studies. In a randomized Phase III trial to compare post-operative chemotherapy with cisplatin plus 5-fluorouracil (5-FU) (CF) to surgery alone (JCOG9204), the superiority of post-operative chemotherapy in disease-free survival was demonstrated (3). In the following randomized Phase III trial (JCOG9907), the survival benefit of preoperative chemotherapy with CF over post-operative chemotherapy with the same regimen was confirmed (4). Therefore, preoperative chemotherapy has become the current Japanese standard treatment for locally advanced esophageal cancer.

In Western countries, on the other hand, survival benefits from preoperative chemoradiotherapy over surgery alone have been demonstrated in several clinical trials (5), and now it is accepted as the standard treatment for locally advanced esophageal cancer. However, many Asian physicians are reluctant to introduce these Western evidences directly to their clinical practice because the Western evidences came from trials where the majority of the enrolled patients had adenocarcinoma and the protocol-defined surgery was mostly transhiatal esophagectomy at least in part. The prognosis observed in the Western trials were usually poorer than that in Asia, and Asian physicians believe that their transthoracic esophagectomy with regional lymphadenectomy can achieve better local control (6). In addition, adenocarcinoma occupies only 1.5% of all esophageal cancers in Japan and a series of JCOG trials have included only squamous cell carcinoma. Nevertheless, the fact that local recurrences were observed among not a few patients in JCOG9907 indicates the possible need for more intensive local control. Thus, the first purpose of this trial is to investigate whether preoperative chemoradiotherapy with radical surgery is effective even for 'Eastern' esophageal cancer.

Reinforcement of systemic control with more intensive preoperative chemotherapy is another strategy to improve survival for locally advanced esophageal cancer. Docetaxel is one of the most promising drugs for esophageal cancer and recently preoperative chemotherapy with docetaxel plus CF (DCF) has been investigated in some exploratory trials. Hara et al. (7) conducted a feasibility study of this regimen for 44 patients with locally advanced esophageal cancer and showed a good response rate (60.0%) with no treatment-related death. Thus, the second purpose of this trial is to investigate whether DCF has better survival benefits over CF as a preoperative chemotherapy for locally advanced esophageal cancer.

Based on these backgrounds, we have launched a three-arm randomized controlled trial to confirm the superiority of DCF and the superiority of chemoradiotherapy with CF (CF-RT) in overall survival over CF as preoperative therapy for locally advanced esophageal cancer.

The JCOG Protocol Review Committee approved this study protocol in November 2012 and patient enrollment was started in December 2012. In each institution, approval by the institutional review board is obtained before starting patient accrual. This trial was registered at the UMIN Clinical Trials Registry as UMIN000009482 (http://www.umin.ac.jp/ctr/index.htm).

## PROTOCOL DIGEST OF THE JCOG1109

### Objectives

The purpose of this study is to confirm the superiority of DCF and the superiority of CF-RT in overall survival over CF as preoperative therapy for locally advanced esophageal cancer.

### Study Setting

A multi-institutional three-arm open label randomized Phase III study.

### Funding

This study was supported by the National Cancer Center Research and Development Fund (23-A-16, 23-A-19).

### Endpoints

The primary endpoint is overall survival in all randomized patients. Overall survival is defined as the number of days from randomization to death from any cause, and it is censored at the last day the patient is alive. The secondary endpoints are progression-free survival (PFS), %R0 resection, response rate, pathologic complete response rate and adverse events.

PFS is defined as the number of days from randomization to progression or death from any cause, and it is censored at the latest day the patient is alive without any evidence of progression. Disease progression during preoperative therapy is not regarded as an event of PFS if R0/R1 resection is conducted. In cases of R2 resection, radiologically confirmed progression after surgery is regarded as a PFS event (8).

Adverse events include those during preoperative therapy, surgical morbidity, late radiation toxicity and serious adverse events.

