The role of the collagen gel droplet embedded culture-drug sensitivity test (CD-DST) to assess the sensitivity to chemo drugs for gastric cancer in combination with other cancer therapeutic drugs

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Running Title
The role of CD-DST for gastric cancer
Summary

Background: Chemosensitivity tests have been a widely discussed research topic for many years. Our group performed the collagen gel droplet embedded culture-drug sensitivity test (CD-DST) in patients with advanced gastric cancer over the period from December 2012 to December 2017. By considering how the sensitivities to cisplatin (CDDP), docetaxel (DOC), paclitaxel (PTX), and CPT11 correlated with the clinical outcome, we sought mainly to verify how the CD-DST should be used.

Methods: Patients with advanced gastric cancer underwent gastrectomy with lymph node dissection, and the surgical samples were retrospectively examined by the CD-DST to assess chemosensitivity in Nippon Medical School Tama Nagayama hospital. The patients went on to receive postoperative adjuvant chemotherapies either as standard adjuvant therapies or chemotherapies. CD-DST test was not performed for S1 because S-1 is a key drug commonly used in chemotherapy for gastric cancer. While oxaliplatin has also become a key drug for advanced gastric cancer recently, it was still not adopted for gastric cancer in 2012 so CD-DST test was not performed.

The χ² test was used for all statistical analyses. A p-value of <0.05 was assumed to indicate statistical significance. 3-year survival rates were estimated using the Kaplan-Meier method, and the log-rank test was used to compare the obtained curves.

Results: Seventy-seven percent (77/115) of the tumors derived from gastric cancer patients could be cultured in this study. The rate of sensitivity was 41% (30/73) for CDDP, 83% (57/69) for DOC, 83% (58/70) for PTX, and 49% (33/67) for CPT11. No correlation between CDDP sensitivity and outcome was observed in patients who underwent the CDDP. Likewise, the sensitivities to CDDP, DOC, PTX, and CPT11 were not found to be correlated with the various patient characteristics. Patients with poorly differentiated adenocarcinoma tended to be sensitive to the CDDP (P=0.051).

Conclusions: No difference between CDDP sensitivity or outcome was observed in the patients administered CDDP. The CD-DST demonstrated a high rate of sensitivity to DOC and PTX in the patients studied.
Introduction

The tailored selection of appropriate drugs for individual patients is a key step in anticancer therapy. Several anticancer agents recently introduced, such as molecular target drugs like trastuzumab and ramucirumab, and immune checkpoint blockades like nivolumab and pembrolizumab, offer better hope for improved chemotherapy outcomes. With the development of various new anticancer drugs for the treatment of gastric cancer (GC) in recent years, the wider selection of drugs now available is expected to improve the therapeutic outcomes of the disease.

Personalized therapies guided by more selective chemosensitivity testing can be hypothesized to lead to better outcomes than the empirical therapy. The collagen gel droplet embedded culture-drug sensitivity test (CD-DST) is likely to be an important component of tailored therapies integrating chemosensitivity testing.

The combination of cisplatin (CDDP) and 5-fluorouracil (5-FU) has been the commonly applied regimen as a first-line chemotherapy for GC both in clinical practice and as a reference arm in phase III trials. The same combination has also been widely applied as a first-line adjuvant chemotherapy for GC postoperatively, and as a chemotherapy against unresectable or recurrent GC. S-1 is an important therapeutic agent for advanced GC and an important key drug in GC treatment. Some patients, however, require stronger antitumor effects. Platinum-containing agents like cisplatin and oxaliplatin, moreover, confer strong emetic effects. While only cisplatin is potentially nephrotoxic, oxaliplatin does induce sensory neuropathy in some cases.

Careful deliberation is therefore needed when selecting a drug to be added in combination with S-1. While platinum-containing agents have been shown to enhance 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) has been found to be effective both as a monotherapy and in combination with S-1 or 5-FU plus CDDP plus docetaxel, compared to S-1 alone. These regimens were compared in patients with pathologic stage III gastric cancer in a randomized phase III study by Yoshida et al. (JACCRO GC-07). 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) list paclitaxel (PTX) and irinotecan (CPT-11) as anticancer drugs in second- and third-line chemotherapies.

The sensitivity to clinical drugs can vary widely in individuals diagnosed with tumors of similar histopathological grades. For this reason, clinicians have developed several in vitro drug sensitivity tests to tailor chemotherapies for individual cancer patients.

