	0	0**	22	0	2 (9)**	14	1 (7)	4 (29)
CPM	1	Alkylat	12	0	1 (8)**	10	0	1 (10)*	21	0	4 (19)*	13	1 (8)	1 (8)**
MCNU	2	Alkylat	14	0	1 (7)**	10	0	1 (10)*	22	0	3 (14)**	14	0*	2 (14)**
CDDP	0.5	Alkylat	10	0	0***	10	0	0**	20	0	2 (10)**	11	0*	4 (36)
BUS	0.8	Alkylat	5	0	0*	10	0	1 (10)*	20	0	2 (10)**	8	1 (13)	2 (25)
IFOS	1	Alkylat	12	0	0***	10	0	0**	21	0	3 (14)**	13	0*	1 (8)**

a) N.D., Not done. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with TAS-103 (5 $\mu\text{g/ml}$) group.

103 was estimated to be 20 mg/m² and the MTD was expected to be 7n to 9n at the modified Fibonacci's dose-escalation scheme,³⁷⁾ 140 to 180 mg/m². These results, together with the aforementioned results of a good correlation between the TAC and PPC of 14 clinically available anticancer drugs, suggest that the TAC method advanced by Schabel *et al.*¹⁹⁾ can predict the optimal concentrations of the investigational new drugs which are employed in the *in vitro* chemosensitivity test.

However, it was shown in this clinical study that the PPCs of TAS-103 after intravenous administration of 130, 160 and 200 mg/m² were 1.5 to 2.2 µg/ml, not 50 µg/ml, and high protein binding of this drug was speculated to concern.⁴⁵⁾ It is interesting to know whether TAS-103 may attain to the concentration of 50 µg/ml and show any significant antitumor activity when given on another treatment schedule (for example, one-shot intravenous injection). Furthermore, development of a new drug delivery system of TAS-103, such as encapsulation into liposome,⁴⁶⁾ may give a possibility that TAS-103 shows its inherent promising antitumor activities against a wide range of human tumors.

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