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# Role of Collagen Gel Droplet-Embedded Culture-Drug Sensitivity Testing (CD-DST) for Assessing the Sensitivity of Gastric Cancer to Chemotherapy Drugs Combined with Other Cancer Therapeutic Drugs

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**Background:** Chemosensitivity tests have long been a widely discussed research topic. Our group performed collagen gel droplet-embedded culture-drug sensitivity testing (CD-DST) of patients with advanced gastric cancer during the period from December 2012 to December 2017. To verify how CD-DST should be used, we invested correlations of sensitivities to cisplatin (CDDP), docetaxel (DOC), paclitaxel (PTX), and CPT11 with clinical outcome.

**Methods:** Patients with advanced gastric cancer underwent gastrectomy with lymph node dissection at Nippon Medical School Tama Nagayama Hospital, and surgical samples were retrospectively examined by CD-DST to assess chemosensitivity. The patients later received adjuvant chemotherapy as standard adjuvant therapy or chemotherapy. The CD-DST test was not performed for S-1 because it is commonly used in chemotherapy for gastric cancer. Although oxaliplatin has also recently become a key drug for advanced gastric cancer, it had not been adopted for gastric cancer in 2012, so CD-DST testing was not performed. The $\chi^2$ test was used for all statistical analyses. A p-value of $<0.05$ was assumed to indicate statistical significance. Three-year survival rates were estimated using the Kaplan-Meier method, and the log-rank test was used to compare the obtained curves.

**Results:** Of the tumors from gastric cancer patients, 67.0% (77/115) could be cultured. The rate of sensitivity was 41.1% (30/73) for CDDP, 82.6% (57/69) for DOC, 82.8% (58/70) for PTX, and 49.2% (33/67) for CPT11. CDDP sensitivity and outcome were not correlated in patients who received CDDP. Sensitivities to CDDP, DOC, PTX, and CPT11 were not correlated with any patient characteristic. Patients with poorly differentiated adenocarcinoma tended to be sensitive to CDDP (P=0.051).

**Conclusions:** No difference between CDDP sensitivity or outcome was observed in patients receiving CDDP. The CD-DST showed a high sensitivity to DOC and PTX in the present patients.

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**Key words:** CD-DST, gastric cancer, chemotherapy

## Introduction

Individualized selection of appropriate drugs for patients is a key step in anticancer therapy. Several recently introduced anticancer agents, such as molecular targeted drugs like trastuzumab and ramucirumab, and immune checkpoint blockades such as nivolumab and pembrolizumab, offer greater hope for improved chemotherapy outcomes. The recent development of new anticancer drugs for treatment of gastric cancer (GC) is expected to improve therapeutic outcomes for GC. Personalized therapies guided by more selective chemosensitivity testing may lead to better outcomes than empirical therapy.

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The collagen gel droplet-embedded culture-drug sensitivity test (CD-DST) is likely to be an important component of tailored therapies integrating chemosensitivity testing[1].

The combination of cisplatin (CDDP) and 5-fluorouracil (5-FU) has been the standard first-line chemotherapy for GC in clinical practice and as a reference arm in phase III trials[2–4]. The same combination has also been widely used as a first-line adjuvant chemotherapy for GC postoperatively and as chemotherapy for unresectable or recurrent GC. S-1 is an important therapeutic agent for advanced GC[5] and a key drug in GC treatment. Some patients, however, require stronger antitumor effects, but platinum-containing agents like cisplatin and oxaliplatin have strong emetic effects[6–8]. While only cisplatin is potentially nephrotoxic, oxaliplatin induces sensory neuropathy in some cases. Careful consideration is therefore needed when selecting a drug to be combined with S-1. Although platinum-containing agents enhanced the effects of 5-FU and S-1 in clinical trials, these combinations can elicit adverse effects that preclude their use.

In a postoperative adjuvant therapy setting, docetaxel (DOC) was more effective as monotherapy[9], and in combination with S-1 or 5-FU plus CDDP plus docetaxel, than S-1 alone. A randomized phase III study (JACCRO GC-07) compared these regimens in patients with pathologic stage III gastric cancer. In addition to DOC, the *Guidelines for Diagnosis and Treatment of Carcinoma of the Stomach* (January 2018 edition, edited by the Japanese Gastric Cancer Society) lists paclitaxel (PTX) and irinotecan (CPT-11) as anticancer drugs for second- and third-line chemotherapies.

