Potential of the Histoculture Drug-Response Assay to Contribute to Cancer Patient Survival[1]

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ABSTRACT

The histoculture drug-response assay (HDRA) was recently evaluated in a retrospective clinical trial and was found to correlate to drug sensitivity, resistance, and patient survival. To further investigate the potential of HDRA to contribute to patient survival, 215 patients with gastric cancer from 45 medical centers were tested with the HDRA in a blinded study after resection of the primary lesion. One hundred sixty-eight patients received at least 20 mg/m^2 of mitomycin C and a minimum of 30 g UFT, a mixture of tegafur and uracil at a molar ratio of 1:4, thereby making them eligible for the study. Of these cases 128 were evaluable by the HDRA. The evaluable patient tumors were tested by the HDRA with the [^{3}H]thymidine incorporation end point measured by microautoradiography to be drug "sensitive" or "resistant." The *in vitro* conditions for distinguishing sensitivity and resistance that matched the response rates for historical controls for gastric carcinoma were 90% inhibition rate and 0.12 μg/ml for mitomycin C and 70% inhibition rate and 1 μg/ml for 5-fluorouracil, respectively. Most importantly in the blinded study, the overall and disease-free survival rates of the HDRA-sensitive group were found to be significantly higher than those of the HDRA-resistant group tested under the above conditions. The data further indicate the importance of three-dimensional tumor culture for obtaining accurate clinical information. The results demonstrate that the HDRA response correlates to patient survival, which suggests the potential of the HDRA to contribute to patient survival in gastric cancer when used prospectively.

INTRODUCTION

To increase the response and survival rate of cancer patients by optimizing and individualizing treatment, we have developed the HDRA[3] (1–3). The HDRA, which was developed by Vescio *et al.* (4) took advantage of the collagen sponge-gel matrix three-dimensional culture system of Leighton (5). Sponge-gel matrix culture, also termed histoculture, allows the culture of patient tumor tissue with maintenance of native tissue-three-dimensional architecture, which is necessary for accurate determination of drug response. The critical importance of maintaining three-dimensional tumor architecture for accurate drug sensitivity determinations is reviewed by Hoffman (6–8).

A recent retrospective study indicated that the HDRA with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide end point is of clinical value to choose optimal chemotherapy for response as well as for survival (9). The present study is a multicenter, blinded, semiprospective trial of the HDRA on 215 patients surgically treated for gastric cancer from 45 institutions to evaluate the potential of the HDRA to contribute to survival of gastric cancer patients.

PATIENTS AND METHODS

Patients. Two hundred fifteen patients with advanced gastric cancer treated in 45 institutions between August 1990 and June 1993 were entered into this trial. The eligibility criteria to enter the trial are listed in Table 1.

Drugs. Commercially available MMC (Kyowa Hakko Kogyo, Co. Ltd., Tokyo, Japan) and UFT, a mixed compound of tegafur and uracil at a molar ratio of 1:4 (Taiho Pharmaceutical Co. Ltd., Tokyo), were used throughout the study.

HDRA with [^{3}H]Thymidine End Point Measured by Autoradiography. Fresh tissue blocks weighing between 0.5 and 5 g were aseptically obtained from the carcinoma lesions of surgical specimens in each patient. One slice, 3-mm thick, was cut, fixed in 10% formalin, and prepared for histological study. Pathological diagnosis and grading were made with the review and consensus of three pathologists (H. S., H. N. and N. S.). The remainder of each specimen was stored in HBSS containing 250 units penicillin, 250 μg streptomycin, 0.62 μg amphotericin B, and 100 μg gentamicin/ml. The stock solution was kept at 4°C and sent from Tokyo to AntiCancer, Inc. (San Diego, CA) within 48 h of the resection. This stock solution had been regarded as optimal for transfer compared with RPMI 1640 with 10% FCS or 0.9% NaCl in our previous study (10). Histopathological analysis was carried out without the knowledge of the patients' background or assay results.

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[3] The abbreviations used are: HDRA, histoculture drug-response assay; MMC, mitomycin C; UFT, a mixed compound of tegafur and uracil at a molar ratio of 1:4; 5-FU, 5-fluorouracil; IR, inhibition rate.