### Eligibility Criteria

#### *Inclusion criteria*

(i) Histologically proven squamous cell carcinoma, adenosquamous carcinoma or basaloid cell carcinoma.
(ii) All lesions are located in the thoracic esophagus.
(iii) Clinical stages IB, II and III (excluding T4) based on the 7th UICC-TNM classification.
(iv) 20–75 years of age.
(v) ECOG performance status of 0 or 1.
(vi) Measurable lesions not required.
(vii) No prior therapy against esophageal cancer except for complete resection by endoscopic mucosal resection/endoscopic submucosal dissection with either pM1/M2 disease or pM3 disease without vascular infiltration.
(viii) No prior chemotherapy, radiotherapy or hormonal therapy against any cancers except for hormonal therapy for prostate cancer with more than 5 years of disease-free interval.

(ix) Adequate organ function.
(x) R0 esophagectomy is expected by open (or laparoscopic) thoracotomy and laparotomy.
(xi) Written informed consent.

*Exclusion criteria*

(i) Synchronous or metachronous (within 5 years) double cancers, except for intramucosal tumor curatively resected by local therapy.
(ii) Active infection requiring systemic therapy.
(iii) Positive hepatitis B surface antigen, hepatitis C virus antibody or human immunodeficiency virus antibody.
(iv) Pregnant or lactating women or women of childbearing potential.
(v) Psychiatric disease.
(vi) Patients requiring systemic steroid medication.
(vii) Requiring continuous administration of flucytosine, phenytoin or warfarin potassium.
(viii) Iodine hypersensitivity.
(ix) Hypersensitivity for docetaxel, cisplatin or polysorbate 80-containing drugs.
(x) Diabetes mellitus with HbA1c of 6.5% or higher.
(xi) Severe emphysema or pulmonary fibrosis.
(xii) Poorly controlled hypertension.
(xiii) Unstable angina within 3 weeks or with a history of myocardial infarction within 6 months.

## Randomization

After the confirmation of the eligibility criteria, registration is made by telephone, fax or web-based system to the JCOG Data Center. Patients are randomized to any of the three arms by minimization method balancing the arms with institution and tumor depth (T1–2 versus T3). The three arms consist of arm A (preoperative CF), arm B (preoperative DCF) and arm C (preoperative CF-RT).

## Treatment Methods

Patients in arm A receive two courses of preoperative CF (cisplatin, 80 mg/m$^2$/day, day 1; 5-FU, 800 mg/m$^2$/day, days 1–5) repeated every 3 weeks. Patients in arm B receive three courses of preoperative DCF (docetaxel, 70 mg/m$^2$/day, day 1; cisplatin, 70 mg/m$^2$/day, day 1; 5-FU, 750 mg/m$^2$/day, days 1–5) repeated every 3 weeks. Patients in arm C receive preoperative chemoradiotherapy (41.4 Gy/23 fractions) with two courses of CF (cisplatin, 75 mg/m$^2$/day, day 1; 5-FU, 1000 mg/m$^2$/day, days 1–4) repeated every 4 weeks.

Radiotherapy in arm C is delivered with 6–10 MV photons to a total dose of 41.4 Gy in 23 fractions over 5 weeks. Three-dimensional treatment planning is required. The gross tumor volume is defined as the volume of the primary tumor and the metastatic lymph nodes measuring $\geq$5 mm along the short axis. The clinical target volume (CTV) includes the primary tumor with a 2-cm cranio-caudal margin, metastatic lymph nodes and regional lymph nodes. The regional lymph nodes include bilaterally supraclavicular fossae and superior mediastinal lymph nodes for carcinoma of the upper thoracic esophagus and mediastinal lymph nodes for carcinoma of the middle or lower thoracic esophagus. Perigastric and celiac axis lymph nodes are not included as elective regional lymph nodes with consideration for anastomotic leak. The planning target volume is defined as CTV plus a 0.5–1 cm margin in the lateral direction and a 1–2 cm margin in the cranio-caudal direction to account for respiratory organ motion and daily set-up error.

