Clinical Applications of the Histoculture Drug Response Assay[1]

Toshiharu Furukawa, Tetsuro Kubota,[2] and Robert M. Hoffman

Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjukuku, Tokyo 160, Japan [T. F., T. K.]; AntiCancer, Inc., San Diego, California 92111 [R. M. H.]; and Laboratory of Cancer Biology, Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla, California 92093–0609 [R. M. H.]

ABSTRACT

There is a need for a clinically useful drug-response assay for cancer patients to individualize their chemotherapy. Collagen sponge-gel-supported histoculture has been shown to maintain tissue architecture and function *in vitro* and has been utilized to develop the histoculture drug-response assay (HDRA) for individualizing chemotherapy. In order to evaluate the HDRA with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide end point for clinical use, chemosensitivity to mitomycin C, doxorubicin, 5-fluorouracil, and cisplatin of 107 advanced gastric and 109 advanced colorectal cancers was determined *in vitro* in a correlative clinical trial. Two hundred eight (96.3%) of 216 of the patient specimens were evaluable in the HDRA. Thirty-eight patients with remaining measurable lesions after surgery were evaluable for comparison of the effects of chemotherapy in the HDRA with clinical outcome. Their overall response in the HDRA to all four drugs correlated to published historical data. Twenty-nine patients were treated with drugs shown to be ineffective in the HDRA, and all 29 cases showed clinical chemoresistance. In nine patients treated with drugs shown to be effective in the HDRA, six showed clinical chemoresponse and three showed arrest of disease progression. The correlation rate of the assay to clinical drug-sensitivity response was thus calculated to be 92.1% (35/38), with 100% (29/29) true-negative and 66.7% (6/9) true-positive rates, 100% (6/6) sensitivity, and 90.6% (29/32) specificity. Thirty-two patients with stage III and IV gastric cancer without remaining measurable tumor lesions after surgery were treated with mitomycin C and a fluoropyrimidine adjuvantly. The survival rate of 10 patients whose tumors were sensitive to either mitomycin C and/or 5-fluorouracil in the assay was significantly ($P < 0.005$) better than that of 22 patients whose tumors were shown to be insensitive to both drugs. Twenty-nine patients with stage III and IV colorectal cancer without remaining measurable tumor lesions after surgery were treated with fluoropyrimidines adjuvantly. The recurrence-free survival rate of 7 patients whose tumors were sensitive to 5-fluorouracil in the assay was significantly ($P < 0.05$) better than that of 22 patients whose tumors were insensitive. Thus the HDRA with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide end point should be of clinical value to choose optimal chemotherapy for response as well as for survival.

INTRODUCTION

It is known that individuals with tumors of similar histopathological classification have markedly different clinical drug-response spectra. *In vitro* drug-response assays have been developed for more than four decades in order to individualize chemotherapy for cancer patients (1, 2). The HTCA[3] of Hamburger and Salmon (3) was the first systematically developed drug-response assay that demonstrated clinical relevance. The major clinical studies for the HTCA were carried out by Von Hoff (4) with results indicating that the HTCA was 91% accurate in correlating to clinical drug resistance and 69% accurate in correlating to clinical drug sensitivity.

A modified version of the HTCA developed by Kern and colleagues (5–7) using [^{3}H]thymidine as an end point instead of colony formation was shown to be highly accurate in correlating to clinical drug resistance but less accurate in correlating to clinical sensitivity. Depending on the investigator, the various versions of the HTCA were shown to evaluate only 30–70% of the specimens attempted (3, 6, 8), thus limiting the usefulness of the HTCA.

Weisenthal *et al.* (7) used disaggregated cells and fast green as the reporting dye to distinguish live and dead cells in a chemosensitivity assay. This assay correlates with clinical outcome and may be best suited to hematological tumors rather than solid malignancies since it uses cells in suspension.

Studies by Heppner's group (9–12) and others have demonstrated that the configuration of cells with respect to each other may affect their drug sensitivity, which suggested the idea that maintaining tumor cells in their native three-dimensional histological architecture may confer more accurate correlation to *in vivo* drug sensitivity.

Hoffman and colleagues (1, 13–22) took advantage of the collagen sponge-gel matrix culture system developed by Leighton in the 1950s (23) to culture patient tumor tissue with

Received 8/16/94; accepted 11/17/94.
[1] This work was supported in part by Grant 03770774 from the Ministry of Education, Science and Culture of Japan.
[2] To whom requests for reprints should be addressed, at Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-Ku, Tokyo 160, Japan.