Table 1 Eligibility criteria to enter the clinical trial

1. Macroscopically stage III and IV gastric cancer.
2. No hepatic metastases and/or peritoneal dissemination.
3. Primary operation for gastric cancer without previous cancer therapy.
4. Patient age less than 75 yr old and more than 18 yr old.
5. Performance status = 0–2 (Japanese Society for Cancer Therapy).
6. Expectancy life period > 12 wk.
7. No severe complications or history of other malignancy.
8. Laboratory data
   Peripheral WBC >3,000/mm^3
   Platelet count >100,000/mm^3
   Creatinine level <2.0 mg/dl
   Total bilirubin level <2.0 mg/dl
   Hematocrit > 30%; hemoglobin > 10 g/dl
   Aspartate aminotransferase < 1.5 × normal range
   Alkaline phosphatase < 1.5 × normal range
   Normal electrolytes
   Normal electrocardiogram
9. Informed consent.

The remainder of the each specimen was tested by one of the authors (R. M. H.) at AntiCancer, Inc. without knowledge of the patients' characteristics. The presence of microscopically detected cancer cells in the specimens was also included in the eligibility criteria.

The method of Hoffman *et al.* (1–4, 6–8) was used for the HDRA as reported previously. Collagen sponge gels manufactured from pig skin were purchased from the Upjohn Co. (Kalamazoo, MI). The cancerous tissue of each specimen was scissor minced into pieces approximately 1–2 mm in diameter, which were then placed on each of the prepared collagen surfaces in 6-well plates. Eagle's MEM containing Earle's salts, 10% (v/v) FCS, nonessential amino acids (1:100 dilution of a stock solution from Irvine Scientific), gentamicin (0.1 mg/ml), penicillin G (100 units/ml), and amphotericin B (2.52 μg/ml) were added to culture dishes such that the upper part of the gel was not covered.

The effect of MMC in the HDRA was determined at concentrations of 0.012 (0.1×), 0.12 (1.0×), and 1.2 (10×) μg/ml, and the effects of 5-FU were determined at concentrations of 1 (0.1×), 10 (1.0×), and 100 (10×) μg/ml. The 1.0× concentrations correspond to clinically achievable doses *in vivo* (4). The original cutoffs were IRs equal to or more than 90% at the 1.0× concentration for MMC and 5-FU. After 24-h histoculture, the specimens were exposed to drugs for a subsequent 24 h.

Cells in the histoculture were labeled with [^{3}H]thymidine (4 μCi/ml; 1 Ci = 37 GBq) for 3 additional days after the drugs were removed. Cellular DNA was labeled in any cell undergoing replication within the tissues.

After 3 days of labeling, the cultures were washed with PBS, placed in histological capsules, and fixed in 10% (v/v) formalin. The cultures were then dehydrated, embedded in paraffin, sectioned, and prepared for autoradiography using Kodak NTB-2 emulsion and counterstaining with hematoxylin and eosin.

Replicating cells were identified by the presence of silver grains over their nuclei due to exposure of the NTB-2 emulsion to radioactive DNA. The silver grains were visualized as bright green with an epipolarization lighting system.

The number of [^{3}H]thymidine-labeled cells was counted per field using × 200 magnification. For each drug concentration, the one to three fields containing the highest number of labeled cells were counted to identify the areas in the heterogenous tumor cultures having the least drug response. The control cultures were evaluated in the same manner. Two replicate cultures were evaluated for each drug concentration to determine the *in vitro* response. Percentage IR was calculated as 1 − (treated/control value of [^{3}H]thymidine-labeled cells).

Patient Treatment. All of the registered cases were treated with the same protocol shown in Fig. 1 without knowledge of the results of the HDRA. MMC was administered i.v. on the day of surgery at a dose of 13.3 mg/m^2 with an additional 6.9 mg MMC/m^2 i.v. on the first postoperative day (Fig. 1). MMC was further administered i.v. at a dose of 5.3 mg/m^2 every 4 weeks until the total dose of MMC reached 60 mg. The patients also received 200 mg UFT as tegafur two times daily perorally for 1 year, starting 2 weeks after the operation. To assess the compliance of UFT administration, urine tegafur was detected every 3 months by high performance liquid chromatography (11).