Total or subtotal thoracic esophagectomy and regional lymphadenectomy with right thoracotomy is performed within 56 days of completion of preoperative therapy. Transhiatal esophagectomy is not allowed. Thoracoscopic esophagectomy is acceptable but only the surgeons credentialed by the study chair can be responsible for thoracoscopic surgery. Regional lymph nodes for upper thoracic disease include both cervical and thoracic (paraesophageal, paratracheal, subcarinal and mediastinal) lymph nodes. Those for middle and lower disease include thoracic and perigastric nodes.

## Follow-up

All randomized patients are followed up for at least 5 years after patient accrual is completed while the analysis of primary endpoint is performed at 3 years after accrual completion. Tumor markers (carcinoembryonic antigen and squamous cell carcinoma) are evaluated at least every 3 months for the first year, every 6 months from the second to the fifth year and every year afterwards. Enhanced computed tomography for the cervix, chest and abdomen is evaluated at least 6 months for the first 5 years.

## Study Design and Statistical Analysis

This three-arm randomized trial is designed to confirm the superiority of preoperative DCF and the superiority of preoperative CF-RT over preoperative CF in terms of overall survival. We assumed 3-year survival with preoperative CF to be 63% and expected a 10% increase in 3-year survival for preoperative DCF and preoperative CF-RT. According to the Schoenfeld and Richter's method (9), the sample size was calculated as 161 patients per arm with a study-wise one-sided alpha level of 5%, a power of 70% for each pair-comparison, an expected accrual period of 6.25 years and a follow-up period of 3 years. One-hundred and seventy events were expected for each pair-comparison. We adjusted for multiplicity due to two pair-comparisons with the Bonferroni method to maintain the study-wise one-sided alpha level of 5%. The total sample size was set at 501 patients considering some patients lost to follow-up. Only when the superiorities of both preoperative DCF and CF-RT over preoperative CF are demonstrated, the direct comparison between preoperative DCF and CF-RT is to be conducted with a one-sided alpha of 5% in a closed testing procedure.

All statistical analyses will be conducted at the JCOG Data Center.

### Interim Analysis and Monitoring

We plan to conduct two interim analyses, taking multiplicity into account using the Lan-DeMets method with O'Brien and Fleming type alpha spending function. The first interim analysis will be conducted after half of the planned number of patients are enrolled and the second interim just before the planned patient accrual is completed. The Data and Safety Monitoring Committee of the JCOG will review the interim analysis reports independently from the group investigators and group statistician. If the superiority of only one of the test arms is demonstrated with an adjusted alpha level, the study will be terminated. If the superiorities of both test arms are demonstrated over the preoperative CF arm, the study will be continued only with the two test arms. If either of the test arms is terminated because of futility, the study will be continued with the other two arms.

In-house monitoring will be performed every 6 months by JCOG Data Center to evaluate and improve study progress, data integrity and patient safety.

## PARTICIPATING INSTITUTIONS (FROM NORTH TO SOUTH)

Hokkaido University, Iwate Medical University, Tohoku University, Ibaragi Prefectural Central Hospital, Tochigi Cancer Center, Saitama Cancer Center, National Cancer Center Hospital East, Chiba Cancer Center, Chiba University, Tokyo Dental College Ichikawa General Hospital, National Canter Center Hospital, Tokyo Women's Medical University, National Hospital Organization Tokyo Medical Center, Keio University, Showa University, Tokyo Medical and Dental University, Cancer Institute Hospital, Toranomon Hospital, Tokai University, Yokohama Municipal Citizen's Hospital, Niigata Cancer Center, Niigata University, Toyama University, Shizuoka Prefectural General Hospital, Shizuoka Cancer Center, Aichi Cancer Center, Kyoto University, Osaka University, Osaka Medical Center for Cancer and Cardiovascular Disease, National Hospital Organization Osaka Medical Center, Osaka City General Hospital, Osaka Medical College, Kobe University, Hyogo Cancer Center, Hiroshima University, Hiroshima City Asa Hospital, National Hospital Organization Shikoku Cancer Center, Kochi Medical Center, National Hospital Organization Kyushu Cancer Center, Kurume University, Kyushu University.