[3] The abbreviations used are: HTCA, human tumor clonogenic assay; HDRA, histoculture drug-response assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MMC, mitomycin C; DXR, doxorubicin; 5-FU, 5-fluorouracil; DDP, cisplatin; CR, complete response; PR, partial response.

maintenance of native tissue architecture to determine drug response. This approach was termed histoculture by Sherwin *et al.* (24). The critical importance of maintaining tumor architecture for accurate drug sensitivity determinations is reviewed by Hoffman (1, 2, 20, 25).

Comparisons of drug-response spectra of human tumor xenografts in the HDRA and in nude mice, using reduction of MTT as an end point *in vitro*, showed that both drug resistance and sensitivity *in vitro* highly correlated to the *in vivo* response at approximately 90% (21). A subsequent study with gastrointestinal cancer demonstrated that the HDRA with the MTT end point correlated highly to historical clinical drug response (22).

We recently compared the clinical effects of cisplatin in the HDRA with the thymidine incorporation end point in 23 patients with head and neck cancers (26). The study was comprised of 21 patients with carcinomas and 2 patients with sarcoma. Ten of 12 patients with HDRA *in vitro*-sensitive tumors had either complete or partial response clinically. The overall accuracy of the HDRA was 74% in this correlative clinical trial; the predictive-positive value was 83%, the sensitivity was 71%, and the specificity was 78%. These results formed the basis to evaluate the HDRA with the MTT end point in a retrospective correlative clinical trial which is described here for advanced gastric and colorectal cancers. The data indicate that the HDRA highly correlates to clinical resistance, clinical sensitivity, and survival of patients whose tumors were tested.

PATIENTS AND METHODS

Patients. One hundred seven patients with advanced gastric cancer and 109 patients with advanced colorectal cancer were included in the study. Charts were reviewed to obtain information on the patients by a person who had no knowledge of the assay results.

Drugs. Four anticancer drugs were used: MMC, DXR, and 5-FU were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) and DDP was purchased from Bristol-Myers Squibb K.K. (Tokyo, Japan).

HDRA with the MTT End Point. The method of Hoffman and colleagues (14–18) was utilized employing the MTT end point as reported previously (21, 22). Collagen sponge gels manufactured from pig skin were purchased from Health Design Inc. (Rochester, NY). The cancerous portions of the specimens were scissor-minced into pieces approximately 0.5 mm in diameter, which were then placed on each of the prepared collagen surfaces in 24-well plates. The plates were incubated for 7 days at 37°C with the drugs dissolved in RPMI 1640 medium containing 20% FCS in a humidified atmosphere containing 95% air-5% CO_2. The cutoff concentrations of the drugs used to distinguish *in vitro* sensitivity and resistance were 7.5 μg/ml for MMC, 15 μg/ml for DXR, 300 μg/ml for 5-FU, and 20 μg/ml for DDP (21, 22).

After histoculture, 100 μl HBSS containing 0.1 mg/ml collagenase (type I; Sigma) and 100 μl MTT (Dojindo Laboratories, Kumamoto, Japan) solution, dissolved in 5 mg/ml PBS were added to each culture well and incubated for another 8 h. After extraction with DMSO, the absorbance of the solution in each well was read at 540 nm. The absorbance/g of histocultured tumor tissue was calculated from the mean absorbance of tissue from four culture wells and tumor-tissue weight determined prior to culture.

The inhibition rate was calculated using the formula:

$$\text{Inhibition rate (\%)} = (1 - \text{mean absorbance of treated tumor/g} / \text{mean absorbance of control tumor/g}) \times 100.$$

When the inhibition rate was 50% or more, the chemosensitivity of tumors to drugs was scored as positive (21, 22). Each drug concentration was tested in at least three culture wells. The HDRA was applicable when the tumor specimens were 150 mg or larger, which is equivalent to 10 mg tissue/well in each of 15 wells consisting of controls and cultures treated with all four drugs used. The HDRA was regarded as evaluable when the mean absorbance of extracted formazan at 540 nm of the control tumor was 15 or more per g (22).

Patient Treatment. Patients who had remaining measurable tumor lesions after surgery were administered drugs which were shown to be effective in the HDRA of their individual tumors. In cases where all four drugs showed negative antitumor activity in the HDRA, the patients were treated with some combination of the four drugs. The doses administered are listed in Table 2. Patients over 75 years old and those with moderate or severe cardiac, respiratory, renal, or hepatic dysfunctions were excluded from the clinical correlation study without regard to the clinical stage of their cancer.

Patients with advanced gastric and colorectal cancers without remaining measurable tumor lesions underwent postoperative adjuvant chemotherapy. Patients with stages I and II were excluded from the clinical correlation study because adjuvant chemotherapy of these patients was only occasionally performed at the discretion of the clinician since their expected prognosis was favorable. Stage III and IV gastric cancer patients without remaining measurable tumor lesions were given 30 mg/m^2 MMC and 400 mg/body/day UFT, a combination of tegafur and uracil in a molar ratio of 1:4 (27). Stage III and IV colorectal cancer patients without remaining measurable tumor lesions were treated adjuvantly with 400 mg/body/day UFT or 1-hexylcarbamoyl-5-FU (28).