To investigate this prospect further in this study, we performed retrospectively the CD-DST to guide the 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 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

This study targeted 115 patients who were diagnosed with stage IB, II, III, or IV gastric cancer classified as curability A, B or C and underwent surgical treatment and lymph node dissection.

Samples resected from 73 patients, (52 males and 21 females; age range, 39-89 years, median age of 72.2 years) who underwent surgery for advanced GC between January 2012 and March 2017 were used. The patients’ clinicopathological characteristics are summarized in Table 1a.

Of the 29 patients who received CDDP, 9 survived and 20 died. Patient sensitivity to CDDP and various patient characteristics (sex, disease stage, administered medicine, and tumor histologic type) 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 a culture medium at 4°C, and the CD-DST was promptly commenced.

All of the CD-DST assays to evaluate sensitivities to CDDP, DOC, PTX, and CPT11 were performed at LSI Medience Corporation. The evaluations were 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 a PCM-1 pre-culture medium (Primaster® Content) containing 10% fetal bovine serum at 37°C in 5% CO2. 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), a 10× concentrated F-12 medium, and a 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 1×10^5 cells/ml. The 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 CO2 incubator at 37°C to obtain a final cell concentration of about 3×10^3 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 hour 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, 0.1μg/ml, 0.1μg/ml, and 0.03μg/ml and incubated for 24 hours. The AUCs of the drugs at the selected concentrations
cultured in the culture medium were the same as the AUCs observed in serum during the 24 hr 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 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 table of the chemotherapy regimen is shown in Table 3.

**Clinical assessments in first line adjuvant chemotherapy**

Out of 73 patients, 49 started adjuvant chemotherapies within approximately six postoperative weeks. Patients at this hospital were administered S-1 chemotherapy for 1 year according to the following cycled schedule: two doses (40 mg per m² of body-surface area) per day for 4 weeks, followed by 2 weeks of no chemotherapy or per day for 2 weeks, followed by 1 week of no chemotherapy.

The patients were divided into three dosage groups according to their body sizes: 80 mg, 100 mg, and 120 mg daily for patients with body surface areas of >1.25 m², 1.25 m² or more but less than 1.5 m², and 1.5 m² or more, respectively.

From 2016 onward, patients with pathological stage II or higher received the following treatment postoperatively for 6 months: SP (S-1 for 3 weeks and 60 mg/m² CDDP on day 8, every 5 weeks) or SOX (80-120 mg/day S-1 for 2 weeks and 100 mg/m² oxaliplatin (OXP) on day 1, every 3 weeks). One patient was administered capecitabine in place 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 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 in
relapse during or after the first-line adjuvant chemotherapy (S-1 or SP and SOX) received DOC, PTX, and CPT11 as second- and third-line chemotherapies. After the RAINBOW test\textsuperscript{14} and REGARD test\textsuperscript{15} in 2014, patients in relapse after the first-line chemotherapy received ramucirumab with or without PTX as a second-line chemotherapy. After the ATTRACTION-2 trial\textsuperscript{16} in 2017, they received nivolumab as a third-line chemotherapy.

**Statistical analysis**

Data were analyzed using SPSS Statistics ver. 23 (IBM). Baseline characteristics and outcome data between two groups were compared using the chi-squared test or Fisher’s exact test for categorical variables and the Mann-Whitney U test for continuous variables. A p-value of <0.05 was considered statistically significant.

3-year survival rates were estimated using the Kaplan-Meier method, and the log-rank test was used to compare the obtained curves.
Results
CD-DST test
Seventy-three of 115 (67%) tumors in this study could be cultured.
The rate of sensitivity to the chemo agents is summarized in Table 4.
Seventy-seven percent (77/115) of the tumors could be cultured. CD-DST could not be performed on 4, 3, and 6 of the tumors for DOC, PTX, and CPT11, respectively. The rates of sensitivity were 41.1% (30/73) for CDDP, 78.1% (57/73) for DOC, 79.5% (58/73) for PTX, and 45.2% (33/73) for CPT11. Drug sensitivity was observed in 94% (54/57) of the DOC-sensitive and 93% (54/58) of the PTX-sensitive patients in common.