Statistical Analysis. Tables 2–4 summarize the characteristics of the patients in terms of sex, age, microscopic stage classification, microscopic serosal invasion (T category), microscopic lymph node metastasis (N category), histological differentiation, type of operation, and microscopic curability categorized by the patients' tumor sensitivity in the HDRA to MMC and/or 5-FU. These background factors are considered to be risk factors for survival of the patients with gastric cancer (12). The surgical and pathological classifications were made according to "The General Rules for the Gastric Cancer Study in Surgery and Pathology" in Japan (13). Background factors were compared using the Mann-Whitney U test, χ^2 test, or Fisher's direct probability test.

The effect of adjuvant chemotherapy was assessed by actuarial 3-year survival rates, which were calculated using the Kaplan-Meier method (14), and examined by the log rank test (15) for statistical significance. The multiple comparisons were adjusted in levels of significance based on the total number of comparisons performed (16). All P values are calculated from the two-sided test of significance.

Multivariate analysis was examined according to Cox's proportional hazard model (17) using SAS Release 6.07 (Sun OS 4.1.1) software to evaluate whether *in vitro* sensitivity was an independent risk factor for decreased overall survival.

RESULTS

Eligibility and Evaluability. Eligibility and evaluability were assessed independently by a Committee consisting of three registered surgeons listed in the "Appendix" who did not participate in the study. Two hundred six cancer specimens were submitted for the HDRA analysis with an evaluability rate of 83.5% (172/206). In the clinic, 128 cases were evaluable of 168 eligible cases. Unevaluable cases included 35 patients given incomplete doses of drugs, which meant <20 mg/m^2 MMC and/or 30 g UFT as tegafur. Urine tegafur was assessed in 106 cases for compliance with a positive rate of 91.9%.

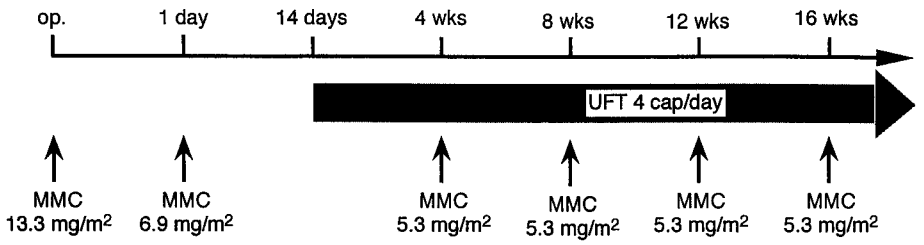

Fig. 1 Patient treatment. MMC was administered i.v. on the day of surgery (*op.*) at a dose of 13.3 mg/m^2 with the addition of 6.9 mg MMC/m^2 i.v. on the first postoperative day. MMC was also administered i.v. at a dose of 5.3 mg/m^2 every 4 weeks until the total dose of MMC reached 60 mg. The patients also received 200 mg UFT as tegafur two times daily perorally for 1 year, starting 2 weeks after surgery. To assess the compliance of UFT administration, urine tegafur was detected every 3 months by high performance liquid chromatography.

Table 2 Comparison of clinical and pathological characteristics between patients with tumors sensitive to MMC in the HDRA and tumors resistant to MMC in the HDRA

Clinical and pathological characteristics	Sensitive group[a]	Resistant group[b]
Age[c]		
<50 years old	5 (20)[d]	19 (19)
>50 years old	20 (80)	79 (81)
Sex[e]		
Male	16 (64)	61 (62)
Female	9 (36)	37 (38)
Stage[c]		
I	2 (8)	6 (6)
II	6 (24)	21 (21)
III	12 (48)	45 (46)
IV	5 (20)	26 (27)
T category[c]		
t2	12 (48)	37 (38)
t3	10 (40)	49 (50)
t4	3 (12)	12 (12)
N category[c]		
n(−)	7 (28)	16 (17)
n1	7 (28)	32 (33)
n2	8 (32)	34 (35)
n3	3 (12)	6 (6)
n4	0 (0)	9 (9)
Differentiation of tumors[e]		
Undifferentiated	11 (44)	57 (58)
Differentiated	14 (56)	41 (42)
Type of operation[c]		
Total gastrectomy	12 (48)	45 (46)
Distal gastrectomy	13 (52)	52 (53)
Proximal gastrectomy	0 (0)	1 (1)
Curability of surgery[c]		
Curative	22 (88)	76 (78)
Noncurative	3 (12)	22 (22)

[a] Patients with tumors sensitive to MMC.
[b] Patients with tumors resistant to MMC.
[c] There were no significant differences in the clinicopathological characteristics between the HDRA-sensitive and -resistant groups by the Mann-Whitney *U* test.
[d] Numbers in parentheses, percentage of each item.
[e] Not significant by the χ^2 test and Fisher's direct probability test.