Clinical Response to Chemotherapy. Patients with remaining measurable tumor lesions were eligible for retrospective evaluation of the assay results by comparison to the clinical effects of chemotherapy. A CR was defined as the total disappearance of the tumor for at least 4 weeks. A PR was defined as at least a 50% decrease in product of the longest perpendicular diameters of measurable lesions. No change was defined as less than partial response without evidence of disease progression. Progressive disease was defined as any increase (>25%) in measurable lesions or the appearance of new lesions.

For patients without remaining measurable tumor lesions, the survival rates and the recurrence-free survival rates of Kaplan-Meier were used for the retrospective evaluation of the assay results and the effects of postoperative adjuvant chemotherapy.

Table 1 *In vitro* chemosensitivity determined by the HDRA for each tumor type

Tumor type	Drugs				Total
	MMC	DXR	5-FU	DDP	
Stomach	32/102[a] (31.4%)	15/102 (14.7%)	19/102 (18.6%)	22/102 (21.6%)	88/408 (21.6%)
Colorectal	18/106 (17.0%)	8/106 (7.5%)	26/106 (24.5%)	4/106 (3.7%)	56/424 (13.2%)
Total	50/208 (24.0%)	23/208 (11.1%)	45/208 (21.6%)	26/208 (12.5%)	144/832 (17.3%)

[a] Data are shown as number of sensitive cases/number of evaluable cases.

RESULTS

Evaluability of the Assay. One hundred two of 107 gastric cancers and 106 of 109 colorectal cancers were found to be evaluable in the HDRA (96.3%). All 107 gastric and 109 colorectal tumor specimens weighed more than 150 mg and were applicable for the HDRA, resulting in a total evaluability rate of 96.3% (208/216).

Tumor Types and *in Vitro* Chemosensitivity. Table 1 gives the overall *in vitro* chemosensitivity determined by the HDRA with the MTT end point for each tumor type. MMC showed a 31.4% efficacy rate against stomach cancer with a total efficacy rate of 24.0%, which was the highest efficacy rate among the four drugs used. 5-FU showed a 24.5% efficacy rate against colorectal cancer, which was the highest against this type of tumor.

Correlation of the Results of the Assay and the Clinical Effects of Chemotherapy. The assay results and clinical effects of chemotherapy for each of 22 patients with gastric cancer and 16 with colorectal cancers with remaining measurable tumor lesions are listed in Table 2. For patients with gastric cancer, six patients whose tumors were sensitive to at least one of the drugs tested in the assay were treated with the *in vitro*-sensitive drugs. Three of these patients had drug responses (one CR and two PRs), while the remaining three showed stable disease. Sixteen patients whose tumors were insensitive to all four drugs tested in the assay were treated with some combination of the four drugs, and all of them showed drug resistance. There were no significant differences in the total doses of the drugs administered between *in vitro*-sensitive and -insensitive cases or clinically responsive and resistant cases (Table 2).

For patients with colon cancer, 5-FU was included in the treatment of all three patients who were sensitive to at least one of the drugs tested in the HDRA and was effective in all of these cases (one CR and two PRs). Thirteen patients whose tumors were insensitive to all four drugs tested in the HDRA were treated with some combination of the four drugs, and all of them showed drug resistance. There were no significant differences in the total doses of the drugs administered between *in vitro*-sensitive and -insensitive cases or clinically responsive and resistant cases (Table 2).

In total, there were 6 true-positive, 3 false-positive, and 29 true-negative cases, giving a correlation rate of the HDRA to the clinical effects of chemotherapy of 92.1% (35/38), with 66.7% (6/9) true-positive and 100% (29/29) true-negative rates, and 100% (6/6) sensitivity and 90.6% (29/32) specificity (Table 3).

Correlation of the Results of the Assay and the Effects of Postoperative Adjuvant Chemotherapy. There were 10 patients (group A) with advanced gastric cancer whose tumors were sensitive to MMC and/or 5-FU, and 22 patients (group B) whose tumors were insensitive to both MMC and 5-FU. There were no significant differences in terms of clinical and pathological characteristics between these two groups (Table 4). Postoperative cancer recurrence was identified in three patients in group A, and one of them died of cancer. In group B, cancer recurrence was identified in 18 patients, and 16 of them died of cancer. The survival and recurrence-free survival rates evaluated according to Kaplan-Meier were significantly ($P < 0.005$ by log rank test) better in group A than group B (Fig. 1).