Statistical analysis
The patients’ clinicopathological characteristics and sensitivities to the drugs CDDP, DOC, PTX, and CPT11 are summarized in Table 1. a, b, c, d. The CDDP, DOC, PTX, and CPT11 sensitivities showed no correlations with the patient characteristics (sex, disease stage, survival, and tumor histologic type) (Table 1. a, b, c, d.).
No correlation between CDDP sensitivity and patient outcome was observed in the patients. (Table. 2)
No correlation between CDDP sensitivity and outcome was observed in patients administered CDDP. Patients with poorly differentiated adenocarcinoma tended to show sensitivity to CDDP. (P=0.051) (Table 2)
3-year OS rate from operation was 50% in the CDDP sensitive group and 46.7% in the non-sensitive group (P=0.995).
No difference in the 3-year OS rates was observed between the CDDP sensitive group and the non-sensitive group. (Fig.1)
In the CDDP sensitivity group 3-year OS rate was 50% in the CDDP treated group and 50% in the non-treated group (P=0.835; Fig.2A). In the CDDP non-sensitive group, 3-year OS rate was 38.5% in the CDDP treated group and 52.9% in the non-treated group (P=0.825; Fig.2B) as well.
No difference in the 3-year OS rates within the CDDP sensitive group and non-sensitive group was observed between CDDP treated group and non-treated group.
Discussion

The development of technologies that enable early diagnosis and advances in surgical methods and perioperative management have improved the prognosis for GC patients. The prognosis of the disease remains extremely poor, however, in cases with locally advanced, recurrent, or distant metastasis.

DOC, PTX and CPT11 are also listed as cancer therapeutic drugs in 2nd and 3rd line adjuvant chemotherapy for GC. The aim of this study is whether CDDP, DOC, PTX and CPT11 sensitivity by investigating CD-DST in patients with GC correlates with clinical outcome or not.

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 and ATP assays and other conventional methods. The effects of anticancer drugs can be evaluated at physiological concentrations using 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. speculated that patients would be less responsive to randomly selected anticancer drugs than to anticancer drugs to which they were expected to be sensitive. Working on that hypothesis, they performed a nonrandomized analysis comparing patients allocated to personalized anticancer drugs identified in advance by CD-DST. The subjects were divided into two groups: patients showing GC sensitivity to S-1, DOC, or CPT11 (T/C ratio<60%) in the CD-DST and patients showing resistance to all three of the agents. The 1-year survival rate was significantly higher in the sensitive group than in the resistant group (P = 0.019), whereas the time to progression (TTP) was significantly longer in the sensitive group than in the resistant group (P = 0.023). The evaluation of chemosensitivity by the CD-DST appeared to reliably predict the outcome in patients receiving chemotherapy for advanced GC.

According to a study by Maejima et al., a correlation was observed between the outcome of patients receiving S-1 postoperatively and the CD-DST test results for 5-FU and 5-chloro-2, 4-dihydroxypyridine (CDHP).

Regimens including S-1, 5Fu and CDDP, OXP

S-1 is an important therapeutic agent among 5-FU for advanced GC, and as adjuvant chemotherapy for GC postoperatively. However, some patients need more powerful antitumor effects. According to the SPIRITS trial in 2008, adding cisplatin to S-1 led to longer overall (OS) and progression-free (PFS) survival compared to S-1 alone. The most commonly used regimen globally, both in the clinical practice and as a reference arm in phase III trials, is a combination of CDDP and 5-FU. Consequently, a combination of CDDP and 5-FU has been first line standard chemotherapy for GC regardless of CDDP sensitivity.

While 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 has been reported to be effective against
colorectal cancer\textsuperscript{23-25} and advanced GC. These results have been expanded and confirmed in a phase III study comparing SOX with SP as a first-line chemotherapy for advanced GC.

In CD-DST studies on surgical CD samples by Kanazawa et al., the addition of 5-FU to oxaliplatin showed an enhanced synergistic effect, albeit with two limitations: (i) the effect only appeared in vitro; (ii) the effect was unlikely to appear in GC patients sensitive to 5-FU\textsuperscript{26}.

**Regimens including DOC or PTX for GC listed in Japanese Gastric Cancer Treatment Guidelines**

Yoshida et al. reported that docetaxel added to S-1 is a safe and relatively effective combination therapy for patients with stage III gastric cancer\textsuperscript{27}. The addition of docetaxel to S-1 in their study brought about 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 gastric cancer. This regimen has been conditionally recommended as first-line GC drug therapy in the 6th edition of the Japanese Gastric Cancer Treatment Guidelines\textsuperscript{28}.

In the REGARD trial\textsuperscript{15} and RAINBOW trial,\textsuperscript{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 edition of the Japanese Gastric Cancer Treatment Guidelines\textsuperscript{28}.