Table 3 Comparison of clinical and pathological characteristics between patients with tumors sensitive to 5-FU in the HDRA and tumors resistant to 5-FU in the HDRA

Clinical and pathological characteristics	Sensitive group[a]	Resistant group[b]
Age[c]		
<50 years old	1 (5)[d]	21 (21)
>50 years old	19 (95)	78 (79)
Sex[e]		
Male	12 (60)	64 (65)
Female	8 (40)	35 (35)
Stage[c]		
I	3 (15)	5 (5)
II	3 (15)	23 (23)
III	7 (35)	47 (48)
IV	7 (35)	24 (24)
T category[c]		
t2	9 (45)	38 (38)
t3	7 (35)	50 (51)
t4	4 (20)	11 (11)
N category[c]		
n(−)	6 (30)	15 (15)
n1	7 (35)	32 (32)
n2	5 (25)	36 (37)
n3	1 (5)	8 (8)
n4	1 (5)	8 (8)
Differentiation of Tumors[f]		
Undifferentiated	6 (30)	61 (62)
Differentiated	14 (70)	38 (38)
Type of operation[e]		
Total gastrectomy	7 (35)	47 (48)
Distal gastrectomy	12 (60)	52 (52)
Proximal gastrectomy	1 (5)	0 (0)
Curability of surgery[c]		
Curative	17 (85)	77 (78)
Noncurative	3 (15)	22 (22)

[a] Patients with tumors sensitive to 5-FU.
[b] Patients with tumors resistant to 5-FU.
[c] There were no significant differences in the clinicopathological characteristics between the HDRA-sensitive and -resistant groups by the Mann-Whitney *U* test.
[d] Numbers in parentheses, percentage of each item.
[e] Not significant by the χ^2 test and Fisher's direct probability test.
[f] Statistically significant at $P = 0.004$ by χ^2 test.

Correlation of the Results of the Assay with the Effects of Postoperative Adjuvant Chemotherapy. The sensitivity of tumor specimens to MMC and 5-FU in the HDRA is shown in Table 5 as a function of cutoff IR determined at three concentrations of each drug. There was a concentration-dependent antitumor effect on the histoculture tumor specimens for both MMC and 5-FU.

At the 1× concentration of MMC, which was determined

Table 4 Comparison of clinical and pathological characteristics between patients with tumors sensitive to MMC or 5-FU in the HDRA and tumors resistant to both of the drugs in the HDRA

Clinical and pathological characteristics	Sensitive group[a]	Resistant group[b]
Age[c]		
<50 yr old	6 (16)[d]	19 (21)
>50 yr old	32 (84)	70 (79)
Sex[e]		
Male	23 (61)	57 (64)
Female	15 (39)	32 (36)
Stage[c]		
I	4 (11)	5 (6)
II	7 (18)	21 (23)
III	17 (45)	41 (46)
IV	10 (26)	22 (25)
T category[c]		
t2	17 (45)	34 (38)
t3	16 (42)	45 (51)
t4	5 (13)	10 (11)
N category[c]		
n(−)	10 (26)	15 (17)
n1	12 (31)	28 (31)
n2	11 (29)	32 (36)
n3	4 (11)	6 (7)
n4	1 (3)	8 (9)
Differentiation of tumors[f]		
Undifferentiated	14 (37)	55 (62)
Differentiated	24 (63)	34 (38)
Type of operation[e]		
Total gastrectomy	16 (42)	41 (46)
Distal gastrectomy	21 (55)	48 (54)
Proximal gastrectomy	1 (13)	0 (0)
Curability of surgery[c]		
Curative	32 (84)	69 (78)
Noncurative	6 (16)	20 (22)

[a] Patients with tumors sensitive to MMC or 5-FU.
[b] Patients with tumors resistant to both MMC and 5-FU.
[c] There were no significant differences in the clinicopathological characteristics between the HDRA-sensitive and -resistant groups by the Mann-Whitney *U* test.
[d] Numbers in parentheses, percentage of each item.
[e] Not significant by the χ^2 test and Fisher's direct probability test.
[f] Statistically significant at $P = 0.005$ by the χ^2 test.