Sensitivity to clinical drugs varies widely in patients with tumors of similar histopathological grades. For this reason, clinicians have developed several in vitro drug sensitivity tests to individualize chemotherapy[10,11]. We retrospectively performed the CD-DST to guide treatment of advanced GC with the agents CDDP, DOC, PTX, and SN38 (the active metabolite of irinotecan [CPT-11]).

## Methods

### Ethical Statement

The study was approved by the institutional ethics board of Nippon Medical School, Tama Nagayama Hospital (approval ID number is 669), and written informed consent was obtained from all patients.

### Patients and Clinical Samples

We analyzed data from 115 patients with stage IB, II, III, or IV GC (curability A, B, or C) who underwent surgical treatment and lymph node dissection, and used samples resected from 73 patients (52 men and 21 women; age range, 39-89 years; median age, 72.2 years) who underwent surgery for advanced GC between January 2012 and March 2017. The patients' clinicopathological characteristics are summarized in **Table 1a**. Of the 29 patients who received CDDP, 9 survived and 20 died. Selected patient characteristics (sex, disease stage, treatment, and tumor histologic type) and sensitivity to CDDP are shown in **Table 2**.

### CD-DST Procedure

A viable portion of the tumor was identified immediately after tumor resection. The tumor was stored in culture medium at 4°C, and the CD-DST was promptly started. All CD-DST assays to evaluate sensitivities to CDDP, DOC, PTX, and CPT11 were performed at LSI Medience Corporation. Testing was performed according to the CD-DST method reported by Kobayashi et al.[12,13], the method's inventor, using a human tumor cell primary culture system (Primaster; Kurabo Industries Ltd., Osaka, Japan).

Thinly sliced sections from a portion of each tumor sample were treated with a dispersed enzyme cocktail EZ (Primaster Reagent; Kurabo Industries, Osaka, Japan) to obtain cell suspensions. The suspensions were then transferred into collagen-coated flasks (CG Flasks; Kurabo Industries) and cultured overnight in PCM-1 preculture medium (Primaster Content) containing 10% fetal bovine serum at 37°C in 5% $CO_2$. The collagen gel was then dissolved with 0.05% EZ to obtain viable cancer cells. Type I collagen (Cellmatrix Type CD; Kurabo Industries Ltd. Osaka, Japan), 10× concentrated F-12 medium, and reconstitution buffer were mixed into an ice water bath at a ratio of 8:1:1 (Primaster content). Each cancer cell suspension was added to the collagen solution at a final density of $1 \times 10^5$ cells/mL. Tumor cells in the collagen gel droplets were then exposed to the anticancer agents at concentrations corresponding to the area under the curve (AUC) for drug concentration and time. Three drops of the collagen-cell mixture (30 µL/droplet) were transferred into each well of a 6-well plate on ice and allowed to gel in a $CO_2$ incubator at 37 ˚C to obtain a final cell concentration of approximately $3 \times 10^3$ cells per droplet. Dulbecco's modified Eagle's medium and F-12 medium (Gibco) containing 10% fetal bovine serum were dispensed over each well 1 h later, and the plates were incubated overnight at 37°C.

CDDP, DOC, PTX, and CPT-11 were added at final concentrations of 0.2 µg/mL, 0.1 µg/mL, 0.1 µg/mL, and 0.03 µg/mL and incubated for 24 h. The AUCs of the

Table 1 Drug sensitivity and characteristics

a. CDDP sensitivity and characteristics

| | | negative n=43 | | positive n=30 | | p |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Sex | Female | 10 | 47.6 | 11 | 52.4 | 0.213 |
| | Male | 33 | 63.5 | 19 | 36.5 | |
| Stage | 1 | 3 | 42.9 | 4 | 57.1 | 0.802 |
| | 2 | 11 | 61.1 | 7 | 38.9 | |
| | 3 | 20 | 62.5 | 12 | 37.5 | |
| | 4 | 9 | 56.3 | 7 | 43.8 | |
| Alive 0, dead 1 | 0 | 14 | 60.9 | 9 | 39.1 | 0.914 |
| | 1 | 22 | 59.5 | 15 | 40.5 | |
| papillary | | 1 | 50 | 1 | 50 | 0.656 |
| tubular | | 26 | 56.5 | 20 | 43.5 | 0.589 |
| poorly differentiated | | 28 | 53.8 | 24 | 46.2 | 0.167 |
| signet ring cell ca | | 6 | 50 | 6 | 50 | 0.493 |
| mucinous | | 7 | 70 | 3 | 30 | 0.443 |