## Conflict of interest statement

Yuko Kitagawa received non-specific support in research from SANOFI in 2012.

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# Impact of excision repair cross-complementing gene 1 (ERCC1) on the outcomes of patients with advanced gastric cancer: correlative study in Japan Clinical Oncology Group Trial JCOG9912

Y. Yamada[1*], N. Boku[2], T. Nishina[3], K. Yamaguchi[4], T. Denda[5], A. Tsuji[6], Y. Hamamoto[7], K. Konishi[8], Y. Tsuji[9], K. Amagai[10], S. Ohkawa[11], Y. Fujita[12], H. Nishisaki[13], H. Kawai[14], A. Takashima[1], J. Mizusawa[15], K. Nakamura[15] & A. Ohtsu[16]

*[1]Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo; [2]Department of Clinical Oncology, St. Marianna University School of Medicine, Kawasaki; [3]Department of Gastrointestinal Medical Oncology, Shikoku Cancer Center, Matsuyama; [4]Department of Gastroenterology, Saitama Cancer Center, Kita-adachi; [5]Department of Gastroenterology, Chiba Cancer Center, Chiba; [6]Department of Clinical Oncology, Kobe City Medical Center General Hospital, Kobe; [7]Department of Gastroenterology, Keio University, School of Medicine, Tokyo; [8]Department of Gastroenterology, Showa University, School of Medicine, Tokyo; [9]Department of Clinical Oncology, Tonan Hospital, Sapporo; [10]Department of Gastroenterology, Ibaraki Prefectural Central Hospital, Kasama; [11]Department of Hepatobiliary and Pancreatic Oncology, Kanagawa Cancer Center, Yokohama; [12]Department of Gastroenterology, Yokohama Municipal Citizen's Hospital, Yokohama; [13]Department of Gastroenterological Oncology, Hyogo Cancer Center, Akashi; [14]Department of Clinical Oncology, Aichi Cancer Center Hospital, Nagoya; [15]JCOG Data Center/Operations Office, National Cancer Center, Tokyo; [16]National Cancer Center, Exploratory Oncology Research and Clinical Trial Center, Kashiwa, Japan*

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**Background:** Since the best chemotherapy regimen for each patient with advanced gastric cancer is uncertain, we aimed to identify molecular prognostic or predictive biomarkers from biopsy specimens in JCOG9912, a randomized phase III trial for advanced gastric cancer.

**Patients and methods:** Endoscopic biopsy specimens from primary lesions were collected in 445 of 704 randomized patients in JCOG9912. We measured the mRNA expression of excision repair cross-complementing group 1 (ERCC1), thymidylate synthase, dihydropyrimidine dehydrogenase, and five other genes, then, categorized them into low and high groups relative to the median, and examined whether gene expression was associated with efficacy end point.

**Results:** Multivariate analyses showed that high ERCC1 expression [HR 1.37; 95% confidence interval (CI) 1.08–1.75; $P = 0.010$], performance status ≥1 (HR 1.45; 95% CI 1.13–1.86; $P = 0.004$), and number of metastatic sites ≥2 (HR 1.66; 95% CI 1.28–1.86; $P < 0.001$) were associated with a poor prognosis, and recurrent disease (versus unresectable; HR 0.75; 95% CI 0.56–1.00; $P = 0.049$) was associated with a favorable prognosis. None of these molecular factors were a predictive marker for choosing irinotecan plus cisplatin or 5-fluorouracil rather than S-1.

**Conclusion:** These correlative analyses suggest that ERCC1 is an independent prognostic factor for overall survival in the first-line treatment of gastric cancer.

**Clinical Trial Number:** C000000062, www.umin.ac.jp.

**Key words:** dihydropyrimidine dehydrogenase, excision repair cross-complementing gene 1, gastric cancer, prognostic factor, thymidylate synthase, vascular endothelial growth factor

## introduction

Fluoropyrimidine and platinum-based combination therapies are the most commonly used and acceptable first-line therapies all over the world. Poor performance status (PS), liver metastases, peritoneal metastases, and higher value of plasma alkaline phosphatase have been identified as clinical prognostic factors for local and advanced gastric cancer [1]. However, these prognostic factors are not predictive markers for selecting the optimal regimens for systemic chemotherapy. Therefore, we need to have a better understanding of biological prognostic markers of conventional cytotoxic agents to so that we can give patients the optimal drugs to prolong their survival and improve their quality of life, since cytotoxic drugs are not effective in every patient and often have severe adverse effects.