from peak plasma concentration (18), the drug had an *in vitro* efficacy rate of 20.3% for the patient tumors tested in this study at an original cutoff IR of 90%. The *in vitro* efficacy of MMC in the HDRA under these conditions corresponded to historical clinical data (19). However, the efficacy of 5-FU at the 1× concentration at an original cutoff inhibition rate of 90% was 43.7%, which was two times the reported clinical efficacy rate of this drug (19). Since the 1× concentration was thought to overestimate the drug's effect, the 0.1× concentration or 1 μg 5-FU/ml was used to distinguish sensitivity and resistance of the histocultured tumors to 5-FU. At the 0.1× concentration using a cutoff at a 70% IR, 5-FU had an efficacy rate of 16.8%, which was equivalent to the efficacy rate reported clinically (19).

At the cutoff IR of 90% at the 1× concentration of MMC in the HDRA, there were 25 advanced gastric cancer tumors sensitive to MMC (sensitive group), and 98 patient tumors were resistant to MMC (resistant group). There were no significant differences in terms of clinical and pathological characteristics between the resistant and sensitive groups (Table 2). The overall survival rate evaluated according to Kaplan and Meier (14) was significantly better in the sensitive group than in the resistant group ($P = 0.02$ by the log rank test with Bonferroni's adjustment; Fig. 2).

Correlation with patient survival was also correlated to response to 5-FU in the HDRA. At the cutoff IR of 70% at the 0.1× concentration of 5-FU in the HDRA, there were 20 advanced gastric cancer patient tumors sensitive to 5-FU (sensitive group), and 99 patient tumors were resistant to 5-FU (resistant group). There were no significant differences in terms of clinical and pathological characteristics between the resistant and sensitive groups (Table 3). The overall survival rate evaluated according to Kaplan and Meier (14) was significantly better in the HDRA-sensitive group than in the -resistant group ($P = 0.04$ by log rank test with adjustment; Fig. 3).

There were 38 advanced gastric cancer tumors sensitive to MMC or 5-FU in the HDRA (sensitive group) and 89 patient tumors were resistant to both MMC and 5-FU in the HDRA (resistant group) under the above conditions. There were no significant differences in terms of clinical and pathological characteristics between the HDRA-resistant and -sensitive groups (Table 4). The overall survival rate evaluated according to Kaplan and Meier (14) was significantly better in the sensitive group than in the resistant group ($P = 0.0006$ by log rank test; Fig. 4).

Multivariate analysis was examined according to Cox's proportional hazard model (17). Risk ratios are shown for prolonged overall survival in Table 6 for each variable of the gastric cancer patients with tumors sensitive or resistant to MMC and/or 5-FU in the HDRA. The analysis demonstrates that the sensitivity to MMC and/or 5-FU in the HDRA is an independent risk factor for overall survival in each category.

DISCUSSION

The HDRA demonstrated a high rate of evaluability (83.5%), even though all of the specimens were sent from Japan to San Diego for histoculture. The evaluability rate was 10% less than that in our previous study in which all of the specimens were assessed in the same institution and put into culture on the day of operation (9). The high evaluability in the present study suggested that the tumor cells in intact tissue with cell to cell contact have a high survival rate for 2 days in HBSS and can maintain their native three-dimensional tissue architecture and viability longer than disaggregated cell suspensions. The tumor specimens were sent from Tokyo to San Diego in this trial, which could account for the somewhat decreased evaluability rate in this study. Sending specimens from Tokyo to San Diego will not be necessary in the future since functional HDRA laboratories are now in place in Japan and the United States.

The original cutoffs were IRs equal to or more than 90% at 0.12 μg/ml (1×) for MMC and 10 μg/ml (1×) for 5-FU. Since drug sensitivity functions as a continuous variable, we have calculated the efficacy rates of MMC and 5-FU from 10 to 90% cutoff inhibitory rates and at three cutoff concentrations (0.1×, 1×, and 10×). The efficacy rates were dependent on the drug concentrations in which higher concentrations and lower cutoff inhibitory rates resulted in higher efficacy rates. For MMC, *in*