There were seven patients (group C) with stage III and IV colorectal cancer whose tumors were sensitive to 5-FU. Group D consisted of 22 patients whose tumors were insensitive to 5-FU. There were no significant differences in terms of clinical and pathological characteristics between these two groups (Table 5). In group C, all seven patients were still alive without cancer recurrence. In group D, cancer recurrence was identified in eight patients, and three of them died of cancer. The survival and recurrence-free survival rates evaluated according to Kaplan-Meier were better in group C than group D with statistical significance ($P < 0.05$ by log rank test) in the latter (Fig. 2).

DISCUSSION

The HDRA demonstrated a very high rate of evaluability (96.3%) in comparison to a corresponding range of 30–70% for the HTCA (3, 6). This might be due to the fact that the HDRA allows tumor cells to maintain their native three-dimensional tissue architecture and viability longer than the cell suspension assay (1, 2, 22).

The *in vitro* efficacy rates of MMC, DXR, 5-FU, and DDP in the HDRA were 31.4%, 14.7%, 18.6%, and 21.6%, respectively, against gastric cancer and 17.0%, 7.5%, 24.5% and 3.7%, respectively, against colorectal cancer, equivalent to the historical clinical efficacy rates reported elsewhere (29–32).

The MTT end point allows chemosensitivity testing of resting tumor cells which cannot be evaluated by the [^{3}H]thymidine incorporation end point (21). For chemosensitivity testing of gastrointestinal tumors, it seems that the MTT end

Table 2 Correlation of results of the assay and the clinical effects of chemotherapy

Case and tumor type	*In vitro* chemosensitivity and clinically used drugs				Clinical response	Sites of measurable disease	Correlation
	MMC	DXR	5-FU	DDP			
Gastric cancer cases							
1	(−)[a]	(−)[b] 120	(−) 12.9	−	PD[c]	Liver	TN[d]
2	(−) 32	−	−	(−) 200	PD	Lymph nodes	TN
3	−	−	−	(−) 330	PD	Peritoneum	TN
4	(−) 40	−	−	(−) 240	PD	Liver	TN
5	−	(−) 210	−	−	PD	Liver	TN
6	(−) 55	−	−	−	PD	Local tumor	TN
7	(−) 38	(−) 60	(−) 10.8	−	PD	Liver	TN
8	−	−	−	(−) 330	NC	Local tumor	TN
9	−	−	−	(−) 330	PD	Peritoneum	TN
10	−	−	−	(−) 330	NC	Peritoneum	TN
11	+	−	(+) 11.5	(+) 240	PR	Lymph nodes	TP
12	(+) 42	+	−	+	NC	Local tumor, liver	FP
13	−	−	(+) 10.6	(+) 240	PR	Local tumor, peritoneum	TP
14	(−) 32	−	−	(−) 210	NC	Liver	TN
15	(+) 40	−	−	(−) 250	NC	Local tumor, liver	FP
16	(+) 33	−	−	(−) 220	NC	Local tumor, peritoneum	FP
17	(−) 36	(−) 72	(−) 9.5	(−) 200	PD	Liver	TN
18	(+) 32	+	+	(+) 230	PR	Liver	TP
19	(−) 34	(−) 60	−	(−) 210	PD	Liver	TN
20	(−) 36	−	(−) 10.2	(−) 220	NC	Local tumor, peritoneum	TN
21	(−) 41	−	−	(−) 200	NC	Liver	TN
22	(−) 35	−	(−) 12.8	(−) 270	NC	Local tumor, peritoneum	TN
Colorectal cancer cases							
1	−	−	(−) 13.3	−	PD	Liver	TN
2	−	(−) 240	−	−	PD	Liver	TN
3	−	−	(−) 11.8	−	NC	Local tumor	TN
4	−	−	(−) 15.4	−	PD	Liver	TN
5	−	−	(−) 10.7	−	NC	Liver	TN
6	−	−	(−) 14.0	−	PD	Liver	TN
7	−	−	(−) 13.9	−	NC	Liver	TN
8	+	−	(+) 12.6	−	PR	Liver	TP
9	(−) 36	(−) 75	(−) 11.2	−	NC	Liver	TN
10	−	−	(−) 12.6	−	NC	Liver	TN

Table 2 Cont'd.

Case and tumor type	*In vitro* chemosensitivity and clinically used drugs				Clinical response	Sites of measurable disease	Correlation
	MMC	DXR	5-FU	DDP			
11	(−) 32	−	(−) 9.6	−	NC	Local tumor, liver	TN
12	−	−	(−) 15.5	−	NC	Liver	TN
13	−	−	(+) 11.4	−	CR	Liver	TP
14	−	−	(−) 9.8	−	NC	Liver	TN
15	+	−	(+) 11.8	−	PR	Liver	TP
16	−	−	(−) 12.9	−	NC	Liver	TN

[a] + and −, positive and negative antitumor effects, respectively.