CDDP: cisplatin

b. DOC sensitivity and characteristics

| | | negative n=12 | | positive n=57 | | p |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Sex | Female | 1 | 5.6 | 17 | 94.4 | 0.123 |
| | Male | 11 | 21.6 | 40 | 78.4 | |
| Stage | 1 | 3 | 42.9 | 4 | 57.1 | 0.284 |
| | 2 | 2 | 11.8 | 15 | 88.2 | |
| | 3 | 5 | 17.2 | 24 | 82.8 | |
| | 4 | 2 | 12.5 | 14 | 87.5 | |
| Alive 0, dead 1 | 0 | 5 | 26.3 | 14 | 73.7 | 0.236 |
| | 1 | 5 | 13.5 | 32 | 86.5 | |
| papillary | | 0 | 0 | 2 | 100 | 0.680 |
| tubular | | 6 | 14 | 37 | 86 | 0.333 |
| poorly differentiated | | 8 | 16.3 | 41 | 83.7 | 0.715 |
| signet ring cell ca | | 2 | 18.2 | 9 | 81.8 | 0.94 |
| mucinous | | 1 | 11.1 | 8 | 88.9 | 0.594 |

DOC: docetaxel

c. PTX sensitivity and characteristics

| | | negative n=12 | | positive n=58 | | p |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Sex | Female | 3 | 16.7 | 15 | 83.3 | 0.950 |
| | Male | 9 | 17.3 | 43 | 82.7 | |
| Stage | 1 | 3 | 42.9 | 4 | 57.1 | 0.150 |
| | 2 | 2 | 11.1 | 16 | 88.9 | |
| | 3 | 6 | 20.7 | 23 | 79.3 | |
| | 4 | 1 | 6.3 | 15 | 93.8 | |
| Alive 0, dead 1 | 0 | 4 | 19 | 17 | 81 | 0.971 |
| | 1 | 7 | 19.4 | 29 | 80.6 | |
| papillary | | 0 | 0 | 2 | 100 | 0.684 |
| tubular | | 6 | 13.3 | 39 | 86.7 | 0.257 |
| poorly differentiated | | 7 | 14.3 | 42 | 85.7 | 0.333 |
| signet ring cell ca | | 2 | 20 | 8 | 80 | 0.796 |
| mucinous | | 1 | 11.1 | 8 | 88.9 | 0.607 |

PTX: paclitaxel

Table 1 Drug sensitivity and characteristics (continued)

d. CPT-11 sensitivity and characteristics

| | | negative n=34 | | positive n=33 | | p |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Sex | Female | 8 | 47.1 | 9 | 52.9 | 0.725 |
| | Male | 26 | 52 | 24 | 48 | |
| Stage | 1 | 3 | 42.9 | 4 | 57.1 | 0.711 |
| | 2 | 10 | 62.5 | 6 | 37.5 | |
| | 3 | 14 | 50 | 14 | 50 | |
| | 4 | 7 | 43.8 | 9 | 56.3 | |
| Alive 0, dead 1 | 0 | 12 | 63.2 | 7 | 36.8 | 0.460 |
| | 1 | 19 | 52.8 | 17 | 47.2 | |
| papillary | | 0 | 0 | 2 | 100 | 0.239 |
| tubular | | 21 | 50 | 21 | 50 | 0.874 |
| poorly differentiated | | 25 | 53.2 | 22 | 46.8 | 0.539 |
| signet ring cell ca | | 6 | 60 | 4 | 40 | 0.526 |
| mucinous | | 4 | 44.4 | 5 | 55.6 | 0.480 |