Table 5 Sensitivity to MMC and 5-FU at various cutoff IR values

Drug[a]	MMC			5-FU		
Concentration (*μg/ml*)	0.012 (0.1×)	0.12 (1×)	1.2 (10×)	1 (0.1×)	10 (1×)	100 (10×)
IR cutoff (%)						
90	9.6[b]	**20.3**[c,d]	62.5	9.2	43.7	71.1
80	13.5	30.1	72.7	12.6	60.5	82.1
70	21.2	42.3	80.5	**16.8**[d,e]	61.3	86.7
60	29.8	52.0	82.8	26.1	67.2	89.1
50	46.2	61.0	85.9	40.3	76.5	92.2
40	54.8	67.5	89.1	48.7	81.5	96.1
30	62.5	73.2	91.4	55.5	84.9	98.4
20	67.3	78.1	92.2	63.0	91.0	99.2
10	72.1	82.9	93.8	72.3	93.3	99.2
n[f]	104	123	128	119	119	128

[a] MMC or 5-FU was incubated with surgical specimens in histoculture for 24 h.
[b] The efficacy rates for the drugs against gastric cancer specimens were calculated at various cutoff IRs.
[c] Sensitive, 25 cases; insensitive, 98 cases.
[d] Statistically significant differences in overall survival rates between HDRA sensitive and insensitive groups at $P < 0.05$.
[e] Sensitive, 20 cases; insensitive, 99 cases.
[f] Number of specimens tested.

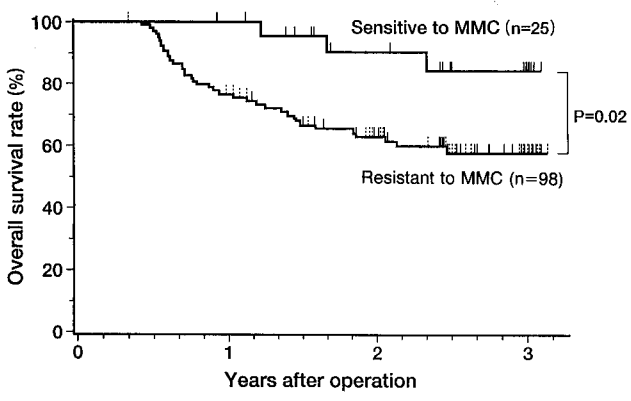

Fig. 2 Correlation of the overall survival rate of MMC- and UFT-treated stage III and IV gastric cancer patients and the HDRA to MMC. The sensitive group consisted of 25 patients whose tumors were sensitive to MMC in the HDRA. The resistant group consisted of 98 patients whose tumors were resistant to MMC in the HDRA. See text for experimental details. The overall survival rate of the sensitive group was better than that of the resistant group ($P = 0.02$ by log rank test with adjustment).

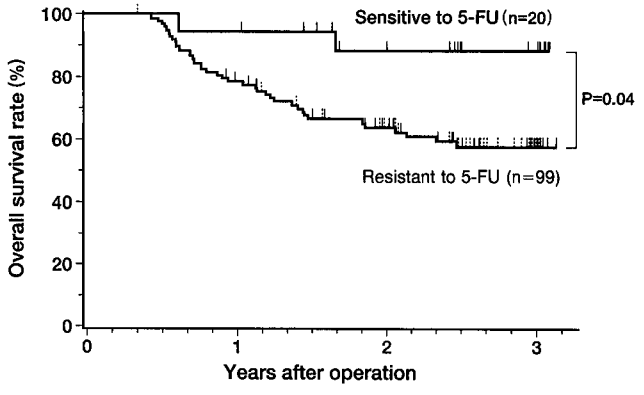

Fig. 3 Correlation of the overall survival rate of MMC- and UFT-treated stage III and IV gastric cancer patients and the HDRA to 5-FU. The sensitive group consisted of 20 patients whose tumors were sensitive to 5-FU in the HDRA. The resistant group consisted of 99 patients whose tumors were insensitive to 5-FU. The overall survival rate of the sensitive group was better than that of the resistant group ($P = 0.04$ by log rank test with adjustment).

vitro efficacy rates correlating to historical control efficacy rates were found at the 0.1× concentration at a 70% inhibitory rate and at the 1× concentration at a 90% inhibitory rate. The original cutoff was used for the statistical analysis of the survival rates. However, the efficacy rate of 5-FU at the 1× concentration was 43.7%, which was two times the reported clinical efficacy rate of this drug (19, 20). There were no statistically significant differences observed between the sensitive and resistant groups to 5-FU at this original cutoff. This suggested that the cutoffs of antimetabolites like 5-FU are not easily estimated from peak plasma concentrations, although this pharmacokinetic estimation is appropriate for cytotoxic agents like MMC. As a result, the cutoff of a 70% inhibitory rate at the 0.1× concentration was used for further analysis of 5-FU. We found in this study that these decision rules correlate the HDRA sensitivity of the tumors to the survival of the patients with statistical significance. Thus, the HDRA-sensitive group had statistically significant greater survival than the HDRA-resistant group (Figs. 2–4).