[b] Symbols in parentheses, drugs administered to patients. Numbers under parentheses, total doses of the drugs used in mg/m^2 for MMC, DXR, and DDP and g/m^2 for 5-FU.

[c] NC, no change; PD, progressive disease.

[d] TP (true-positive) indicates that drugs which showed positive antitumor effects *in vitro* demonstrated a positive response in patients. TN (true-negative) indicates that drugs which showed negative antitumor effects *in vitro* demonstrated a negative response in patients. FP (false-positive) indicates that drugs which showed positive antitumor effects *in vitro* demonstrated a negative response in patients.

Table 3 Correlation of results of the assay and clinical effects of chemotherapy

In vitro drug response	Clinical drug response		
	Positive	Negative	Total
Positive	6[a]	3	9
Negative	0	29	29
Total	6	32	38

[a] Number of cases. True-positive rate (TP/TP + FP): 6/9 (66.7%); true-negative rate (TN/TN + FN): 29/29 (100%); sensitivity (TP/TP + FN): 6/6 (100%); specificity (TN/FP + TN): 29/32 (90.6%); Correlation rate (TP + TN/TP + FP + TN + FN): 35/38 (92.1%). TP (true-positive) indicates that drugs which showed positive antitumor effects *in vitro* demonstrated a positive response in patients. FP (false-positive) indicates that drugs which showed positive antitumor effects *in vitro* demonstrated a negative response in patients. TN (true-negative) indicates that drugs which showed negative antitumor effects *in vitro* demonstrated a negative response in patients. FN (false-negative) indicates that drugs which showed negative antitumor effects *in vitro* demonstrated a positive response in patients.

Table 4 Comparison of clinical and pathological characteristics between groups A and B[a]

Clinical and pathological factors	Group A (n = 10)	Group B (n = 22)
Age[b] (yr)	55.7 (14.4)	59.7 (12.9)
Sex[c]		
Male	4	13
Female	6	9
Stage[b]		
III	4	8
IV	6	14
T category[b]		
T_2	1	3
T_3	6	15
T_4	3	4
N category[b]		
N_1	3	8
N_2	7	14
Differentiation of tumors[c]		
Undifferentiated	7	17
Differentiated	3	5
Operation[c]		
Total gastrectomy	7	16
Subtotal gastrectomy	3	6

[a] Group A consisted of 10 patients whose tumors were sensitive to at least one of the drugs MMC or 5-FU *in vitro* and group B, 22 patients whose tumors were insensitive to both MMC and 5-FU. Patients were treated adjuvantly with MMC and UFT.

[b] Data are shown as mean (SD). There was no significant difference between Groups A and B by Student's t test.

[c] Data show number of cases. Not significant by χ^2 test.

point, which evaluates total tumor cell viability, is more appropriate than a proliferating-cell end point since many cells in these types of tumors may be in the resting stage of the cell cycle.

In the patients with remaining measurable tumor lesions, six true-positive cases were obtained along with three-false positive cases, giving a true-positive rate of 66.7%. There were 29 true-negative cases and no false-negative cases. The high prediction rate (92.1%) demonstrated that the HDRA with the MTT end point is useful for the elimination of ineffective and harmful drugs in individual patients.

An important point of the assay appeared to be that there were no false-negative cases, despite the presence of some false-positive cases. Considering that the overall clinical efficacy rate of current chemotherapy for gastrointestinal cancers is limited (29–32), care should be taken to eliminate false-negative results on the basis of which potentially effective drugs might be abandoned in a clinical setting. In addition, it should be noted that all three false-positive cases were very advanced gastric cancers and had remaining unresectable local tumors, against which MMC was administered, resulting in no change