CPT-11: irinotecan

drugs at the selected concentrations cultured in the culture medium were the same as those observed in serum during the 24-h period after the drugs were administered intravenously at the standard clinical doses. The medium containing the anticancer drugs was then removed from each well, and the wells were rinsed twice with Hanks' balanced saline solution (3 mL) and filled with 4 mL of PCM-2 medium (Nitta Gelatin Inc.). After another 7 days of incubation, a neutral red solution (50 μg/mL) was added to each well for 2 h to stain the colonies in the gel droplets. Each droplet was fixed with 10% neutral buffered formalin, rinsed with water, air dried, and quantified by optical density image analysis by using a Primage System (Solution Systems, Tokyo, Japan). Control samples with optical densities of greater than 3.0 were used for the evaluation. In vitro sensitivity was expressed as the ratio between T, the optical density of the treated samples, and C, the optical density of the controls. The cutoff values of tumor cell inhibition rates were set at 40% for CDDP and 50% for the other drugs. These values corresponded to the percentages of chemo-sensitive patients in the current chemical trial and were close to the clinical response rates to the drugs overall.

**Adjuvant Chemotherapy**

The chemotherapy regimens are detailed in **Table 3**.

**Clinical Assessments of First-line Adjuvant Chemotherapy**

Out of 73 patients, 49 started adjuvant chemotherapies within approximately 6 weeks postoperatively. Patients at our center received S-1 chemotherapy for 1 year, in accordance with the following cycled schedule: 2 doses (40 mg per $m^2$ of body surface area) per day for 4 weeks, followed by 2 weeks of no chemotherapy for 2 weeks, followed by 1 week of no chemotherapy.

Patients were assigned to one of 3 dosage groups according to their body sizes, namely, 80 mg, 100 mg, and 120 mg daily for patients with body surface areas of 1.25 $m^2$ less, 1.25 $m^2$ or more but less than 1.5 $m^2$, and 1.5 $m^2$ or more, respectively. From 2016, patients with pathological stage II or worse disease received the following treatment postoperatively for 6 months: SP (S-1 for 3 weeks and 60 mg/$m^2$ CDDP on day 8, every 5 weeks) or SOX (80-120 mg/day S-1 for 2 weeks and 100 mg/$m^2$ oxaliplatin [OXP] on day 1, every 3 weeks). One patient received capecitabine instead of S-1. One patient with HER2-overexpressing GC responded well to treatment with trastuzumab, an anti-HER2 antibody.

Hematological findings and clinical symptoms were assessed every 3 or 6 weeks. Cancer relapse was determined based on imaging examinations by ultrasonography, computed tomography (CT), and endoscopy. At least one type of imaging exam, usually CT, was performed at 6-month intervals for up to 5 years after surgery, and esophago-gastro-duodenoscopy was performed at 1-year intervals over the same period.

**Second- and Third-line Chemotherapy**

Before the publication of the *Japanese Gastric Cancer Treatment Guidelines*, *5th edition*, patients who experienced relapse during or after first-line adjuvant chemotherapy (S-1 or SP and SOX) received DOC, PTX, and CPT11 as

Table 2 CDDP sensitivity and characteristics in survivors and nonsurvivors

| | | | CDDP sensitivity negative | | CDDP sensitivity positive | | |
|---|---|---|---|---|---|---|---|
| | | | n=16 | | n=13 | | |
| Age | Median (range) | | 72 (42-85) | | 68 (50-86) | | 0.329 |
| Sex | Female | | 5 | 31.3 | 7 | 53.8 | 0.219 |
| | Male | | 11 | 68.8 | 6 | 46.2 | |
| Alive 0, dead 1 | 0 | | 5 | 31.3 | 4 | 30.8 | 0.647 |
| | 1 | | 11 | 68.8 | 9 | 69.2 | |
| Stage | II | | 2 | 12.5 | 1 | 7.7 | 0.715 |
| | III | | 10 | 62.5 | 7 | 53.8 | |
| | IV | | 4 | 25 | 5 | 38.5 | |
| combined chemo agent | S1 | negative | 0 | 0 | 0 | 0 | NA |
| | | positive | 16 | 100 | 13 | 100 | |
| | capecitabine | negative | 15 | 93.8 | 13 | 100 | 0.552 |
| | | positive | 1 | 6.3 | 0 | 0 | |
| | DOC | negative | 12 | 75 | 10 | 76.9 | 0.626 |
| | | positive | 4 | 25 | 3 | 23.1 | |
| | PTX | negative | 15 | 93.8 | 11 | 84.6 | 0.420 |
| | | positive | 1 | 6.3 | 2 | 15.4 | |
| | nivolumab | negative | 16 | 100 | 12 | 92.3 | 0.448 |
| | | positive | 0 | 0 | 1 | 7.7 | |
| | trastuzumab | negative | 16 | 100 | 12 | 92.3 | 0.448 |
| | | positive | 0 | 0 | 1 | 7.7 | |
| histological type | papillary | negative | 15 | 93.8 | 12 | 92.3 | 0.704 |
| | | positive | 1 | 6.3 | 1 | 7.7 | |
| | tubular | negative | 6 | 37.5 | 7 | 53.8 | 0.379 |
| | | positive | 10 | 62.5 | 6 | 46.2 | |
| | poorly differentiated | negative | 8 | 50 | 2 | 15.4 | 0.051 |
| | | positive | 8 | 50 | 11 | 84.6 | |
| | signet ring cell ca | negative | 12 | 75 | 10 | 76.9 | 0.66 |
| | | positive | 4 | 25 | 3 | 23.1 | |
| | mucinous | negative | 12 | 75 | 12 | 92.3 | 0.236 |
| | | positive | 4 | 25 | 1 | 7.7 | |