Excision repair cross-complementation group 1 (ERCC1) is an important component of the nuclear excision repair pathway

*Correspondence to: Dr. Yasuhide Yamada, Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 1040045, Japan. Tel: +81-3-3542-2511; Fax: +81-3-3542-3815; E-mail: yayamada@ncc.go.jp

which repairs DNA intrastrand, interstrand, and DNA-protein crosslinks caused by cisplatin. High mRNA levels of ERCC1 in primary gastric cancer may be associated with a lower response to cisplatin and poor survival [2]. The overall survival (OS) in patients with low ERCC1 levels was significantly longer than that in patients with high levels [3]. Several potential predictive factors of the response to 5-fluorouracil (5-FU) or prognostic factors have been reported in the metabolic pathway of 5-FU and folic acid. These include thymidylate synthase (TS), which is a target enzyme of 5-FU for the synthesis of DNA, and the cytosolic enzyme dihydropyrimidine dehydrogenase (DPD), which degrades 5-FU in mainly the liver but also in tumor [4, 5]. High mRNA expression of TS and DPD has been shown to predict a poor clinical outcome of treatment with 5-FU [5, 6].

Some studies have suggested that expression of the ERCC1, TS, and DPD genes is clinically useful for predicting the effects of chemotherapy. Other studies [7, 8], however, have failed to confirm that they are associated with the outcome of chemotherapy. Thus, further larger studies are required to identify predictive and prognostic factors to individualize anti-cancer drugs in patients.

The Japan Clinical Oncology Group (JCOG) trial JCOG9912 was a randomized phase III trial of advanced gastric cancer which revealed the noninferiority of S-1 to 5-FU [hazard ratio (HR) 0.83; 95% confidence interval (CI) 0.68–1.01; $P < 0.001$] with regard to OS, but failed to show the superiority of irinotecan plus cisplatin (IP) (HR 0.85; 95% CI 0.70–1.04; $P = 0.055$) [9].

This study was designed to identify differences in survival and tumor shrinkage after 5-FU, S-1, and IP therapy through the use of molecular markers, and to identify potential prognostic and predictive factors for the clinical outcome from subset analyses in JCOG9912.

## patients and methods

Between 2000 and 2006, 704 patients were enrolled in the JCOG9912 trial [9]. After the primary analysis of JCOG9912, endoscopic biopsy specimens taken before treatment were obtained from patients enrolled in JCOG9912. The tumor response was scheduled to be assessed every 8 weeks according to the RECIST ver1.0. OS was defined as the period from the date of randomization until death from any cause. Progression-free survival (PFS) was calculated as the time from randomization until the first objective evidence of disease progression or death from any cause. Written informed consent to be enrolled in JCOG9912 was obtained before registration and the opportunity to refuse to provide tumor samples for this translational research was provided through web sites of the National Cancer Center (NCC) and JCOG according to the Japanese Ethical Guidelines for Clinical Studies. The protocol of this translational study was approved by the institutional review board of NCC and each participating hospital, and complied with the REMARK, reporting recommendations for tumor marker prognostic studies [10].

### laboratory methods

The tumor cells on the sections of interest were selectively isolated by laser-captured microdissection (P.A.L.M. Microsystem, Leica, Wetzlar, Germany). ERCC1, TS, DPD, orotate phosphoribosyl transferase (OPRT), and methylene tetrahydrofolate reductase (MTHFR), epidermal growth factor receptor (EGFR), topoisomerase I (Topo-1), vascular endothelial growth factor-A (VEGF-A), and an internal reference gene (beta-actin) were quantified with a fluorescence-based real-time detection method (ABI PRISM 7900 Sequence Detection System, TaqMan®, Perkin-Elmer [PE] Applied Biosystems, Foster City, CA). The same primers and probes as previously described were used [7].