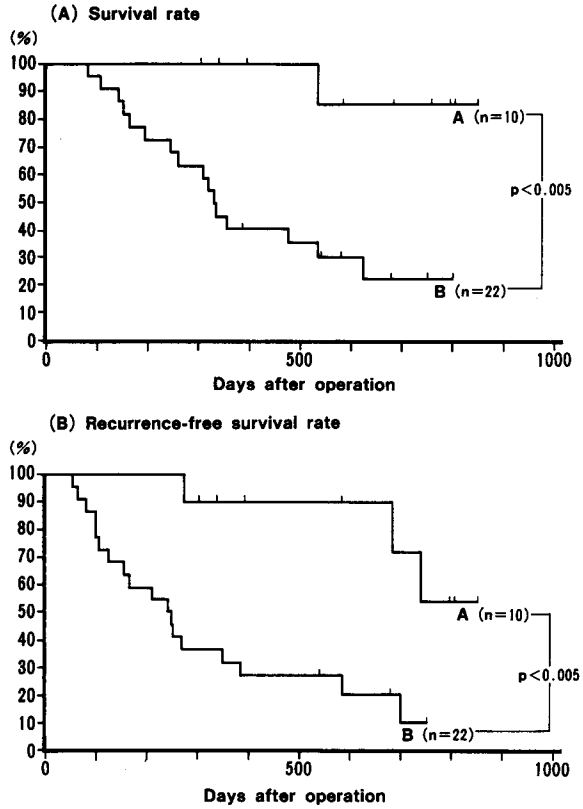

Fig. 1 *A*, Kaplan-Meir survival curves; *B*, recurrence-free survival curves following surgery of stage III and IV gastric cancer patients without remaining measurable tumor lesions who were treated adjuvantly with MMC and UFT. Group A consisted of 10 patients whose tumors were sensitive to MMC and/or 5-FU *in vitro*. Group B consisted of 22 patients whose tumors were insensitive to both MMC and 5-FU. Survival rate and recurrence-free survival rate in group A were better than those in group B ($P < 0.005$ by log rank test).

and not in progressive disease, suggesting that the assay may have given useful information in these cases also. Therefore, the HDRA seems to be effective in identifying drug-sensitive patients as well as drug-resistant patients.

The most important point for *in vitro* chemosensitivity assays is to improve the patient's prognosis (33–35). In the present study, the HDRA was applied to postoperative adjuvant chemotherapy in cases without remaining measurable tumor lesions and demonstrated, although retrospectively, a potential use for selecting effective adjuvant cancer chemotherapy to improve the survival rates of the patients since *in vitro* drug sensitivity in the HDRA significantly correlated with patient survival.

The HDRA with the MTT end point highly correlates with clinical drug response, resistance, and survival in advanced gastric and colorectal cancers. The results of the present retrospective study are a basis for a prospective predictive clinical study of the HDRA to further ensure the reliability of the HDRA to predict clinical response and disease-free and overall survival.

Table 5 Comparison of clinical and pathological characteristics between Groups C and D[a]

Clinical and pathological factors	Group C (n = 7)	Group D (n = 22)
Age[b] (yr)	57.9 (15.2)	54.4 (13.3)
Sex[c]		
Male	5	14
Female	2	8
Stage[b]		
III	3	10
IV	4	12
T category[c]		
T_3	1	5
T_4	6	17
N category[c]		
N_1	3	10
N_2	1	3
N_3	3	9
Tumor location[c]		
Colon	3	13
Rectum	4	9

[a] Group C consisted of 7 patients whose tumors were sensitive to 5-FU *in vitro* and Group D, 22 patients whose tumors were insensitive to 5-FU. Patients were treated adjuvantly with fluoropyrimidines. All colorectal tumors studied were of the differentiated type.

[b] Data are shown as mean (SD). There was no significant difference between Groups C and D by Student's t test.

[c] Data show number of cases. Not significant by χ^2 test.

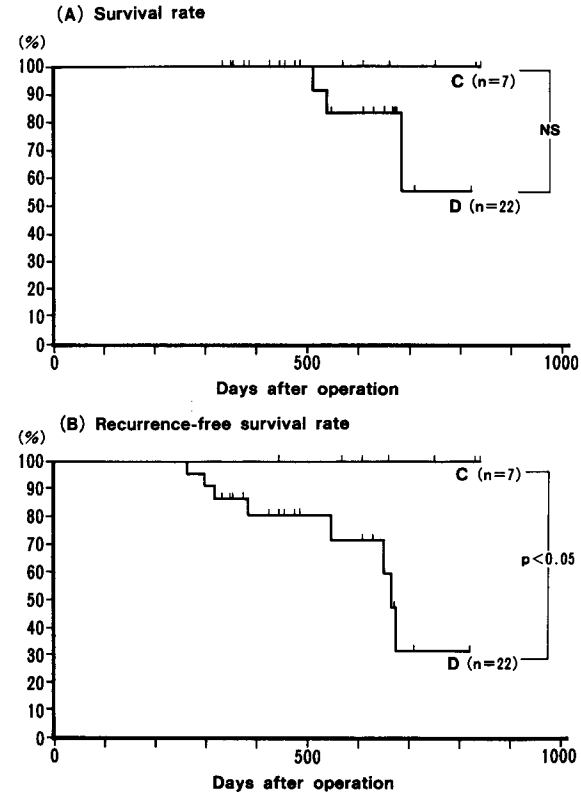

Fig. 2 *A*, Kaplan-Meier survival curves; *B*, recurrence-free survival curves following surgery of stage III and IV colorectal cancer patients without remaining measurable tumor lesions who were treated adjuvantly with fluoropyrimidines. Group C consists of 7 patients whose tumors were sensitive to 5-FU *in vitro*. Group D consists of 22 patients whose tumors were insensitive to 5-FU. The recurrence-free survival rate in group C was better than that in group D ($P < 0.05$ by log rank test.)