CDDP: cisplatin, DOC: docetaxel, PTX: paclitaxel, CPT-11: irinotecan, Mann-Whitney U test

second- and third-line chemotherapies. After the RAINBOW[14] and REGARD studies[15] in 2014, patients with a relapse after first-line chemotherapy received ramucirumab with or without PTX as second-line chemotherapy. After the ATTRACTION-2 trial[16] in 2017, they received nivolumab as third-line chemotherapy.

**Statistical Analysis**

Data were analyzed with SPSS Statistics ver. 23 (IBM). Baseline characteristics and outcome data were compared between 2 groups with the $\chi^2$ test or Fisher exact test, for categorical variables, or with the Mann-Whitney U test, for continuous variables. A p-value of $<0.05$ was considered statistically significant. Three-year survival rates were estimated with the Kaplan-Meier method, and the log-rank test was used to compare the obtained curves.

## Results

**CD-DST Test**

Seventy-three of the 115 (67.0%) tumors in this study could be cultured.

The rate of sensitivity to the chemotherapy agents is summarized in **Table 4**. CD-DST could not be performed on 4, 3, and 6 tumors for DOC, PTX, and CPT11, respectively. The sensitivity rate was 41.1% (30/73) for CDDP, 82.6% (57/69) for DOC, 82.8% (58/70) for PTX, and 49.2% (33/67) for CPT11. Drug sensitivity was observed in 94.7% (54/57) of DOC-sensitive patients and 93.1% (54/58) of PTX-sensitive patients in common.

**Statistical Analysis**

The patients' clinicopathological characteristics and sensitivities to CDDP, DOC, PTX, and CPT11 are summarized in **Table 1**. Sensitivities to CDDP, DOC, PTX, and

Table 3 Drug regimens for gastric cancer patients

| Regimen/drugs | Dose | Schedule |
|---|---|---|
| SP | | every 5 weeks |
| S-1 | 40 mg/m$^2$ | 3 weeks |
| CDDP | 60 mg/m$^2$ | day 8 |
| SOX | | every 3 weeks |
| S-1 | 80-120 mg/day | 2 weeks |
| oxaliplatin (OXP) | 100 mg/m$^2$ | day 1 |
| XEROX | | every 3 weeks |
| capecitabine | 1,000 mg/m$^2$ | 2 weeks |
| oxaliplatin (OXP) | 130 mg/m$^2$ | day 1 |
| DOC | 60 mg/m$^2$ | every 3 weeks |
| S-1+DOC | | every 3 weeks |
| S-1 | 80 mg/m$^2$ | 2 weeks |
| DOC | 40 mg/m$^2$ | day 1 |
| Ramucirumab+ PTX | | every 4 weeks |
| Ramucirumab | 8 mg/kg | days 1, 15 |
| PTX | 80 mg/m$^2$ | days 1, 8, 15 |
| Trastuzumab+SOX | | every 3 weeks |
| Trastuzumab | 8 mg/kg (1$^{st}$),6 mg/kg (2$^{nd}$~) | 2 weeks |
| S-1 | 80 mg/m$^2$ | day 1 |
| oxaliplatin (OXP) | 100 mg/m$^2$ | day 1 |
| | | every 2 weeks |
| Nivolumab | 240 mg/body | day 1 |