In the present study, we used only one arm for postoperative adjuvant cancer chemotherapy consisting of MMC and UFT. MMC at a dose of 13.3 + 6.9 mg/m^2 was administered as induction chemotherapy. Induction chemotherapy is given in the early period after surgery to kill any tumor cells that might have been released into the circulation during the operation (21). A previous clinical trial of adjuvant chemotherapy for gastric carcinoma also revealed that induction therapy with MMC was beneficial for survival (22). UFT is a mixed compound of tegafur and uracil at a molar ratio of 1:4 and was developed to

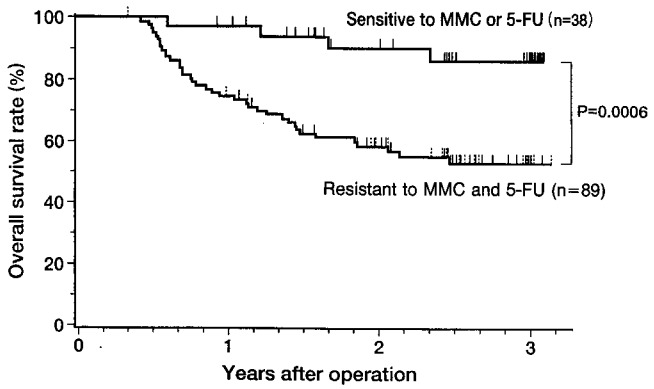

Fig. 4 Correlation of the overall survival rate of MMC- and UFT-treated stage III and IV gastric cancer patients and the HDRA to MMC or 5-FU. The sensitive group consisted of 38 patients whose tumors were sensitive to MMC or 5-FU in the HDRA. The resistant group consisted of 89 patients whose tumors were insensitive to MMC and 5-FU in the HDRA. The overall survival rate of the sensitive group was better than that of the resistant group ($P = 0.0006$ by log rank test).

increase the uptake of tegafur into tumor cells. Tegafur is a 5-FU pro-drug, which was synthesized in an attempt to obtain a more effective fluoropyrimidine antitumor agent (22, 23). It is active against several adenocarcinomas including gastric cancer, being metabolized into active 5-FU by microsomal cytochrome P450 in liver and tumor cells. Since the final metabolite of UFT is 5-FU, the sensitivity of the histocultured tumors to 5-FU was assessed and correlated to the efficacy of UFT administered to the patients postoperatively. Although this combined adjuvant cancer chemotherapy is currently "first-choice" treatment after gastric surgery, an effective chemosensitivity test should be able to select the most efficacious therapy for patients with tumors resistant to first-choice medication. A Phase III study of the HDRA will be carried out to confirm this hypothesis by comparing the clinical efficacy of the "HDRA-guided" arm to the standard first-choice arm.

In our previous clinical trial of HDRA, it was reported that the survival rate of patients treated with drugs shown to be effective in the HDRA was significantly better than that of patients given drugs shown to be ineffective in the HDRA (9). The present multicenter semiprospective trial was carried out using a much larger cohort and blinded for surgeons, pathologists, and laboratory scientists. The present study demonstrated that HDRA drug sensitivity could distinguish those patients with increased survival rates.

The current study did not include a surgery-alone arm, because in Japan, it is difficult to prescribe surgery alone for patients with stage III and IV gastric cancer, who almost always have chemotherapy in addition. We have compared the present survival results with historical survival rates (24) of patients with advanced gastric cancer who had received no adjuvant cancer chemotherapy due to their poor general condition due mainly to advanced age. Fifty percent survival of this untreated cohort was approximately 200 days, which was shorter than those of the HDRA-resistant group in the present study. This short survival time was probably due to the poor condition of the historical untreated cohort. Thus, a surgery-alone arm will be included in the Phase III study of the HDRA mentioned above.