ACKNOWLEDGMENTS

We are grateful to Minako Uematsu for her excellent technical assistance.

REFERENCES

1. Hoffman, R. M. In vitro sensitivity assays in cancer: A review, analysis and prognosis. J. Clin. Lab. Anal., *5:* 133–143, 1991.

2. Hoffman, R. M. *In vitro* assays for chemotherapy sensitivity. Crit. Rev. Oncol. Hematol., *15:* 99–111, 1993.

3. Hamburger, A. W., and Salmon, S. E. Primary bioassay of human tumor stem cells. Science (Washington DC), *197:* 461–463.

4. Von Hoff, D. D. He's not going to talk about *in vitro* predictive assays again, is he? J. Natl. Cancer Inst., *82:* 96–101, 1990.

5. Kern, D. H., Campbell, M. A., Cochran, A. J., Burk, M. W., and Morton, D. L. Cloning of human solid tumors in soft agar. Int. J. Cancer, *30:* 725–729, 1982.

6. Kern, D. H., and Wiesenthal, L. M. Highly specific prediction of antineoplastic drug resistance with an *in vitro* assay using supraphar-malogic drug exposure. J. Natl. Cancer Inst., *82:* 582–588, 1990.

7. Weisenthal, L. M., Dill, P. L., Kurnick, N. B., *et al.* Comparison of dye exclusion assays with a clonogenic assay in the determination of drug-induced cytotoxicity. Cancer Res., *43:* 258–264, 1983.

8. Selby, P., Buick, R. N., and Tannock, I. Human tumor skin cell assay (Letter). N. Engl. J. Med., *308:* 1480–1481, 1983.

9. Lawler, E. M., Miller, F. R., and Heppner, G. H. Significance of three-dimensional growth patterns of mammary tissues in collagen gel cultures. In Vitro, *19:* 600–610, 1983.

10. Miller, B. E., Miller, F. R., and Heppner, G. H. Assessing tumor drug sensitivity by a new in vitro assay which preserves tumor heterogeneity and subpopulation interactions. J. Cell. Physiol., *3* (Suppl.)*:* 105–116, 1984.

11. Miller, B. E., Miller, F. R., and Heppner, G. H. Factors affecting growth and drug sensitivity of mouse mammary tumor lines in collagen gel cultures. Cancer Res., *45:* 4200–4205, 1985.

12. Furukawa, T., Kubota, T., Watanabe, M., Kase, S., Saikawa, Y., Nishibori, H., Kuo, T-H., Kodaira, S., Ishibiki, K., and Kitajima, M. Increased drug resistance of cultured human cancer cell lines in a three-dimensional cellular growth assay using collagen gel matrix. J. Surg. Oncol., *49:* 86–92, 1992.

13. Freeman, A. E., and Hoffman, R. M. *In vivo*-like growth of human tumors in vivo. Proc. Natl. Acad. Sci. USA, *83:* 2694–2698, 1986.

14. Vescio, P. H., Redfern, C. H., Nelson, T. J., Ugoretz, S., Stern, P. H., and Hoffman, R. M. *In vivo*-like drug responses of human tumors growing in three-dimensional, gel-supported primary culture. Proc. Natl. Acad. Sci. USA, *84:* 5029–5033, 1987.

15. Hoffman, R. M., Monosov, A. Z., Connors, K. M., Herrera, H., and Price, J. H. A general native-state method for determination of proliferation capacity of human normal and tumor tissues *in vitro*. Proc. Natl. Acad. Sci. USA, *86:* 2013–2017, 1989.

16. Vescio, R. A., Connors, K. M., Youngkin, T., Bordin, G. M., Robb, J. A., Umbreit, J. N., and Hoffman, R. M. Cancer biology for individualizes cancer therapy: correlation of growth fraction index in native-state histoculture with tumor grade and stage. Proc. Natl. Acad. Sci. USA, *87:* 691–695, 1990.

17. Vescio, R. A., Connors, K. M., Bordin, G. M., Robb, J. A., Youngkin, T., Umbriet, J. N., and Hoffman, R. M. The distinction of small cell and non-small cell cancer by growth in native-state histoculture. Cancer Res., *50:* 6095–6099, 1990.