### statistical analysis

To assess the associations of gene expression levels with the response rate (RR), PFS, and OS, the expression levels of each gene were categorized into low and high values with respect to the median. Categorical data were evaluated using Fisher's exact test. The probability of survival was calculated with the Kaplan–Meier method, and differences between curves were evaluated with the log-rank test. Estimates of hazard ratios with 95% CIs based on a Cox proportional hazards model were used to provide quantitative summaries of the gene expression data.

Variables for the multivariate analysis included the genes with expression levels (high or low) that showed associations in the univariate analyses in this study, as well as the patient's background, such as sex, age, tumor status (recurrent versus unresectable), PS, number of metastatic sites, presence or absence of target lesions according to RECIST version 1.0, macroscopic type (Borrmann 0,1,2 versus 3,4,5), histological classification (intestinal/diffuse), and presence or absence of peritoneal metastasis. All reported $P$-values are two sided, and the level of statistical significance was set at $P < 0.05$. All analyses were carried out using the SAS statistical package, version 9.1 or 9.2 (SAS Institute, Inc., Cary, NC).

## results

### patient characteristics and molecular biomarkers

Tissue samples for this gene expression study were collected in 445 of 704 randomized patients in JCOG9912, and assay data were available in 325 (supplementary Figure S1, available at *Annals of Oncology* online). The MST of the 325 patients analyzed in this correlative study was 12.6 months (95% CI 11.5–14.1). The MST was 11.5 months in the 5-FU arm, 14.2 months in the IP arm, and 11.9 months in the S-1 arm. The baseline characteristics were equally distributed among the subsets for each biomarker (supplementary Table S1, available at *Annals of Oncology* online). The numbers of patients assayed were not equal for each biomarker because some samples were not sufficient for all eight assays.

The mRNA expression of ERCC1 and DPD in the diffuse type were higher than those in the intestinal type (Figure 1), while there were no clear associations between histological types and the expression of the other five genes for OPRT, EGFR, MTHFR, Topo-1, and VEGF-A. ERCC1 expression did not show a strong association with TS expression (Spearman's coefficient 0.38) or DPD (0.30). Higher VEGF-A expression was more commonly observed in patients with unresectable disease ($P = 0.060$), target lesions ($P = 0.052$), and liver metastasis ($P = 0.090$) (supplementary Table S2, available at *Annals of Oncology* online).

### value of molecular markers and efficacy in each treatment arm

To better understand the association between mRNA levels of selected biomarkers and treatment outcomes with each chemotherapy regimen, we carried out a subgroup analysis in terms of tumor shrinkage (Table 1). The RR of IP in the low ERCC1 group was significantly higher than that in the high

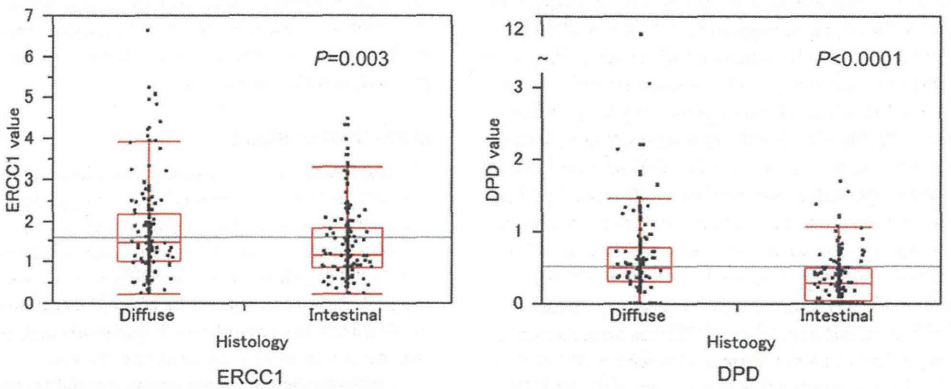


**Figure 1.** Gene expression levels in diffuse type and intestinal type. Intestinal type, papillary and tubular adenocarcinoma; diffuse type, poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous adenocarcinoma; ERCC1, excision repair cross-complementation group 1; DPD, dihydropyrimidine dehydrogenase.