CDDP: cisplatin, DOC: docetaxel, PTX: paclitaxel, CPT-11: irinotecan

Table 4 Rate of drug sensitivity of CD-DST

| Drug | CDDP | DOC | PTX | CPT-11 |
|---|---|---|---|---|
| Rate (%) | 41.1 | 82.6 | 82.8 | 49.2 |

CDDP: cisplatin, DOC: docetaxel, PTX: paclitaxel, CPT-11: irinotecan

CPT11 were not correlated with any patient characteristic (sex, disease stage, survival, and tumor histologic type). CDDP sensitivity was not correlated with patient outcome (**Table 2**). CDDP sensitivity and outcome were not correlated in patients receiving CDDP. Patients with poorly differentiated adenocarcinoma tended to exhibit sensitivity to CDDP (P=0.051) (**Table 2**).

Three-year OS after surgery was 50.0% in patients sensitive to CDDP and 46.7% in those not sensitive to CDDP (P = 0.995); the difference was not significant (**Fig. 1**). In the CDDP sensitivity group 3-year OS rate was 50.0% in the CDDP-treated group and 50.0% in the CDDP-untreated group (P = 0.835; **Fig. 2A**). Among patients not sensitive to CDDP, 3-year OS was 38.5% in the CDDP-treated group and 52.9% in the CDDP-untreated group (P = 0.825; **Fig. 2B**). Three-year OS did not significantly differ in relation to CDDP sensitivity in the CDDP-treated or CDDP-untreated group.

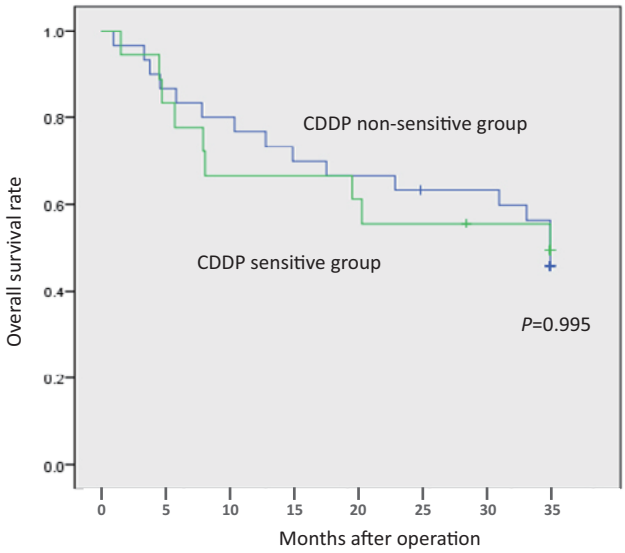


Fig. 1

## Discussion

Development of technologies that enable early diagnosis and advances in surgical methods and perioperative management has improved outcomes for GC patients. The disease prognosis remains poor, however, for pa-