18. Vescio, R. A., Connors, K. M., Kubota, T., and Hoffman, R. M. Correlation of histology and drug response of human tumors grown in native-state three-dimensional histoculture and in nude mice. Proc. Natl. Acad. Sci. USA, *88:* 5163–5166, 1991.

19. Li, L., Margolis, L. B., and Hoffman, R. M. Skin toxicity determined in vitro by three-dimensional, native-state histoculture. Proc. Natl. Acad. Sci. USA, *88:* 1908–1912, 1991.

20. Hoffman, R. M. Three-dimensional histoculture: origins and application in cancer research. Cancer Cells, *3:* 86–92, 1991.

21. Furukawa, T., Kubota, T., Watanabe, M., Takahara, T., Yamaguchi, H., Takeuchi, T., Kodaira, S., Ishibiki, K., Kitajima, M., and Hoffman, R. M. High *in vivo-in vitro* correlation of drug response using sponge-gel-supported three-dimensional histoculture and the MTT end point. Int. J. Cancer, *51:* 489–498, 1992.

22. Furukawa, T., Kubota, T., Watanabe, M., Kase, S., Takahara, T., Yamaguchi, H., Takeuchi, T., Teramoto, T., Ishibiki, K., Kitajima, M., and Hoffman, R. M. Chemosensitivity testing of clinical gastrointestinal cancers using histoculture and the MTT end point. Anticancer Res., *12:* 1377–1382, 1992.

23. Leighton, J. A sponge matrix-method for tissue cultures. Formation of organized aggregates of cells *in vitro*. J. Natl. Cancer Inst., *12:* 545–561, 1951.

24. Sherwin, R. P., Richters, A., Yellin, A. E., and Donavan, A. J. Histoculture of human breast cancers. Surg. Oncol., *13:* 9–20, 1980.

25. Hoffman, R. M. To do tissue culture in two or three dimensions? That is the question. Stem Cells, *11:* 105–111, 1993.

26. Robbins, K. T., Connors, K. M., Storniolo, A. M., Hanchett, C., and Hoffman, R. M. Sponge-gel-supported histoculture drug-response assay for head and neck cancer. Arch. Otolaryngol. Head Neck Surg., *120:* 288–292, 1994.

27. Maehara, Y., Watanabe, A., Kakeji, Y., Baba, H., Kohnoe, S., and Sugimachi, K. Postgastrectomy prescription of mitomycin C and UFT for patients with stage IV gastric carcinoma. Am. J. Surg., *160:* 242–244, 1990.

28. Koyama, Y., and Koyama, Y. Phase I study of a new antitumor drug, I-hexylcarbamoyl-5-fluorouracil (HCFU) administered orally: an HCFU clinical study group report. Cancer Treat. Rep., *64:* 861–867, 1980.

29. Carter, S. K., and Friedman, M. Integration of chemotherapy into combined modality treatment of solid tumors. II. Large bowel carcinoma. Cancer Treat. Rev., *1:* 111–129, 1974.

30. Comis, R. I., and Carter, S. K. Integration of chemotherapy into combined modality treatment of solid tumors. III. Gastric cancer. Cancer Treat. Rev., *1:* 221–283, 1974.

31. Perry, M. C., Green, M. R., Mick, P., and Schein, P. Cisplatin in patients with gastric cancer: a cancer and leukemia group B phase II study. Cancer Treat. Rep., *70:* 415–416, 1986.

32. DeSimone, P. A., Davalia, E., Jochimensen, P. R., and Bartolucci, A. A. High-dose cisplatin in the treatment of advanced adenocarcinoma of the colon and rectum: a southeastern cancer study group trial. Cancer Treat. Rep., *70:* 1229–1230, 1986.

33. Von Hoff, D. D. Selection of cancer chemotherapy for a patient by an *in vitro* assay versus a clinician. J. Natl. Cancer Inst., *82:* 110–116, 1990.

34. Gazdar, A. F., Steinberg, S. M., Russell, E. K., Linnoila, R. I., Oie, H. K., Ghosh, B. C., Cotelingham, J. D., Johnson, B. E., Minna, J. D., and Ihde, D. C. Correlation of *in vitro* drug-sensitivity testing results with response to chemotherapy and survival in extensive-stage small cell lung cancer: a prospective clinical trial. J. Natl. Cancer Inst., *82:* 117–124, 1990.

35. Leone, L. A., Meitner, P. A., Myers, T. J., Grace, W. R., Gajewski, W. H., Fingert, H. J., and Rotman, B. Predictive value of the fluorescent cytoprint assay (FCA): a retrospective correlation study of *in vitro* chemosensitivity and individual responses to chemotherapy. Cancer Invest., *9:* 491–503, 1991.