**Molecular targeted drugs and immune checkpoint blockade for GC**

Molecular targeted drugs have recently drawn attention in chemotherapy for GC. The HER2 oncogene is amplified and the HER2 protein is overexpressed in 17–20% of GC patients\textsuperscript{29,30}. In the randomized controlled TOGA trial\textsuperscript{31}, trastuzumab plus CDDP and capecitabine or 5FU chemotherapy demonstrated a better median overall survival than patients receiving chemotherapy alone.

The 5th and 6th edition of the Japanese Gastric Cancer Treatment Guidelines\textsuperscript{28} also lists an immune checkpoint blockade, anti-PD-1 monoclonal antibody nivolumab and pembrolizumab as a monotherapy for patients with GCs based on the ATTRACTION-2 trial and the KEYNOTE 059 trial\textsuperscript{32}.

A chemotherapy agent in combination with these new drugs is considered appropriate therapy for GC.

**Poorly differentiated GC and chemo-sensitivity**

Poorly differentiated GC cell lines have been found to be the most sensitive to oxaliplatin, and to our knowledge by Eriguchi et al.\textsuperscript{33}, none of the clinical trials performed have sought to determine the effect of oxaliplatin on tumor differentiation\textsuperscript{34-36}.

**Discussion about results in this study**

The proportion of cases deemed to be evaluable by the CD-DST in this study was relatively low (67%) compared to the previous reports (approximately 80%). The low evaluable rate may be attributable to the sample storage conditions, as some of the samples were stored for long periods in the hospital refrigerator.

In this study the drug sensitivity of GC to PTX in the present study was almost the same as that to
DOC, which again was greater than the sensitivity to CDDP. (Table 4) The drug sensitivity of GC to both DOC and PTX in the present study was about 80%, markedly higher than that to CDDP (41%). (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. Ninety-four percent (54/57) of the DOC-sensitive patients and 93% (54/58) of the PTX-sensitive patients were sensitive to both drugs. The sensitivity to these drugs should be analyzed individually.

Patients with GC should initially undergo the 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 which show DOC or PTX sensitivity in the CD-DST results. The CD-DST test should first be carried out for patients with stage III gastric cancer, 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 based on 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 to GC. Clinically, S1 plus CDDP chemotherapy had a synergistic effect for GC, thus suggesting that CD-DST may be useful in clinical practice.

Patients with poorly differentiated adenocarcinoma in the present study tended to show sensitivity to CDDP (P=0.051). (Table 1) An analysis of more tumors, or a meta-analysis would be helpful.

The drug sensitivity of GC to CPT11 was 49% in the present study, which again was higher than that to CDDP (45%). (Table 1) The authors recommend selecting CPT11 as a second- or third-line chemotherapy for tumors sensitive to CPT11.

**Limitation**

In the present study CD-DST for choosing chemotherapy drug of postoperative adjuvant chemotherapy in patients with GC on a single institution was retrospectively examined. The present study had a retrospective design in Nippon Medical School Tama Nagayama hospital and had several limitations.

**Conclusion**

No correlation between CDDP sensitivity or outcome was observed in the patients receiving CDDP. While the use of S1 and CDDP may have had a synergistic effect against tumors clinically, no such effect was observed in vitro.

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We would like to express our appreciation to LSI Medience Corporation in Japan for performing the CD-DST analyses for gastric cancer patients. The authors declare that no conflicts of interest
occurred during the analyses.

Footnote

Informed Consent

Informed consent was obtained from individual participants included in the study or in the withdrawal form posted on the website. Persons who failed to provide consent were excluded. The study protocol conformed with the ethical guidelines established by the Declaration of Helsinki and was approved by the institutional review board of Nippon Medical School.

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Figure 1

Overall survival rate vs. Months after operation for CDDP sensitive and non-sensitive groups. The survival curves show a significant difference between the two groups, with a P-value of 0.995.
**Figure.2**

**A.** CDDP sensitive group

- CDDP treated group
- CDDP non-treated group

*P* = 0.835

**B.** CDDP non-sensitive group

- CDDP treated group
- CDDP non-treated group

*P* = 0.825
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       | 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       | 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 |
| mucious | 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 |       |
| alive0, dead1 |     |    |     |    |     |
| 0       | 4   | 19  | 17 | 81  | 0.971 |
| 1       | 7   | 19.4 | 29 | 80.6 |       |
| papillary |     |    |     |    |     |
| 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