ERCC1 group ($P = 0.045$). IP was also more effective in patients with low DPD compared with high DPD ($P = 0.006$). A similar tendency was seen for 5-FU: the RR was 17.5% in the low ERCC1 group and 2.7% in the high ERCC1 group ($P = 0.058$). The RR in patients with low TS treated with 5-FU (16.7%) seemed to be higher than that in patients with high TS (2.9%) ($P = 0.068$). On the other hand, S-1 showed constant activity in terms of the RRs between low and high ERCC1, TS, DPD, and the five other genes. There were no significant findings regarding the associations between the expression levels of the five other genes and the RR.

Although the RR for IP in the low ERCC1 group was better than that in the high ERCC1 group, there was no difference in PFS of IP regardless of the expression level of ERCC1 (HR 1.04; $P = 0.82$). Similarly, there was no difference in PFS of S-1 between the low and high ERCC1 groups. Patients with high ERCC1 showed substantially worse survival than those with low ERCC1 in both S-1 and IP, as did patients with high TS in IP.

### value of molecular markers as prognostic factors

A univariate analysis of the whole study population showed that both OS and PFS in the low ERCC1 and low TS groups were better than those in the high ERCC1 and high TS groups (supplementary Tables S3 and S4, available at *Annals of Oncology* online). There were no differences in OS or PFS according to the expression of the six other genes.

Multivariate analyses for OS with molecular markers and clinical characteristics showed that ERCC1 (HR 1.37; 95% CI 1.08–1.75, $P = 0.010$), PS, tumor status (recurrent versus unresectable), and the number of metastatic sites were independent prognostic factors for OS (supplementary Table, available at *Annals of Oncology* online). Multivariate analyses for PFS showed that recurrent disease and a histological classification of intestinal type were independent favorable prognostic factors.

### value of molecular markers as predictive factors

Supplementary Table S5, available at *Annals of Oncology* online, shows the predictive values of ERCC1, TS, and DPD for choosing 5-FU or IP rather than S-1. Although marginal interaction was seen between ERCC1 and PFS after 5-FU or S-1, S-1 was superior to 5-FU regardless of the expression level of ERCC1. Thus, ERCC-1 cannot be a predictive marker for choosing S-1 or 5-FU from the perspective of PFS. The hazard ratios of IP compared with S-1 for PFS and OS in the low DPD group were 0.87 and 0.84, and those in the high DPD group were 1.13 and 1.21, which suggested that there might be some interaction between DPD and the treatment arm of IP or S-1. Furthermore, ERCC1, TS, and the five other genes had no predictive value for choosing IP rather than S-1 from the perspective of either PFS or OS.

## discussion

This study shows that low ERCC1 expression was a significant independent favorable prognostic factor in patients with advanced gastric cancer who were receiving first-line chemotherapy regardless of the treatment regimen in JCOG9912. High ERCC1 expression confers cisplatin resistance and reconstitutes the cell's ability to remove cisplatin from cellular DNA in an animal model [11]. Furthermore, the aberrant methylation of DNA repair genes has been shown to be indicative of sensitivity to chemotherapeutic agents other than cisplatin [12]. Other studies in ovarian [13], pancreatic [14], lung cancer [15] have also suggested that greater activity of ERCC1 was associated with resistance to platinum compounds. In this study, patients with low ERCC1 showed higher RRs than those with high ERCC1 in both IP and 5-FU, while the RRs were similar regardless of the ERCC1 level among patients treated with S-1.