Table 6 Multivariate analysis of risk factors of gastric cancer patients for prolonged overall survival

Variable	Hazard ratio[a]	*P*
Sensitive or resistant to MMC in the HDRA		
Sensitivity to MMC	0.180[b]	0.0218[c]
Pathological stage	2.801	0.0275[c]
T category	1.040	0.9247
N category	1.166	0.4550
Differentiation	3.118	0.0060[c]
Type of operation	0.715	0.3500
Curability of surgery	0.714	0.5023
Sensitive or resistant to 5-FU in the HDRA		
Sensitivity to 5-FU	0.098	0.0266[c]
Pathological stage	2.822	0.0234[c]
T category	1.560	0.2369
N category	1.083	0.6962
Differentiation	1.901	0.1316
Type of operation	0.561	0.1121
Curability of surgery	0.571	0.2704
Sensitive to MMC or 5-FU or resistant to MMC and 5-FU		
Sensitivity to MMC or 5-FU	0.137	0.0014[c]
Pathological stage	3.441	0.0078[c]
T category	1.133	0.7513
N category	1.152	0.4593
Differentiation	2.027	0.0843
Type of operation	0.754	0.4309
Curability of surgery	0.598	0.2903

[a] Risk ratios for overall survival rate.
[b] Risk ratios were calculated according to Cox's proportional hazard model using SAS Release 6.07 (Sun OS 4.1.1) software.
[c] $P < 0.05$.

From these results, we can conclude that the HDRA has the potential to improve the survival rates of cancer patients with tumors sensitive to any antitumor agents. This will be confirmed in a clinical Phase III study of the HDRA comparing patient survival in "HDRA-guided" therapy *versus* "physicians' choice" chemotherapy.

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APPENDIX

The eligibility and evaluability were assessed by the Committee consisting of Dr. Fumiki Asanuma, Kitasato Institute Hospital, Dr. Yo Isobe, National Tokyo Second Hospital, and Dr. Akihiko Suto, Yamato City Hospital.

The following institutions (Chief) participated in the study: Ashikaga Red Cross Hospital (M. Fujisaki), Chiba Cancer Center (I. Honda), Dokkyo Medical College (S. Shida), Ehime University (N. Ogawa, Controller), Eiju General Hospital (S. Takanosu), Haga Red Cross Hospital (T. Ogiwara), Hino City General Hospital (A. Matsudo), Hiratsuka City Hospital (A. Aoki), Isezaki Municipal Hospital (T. Matsuzawa), Kawasaki Municipal Hospital (T. Nouga), Keio University (O. Abe, Co-chairman, M. Kitajima, and T. Kubota), Kitasato Institute Hos-

pital (E. Kawamura, Co-chairman), Kitasato Institute Medical Center Hospital (S. Ueno), Kitasato University East Hospital (Y. Hiki), Kyorin University (M. Kitajima), Kyoundo Hospital (T. Saito), Maki Hospital (M. Muratani), Metropolitan Komagome Hospital (S. Kitamura), Metropolitan Red Cross Blood Center (I. Nakao, Co-chairman), Mito Corporated Hospital (K. Ishioka), Mito Red Cross Hospital (M. Sakuma), Motojima General Hospital (S. Yamada), National Saitama Hospital (Y. Ushijima), National Mito Hospital (A. Murakami), National Tochigi Hospital (T. Hashimoto and M. Miyakita), Nihon Medical College (H. Niitani and M. Onda), Nihon University (T. Tanaka), Otawara Red Cross Hospital (T. Amemiya), Saitama Cancer Center (Y. Suda), Self-Defense Medical College (T. Ogata), Shimizu City General Hospital (A. Kosaka), Showa University Toyosu Hospital (M. Kurihara), Social Insurance Saitama Chuo Hospital (H. Katai), Suifu Hospital (A. Konoe), Teikyo University (J. Shikata and S. Kodaira), Teikyo University Mizonokuchi Hospital (H. Furue), Tochigi Cancer Center (Y. Ogata), Tokai University (T. Mitomi), Tohoku University (N. Sasano, Chairman), Tohoku University Kosankinbyo Research Institute Hospital (A. Wakui), Tokyo Hospital Matsuhidai (T. Kubota), Tokyo Jikeikai Medical College (K. Sakurai and T. Aoki), Tokyo Medical College (K. Kimura), Tokyo Medical and Dental University (M. Endo), Tokyo Women's Medical College (H. Demura and H. Suzuki), and Urawa City Hospital (Y. Tokura).

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