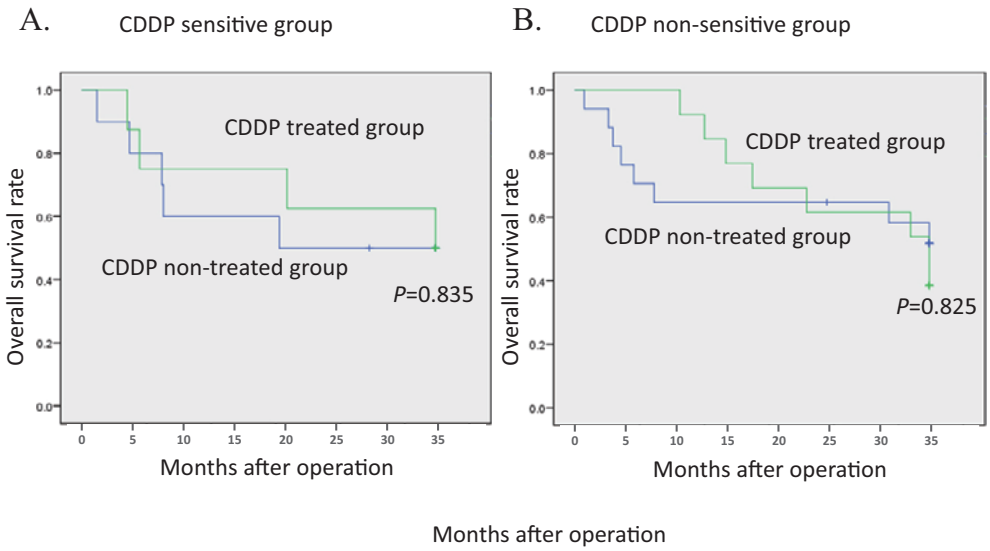


Fig. 2

tients with locally advanced, recurrent, or distant metastasis. DOC, PTX, and CPT11 are listed as cancer therapeutic drugs for second- and third-line adjuvant chemotherapy for GC. This study examined whether CDDP, DOC, PTX, and CPT11 sensitivity, as determined by CD-DST, correlated with clinical outcome in patients with GC.

**Availability of CD-DST**

CD-DST is a three-dimensional culture system used to test the chemosensitivity of isolated tumor cells embedded in collagen droplets. The system has several advantages over MTT[17] and ATP[18] assays and other conventional methods. The effects of anticancer drugs can be evaluated at physiological concentrations in very small samples. With help from an image analysis system, the test eliminates the masking phenomenon that occurs when fibroblasts contaminate the culture.

Naitoh et al. hypothesized that patients would be less responsive to randomly selected anticancer drugs than to anticancer drugs to which they were expected to be sensitive. To test that hypothesis, they performed a nonrandomized analysis comparing patients allocated to personalized anticancer drugs that were identified in advance by CD-DST[19]. The participants were divided into 2 groups: those with GC sensitive to S-1, DOC, or CPT11 (T/C ratio <60%) on a CD-DST and those resistant to all 3 agents. In the former group, the 1-year survival rate was significantly higher (P = 0.019), and time to progression was significantly longer (P = 0.023). Evaluation of chemosensitivity by CD-DST appeared to reliably predict outcomes in patients receiving chemotherapy for advanced GC. Maejima et al. reported a correlation between outcomes of patients receiving S-1 postoperatively and CD-DST test results for 5-FU and 5-chloro-2, 4-dihydroxypyridine (CDHP)[20].

**Regimens Including S-1, 5-FU and CDDP, OXP**

S-1 is an important therapeutic agent among 5-FU drugs for advanced GC[4] and as adjuvant chemotherapy for GC postoperatively[21,22]. However, some patients need more powerful antitumor effects. The SPIRITS trial in 2008 found that adding cisplatin to S-1 increased overall (OS) and progression-free survival, as compared with S-1 alone. The most commonly used regimen globally, both in clinical practice and as a reference arm in phase III trials, is the combination of CDDP and 5-FU[1–3]. Consequently, this combination has been the chemotherapy standard for GC, regardless of CDDP sensitivity.

Although OXP, like cisplatin, contains platinum, it has no nephrotoxic effects and may be less toxic than cisplatin. Along with 5FU and leucovorin or S-1, OXP was reported to be effective against colorectal cancer[23–25] and advanced GC. These results were expanded and confirmed in a phase III study comparing SOX with SP as a first-line chemotherapy for advanced GC. In CD-DST studies of surgical CD samples, addition of 5-FU to oxaliplatin showed an enhanced synergistic effect, although this effect only appeared in vitro and was unlikely to appear in GC patients sensitive to 5-FU[26].

**Regimens Including DOC or PTX for GC Listed in the Japanese Gastric Cancer Treatment Guidelines**

Yoshida et al. reported that docetaxel added to S-1 was a safe and relatively effective combination therapy for patients with stage III GC[27]. The addition of docetaxel to S-1 resulted in a significant clinical benefit. On this basis,

the combination of docetaxel and S-1 can be recommended as a standard postoperative adjuvant chemotherapy for stage III GC. This regimen is conditionally recommended as first-line GC drug therapy in the 6th edition of the *Japanese Gastric Cancer Treatment Guidelines*[28]. In the REGARD trial[15] and RAINBOW trial[14], ramucirumab, anti-vascular endothelial growth factor 2 receptor antibody, and adding PTX improved survival and has been recommended as second-line GC drug therapy in the 5th and 6th editions of the *Japanese Gastric Cancer Treatment Guidelines*[28].