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 |       |
| alive0dead1 |     |    |     |    |     |
| 0       | 12  | 63.2 | 7  | 36.8 | 0.460 |
| 1       | 19  | 52.8 | 17 | 47.2 |       |
| papillary |     |    |     |    |     |
| 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
Table 2. CDDP sensitivity and characteristics in the patients who are alive or dead.

|                | CDDP sensitivity negative | CDDP sensitivity positive |
|----------------|---------------------------|---------------------------|
|                | n=16                      | n=13                      |
| Age            | median (range)            |                           |
|                | 72 (42–85)                | 68 (50–86)                | 0.329 |
| Sex            |                           |                           |
| Female         | 5                         | 7                         | 0.219 |
| Male           | 11                        | 6                         | 0.219 |
| alive, dead    |                           |                           |
| 0              | 5                         | 4                         | 0.647 |
| 1              | 11                        | 9                         | 0.647 |
| Stage          |                           |                           |
| II             | 2                         | 1                         | 0.715 |
| III            | 10                        | 7                         | 0.715 |
| IV             | 4                         | 5                         | 0.715 |
| combined       |                           |                           |
| chemo agent    |                           |                           |
| S1             | negative                  | 0                         | 0 |
|                | positive                  | 16                        | 100 |
|                | negative                  | 15                        | 93.8 |
|                | positive                  | 1                         | 6.3 |
| DOX            | negative                  | 12                        | 75 |
|                | positive                  | 4                         | 25 |
| PTX            | negative                  | 15                        | 93.8 |
|                | positive                  | 1                         | 6.3 |
| nivolumab      | negative                  | 16                        | 100 |
|                | positive                  | 0                         | 0 |
| trastuzumab    | negative                  | 16                        | 100 |
|                | positive                  | 1                         | 6.3 |
| histological   | papillary                 | 15                        | 93.8 |
| type           | positive                  | 1                         | 6.3 |
| tubular        | negative                  | 6                         | 37.5 |
|                | positive                  | 10                        | 62.5 |
| poorly         | differentiating           | 8                         | 50 |
| differentiating| negative                  | 8                         | 50 |
|                | positive                  | 8                         | 50 |

Note: The table compares the CDDP sensitivity and characteristics among patients who are alive or dead.
|                       | negative |   |   |   |   |
|-----------------------|----------|---|---|---|---|
| **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
Table.3. The regimen of drug therapy for gastric cancer patients

| Regimen/drugs          | Dose               | Schedule          |
|------------------------|--------------------|-------------------|
| SP                     |                    |                   |
| S-1                    | 40 mg/m²           | every 5 weeks     |
| CDDP                   | 60 mg/m²           | every 5 weeks     |
|                        | 3 weeks            | day 8             |
|                        | 3 weeks            |                   |
|                        | day 8              |                   |
| SOX                    |                    |                   |
| S-1                    | 80-120 mg/day      | every 3 weeks     |
| oxaliplatin (OXP)      | 100 mg/m²          | 2 weeks           |
|                        | 2 weeks            | day 1             |
| XEROX                  |                    |                   |
| capecitabine           | 1000 mg/m²         | every 3 weeks     |
| oxaliplatin (OXP)      | 130 mg/m²          | 2 weeks           |
|                        | 2 weeks            | day 1             |
| DOC                    |                    |                   |
| S-1+DOC                | 60 mg/m²           | every 3 weeks     |
| DOC                    | 80 mg/m²           | every 3 weeks     |
| S-1                    | 40 mg/m²           | 2 weeks           |
|                        | 2 weeks            | day 1             |
| Ramucirumab+ PTX       |                    |                   |
| Ramucirumab            | 8 mg/kg            | every 4 weeks     |
| PTX                    | 80 mg/m²           | day 1, 15         |
|                        |                    | day 1, 8, 15      |
| Trastuzumab+SOX        |                    |                   |
| Trastuzumab            | 8 mg/kg (1st), 6 mg/kg (2nd~) | every 3 weeks |
| S-1                    | 80 mg/m²           | 2 weeks           |
| oxaliplatin (OXP)      | 100 mg/m²          | day 1             |
|                        |                    | day 1             |
| Nivolumab              | 240 mg/body        | every 2 weeks     |
|                        |                    | day 1             |

CDDP: cisplatin, DOC: docetaxel, PTX: paclitaxel, CPT-11: irinotecan
Table 4. The rate of drug sensitivity of CD-DST

| Drug  | CDDP | DOC | PTX | CPT-11 |
|-------|------|-----|-----|--------|
| Rate(%) | 41   | 83  | 83  | 49     |

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