**Molecular Targeted Drugs and Immune Checkpoint Blockade for GC**

Molecular targeted drugs have drawn attention in chemotherapy for GC. The HER2 oncogene is amplified and the HER2 protein is overexpressed in 17-20% of GC patients[29,30]. In the TOGA randomized controlled trial[31], trastuzumab plus CDDP and capecitabine or 5FU chemotherapy resulted in better median overall survival than did chemotherapy alone. On the basis of findings reported in the ATTRACTION-2 trial and KEYNOTE 059 trial[32], the 5 th and 6th editions of the *Japanese Gastric Cancer Treatment Guidelines*[28] also list an immune checkpoint blockade, anti-PD-1 monoclonal antibody nivolumab, and pembrolizumab as monotherapy for patients with GC. A chemotherapy agent in combination with these new drugs is considered appropriate therapy for GC.

**Poorly Differentiated GC and Chemosensitivity**

Poorly differentiated GC cell lines were most sensitive to oxaliplatin[33]; however, no clinical trial has examined the effect of oxaliplatin on tumor differentiation[34–36].

**Discussion of the Present Results**

The proportion of cases deemed evaluable by CD-DST in this study was lower (67.0%) than in previous reports (approximately 80%), perhaps because of the sample storage conditions, as some samples were stored for an extended period in a hospital refrigerator.

In this study, the drug sensitivity of GC to PTX was similar to that to DOC, which was greater than the sensitivity to CDDP (**Table 4**). The drug sensitivity of GC to DOC and PTX in the present study was about 80%, markedly higher than that to CDDP (41.1%) (**Table 4**). In vitro CD-DST showed sensitivity to DOC or PTX for GC, suggesting that chemotherapy regimens including DOC or PTX may be useful for GC; 94.7% (54/57) of DOC-sensitive patients and 93.1% (54/58) of PTX-sensitive patients were sensitive to both drugs. Sensitivity to these drugs should be analyzed individually.

Patients with GC should initially undergo a CD-DST to aid in the selection of a therapeutic regimen. Since CD-DST can reveal high rates of sensitivity to DOC and PTX in GC, a regimen including DOC or PTX should be administered for GCs that show DOC or PTX sensitivity in the CD-DST results. The CD-DST test should first be carried out for patients with stage III GC, and S-1 and DOC should be selected as the first adjuvant chemotherapy for tumors found to be sensitive to DOC. The CD-DST test should also be first carried out for patients with advanced GC, and PTX and ramucirumab should be selected as the first adjuvant chemotherapy for tumors found to be sensitive to PTX.

In this study, treatment regimen was not determined on the basis of CD-DST results. Consequently, a difference between CDDP sensitivity and outcome in the patients administered CDDP in this study was not observed. The synergistic effect between 5-FU and CDDP, like oxaliplatin, would appear for GC. Clinically, S1 plus CDDP chemotherapy had a synergistic effect for GC, thus suggesting that CD-DST may be useful in clinical practice. The present patients with poorly differentiated adenocarcinoma tended to show sensitivity to CDDP (P= 0.051) (**Table 1**). An analysis of additional tumors, or a meta-analysis, would be helpful.

The drug sensitivity of GC to CPT11 was 49.2% in the present study, which again was higher than that to CDDP (41.1%) (**Table 1**). We recommend selecting CPT11 as a second- or third-line chemotherapy for tumors sensitive to CPT11.

## Limitations

This study was limited the fact that it retrospectively examined CD-DST for selecting postoperative adjuvant chemotherapy drugs for patients with GC at a single center (Nippon Medical School Tama Nagayama Hospital).

## Conclusions

CDDP sensitivity was not correlated with outcome in patients receiving CDDP. Although use of S-1 and CDDP may have had a synergistic effect against tumors clinically, no such effect was observed in vitro.

## Informed Consent

Informed consent was obtained directly from study participants or on the withdrawal form posted on the website. Persons who did not provide consent were excluded.

The study protocol conformed with the ethical guidelines es-

tablished by the Declaration of Helsinki and was approved by the institutional review board of Nippon Medical School.

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**Conflict of Interest:** None.

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