The expression of several DNA repair genes has been shown to be inactivated or decreased in tumors associated with promoter hypermethylation [16], and it has been reported that ERCC1 promoter methylation was inversely associated with mRNA expression [17]. Concurrent hypermethylation of gene promoters is associated with a high microsatellite instability phenotype in gastric cancer [18], and the concordant methylation of CIMP-high is associated with better survival [19]. Overall, in this study, in patients with a high expression of

**Table 1.** Univariate analyses for clinical outcomes in first-line chemotherapy: correlation with mRNA expression levels

| mRNA | Group | All | | | 5-Fluorouracil | | | S-1 | | | Irinotecan plus cisplatin | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n* | RR (%) | *P* | *n* | RR (%) | *P* | *n* | RR (%) | *P* | *n* | RR (%) | *P* |
| ERCC1 | Low | 120 | 34.2 | 0.064 | 40 | 17.5 | 0.058 | 40 | 32.5 | 1.00 | 40 | 52.5 | 0.045 |
| | High | 123 | 22.8 | | 37 | 2.7 | | 42 | 33.3 | | 44 | 29.6 | |
| TS | Low | 120 | 33.3 | 0.15 | 42 | 16.7 | 0.068 | 36 | 41.7 | 0.17 | 42 | 42.9 | 0.82 |
| | High | 120 | 24.2 | | 34 | 2.9 | | 45 | 26.7 | | 41 | 39.0 | |
| DPD | Low | 119 | 31.9 | 0.39 | 46 | 15.2 | 0.24 | 37 | 27.0 | 0.24 | 36 | 58.3 | 0.006 |
| | High | 113 | 26.6 | | 26 | 3.9 | | 41 | 41.5 | | 46 | 26.1 | |

| mRNA | Group | *n* | mPFS (months) | HR (95%CI) | *P* | *n* | mPFS (months) | HR (95% CI) | *P* | *n* | mPFS (months) | HR (95% CI) | *P* | *n* | mPFS (months) | HR (95% CI) | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ERCC1 | Low | 162 | 4.78 | 1 | 0.31 | 53 | 3.81 | 1 | 0.062 | 55 | 5.32 | 1 | 0.87 | 54 | 5.32 | 1 | 0.82 |
| | High | 160 | 3.89 | 1.12 (0.90–1.40) | | 50 | 2.07 | 1.45 (0.98–2.14) | | 55 | 4.29 | 1.03 (0.71–1.51) | | 56 | 4.32 | 1.04 (0.72–1.52) | |
| TS | Low | 159 | 5.10 | 1 | 0.015 | 54 | 3.71 | 1 | 0.093 | 48 | 5.34 | 1 | 0.16 | 57 | 5.59 | 1 | 0.068 |
| | High | 158 | 3.81 | 1.32 (1.06–1.65) | | 47 | 2.10 | 1.40 (0.94–2.10) | | 60 | 4.24 | 1.32 (0.90–1.94) | | 51 | 4.11 | 1.43 (0.97–2.11) | |
| DPD | Low | 154 | 4.22 | 1 | 0.97 | 57 | 2.14 | 1 | 0.60 | 50 | 4.47 | 1 | 0.73 | 47 | 5.72 | 1 | 0.26 |
| | High | 150 | 4.21 | 1.00 (0.80–1.26) | | 37 | 3.58 | 0.89 (0.59–1.36) | | 52 | 4.35 | 0.93 (0.63–1.38) | | 61 | 4.04 | 1.25 (0.85–1.84) | |

| mRNA | Group | *n* | MST (months) | HR | *P* | *n* | MST (months) | HR | *P* | *n* | MST (months) | HR (95% CI) | *P* | *n* | MST (months) | HR | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ERCC1 | Low | 162 | 14.9 | 1 | 0.016 | 53 | 11.8 | 1 | 0.41 | 55 | 15.0 | 1 | 0.10 | 54 | 16.1 | 1 | 0.066 |
| | High | 160 | 11.5 | 1.32 (1.05–1.65) | | 50 | 10.5 | 1.18 (0.79–1.75) | | 54 | 11.0 | 1.39 (0.94–2.07) | | 56 | 11.7 | 1.43 (0.97–2.12) | |
| TS | Low | 159 | 14.2 | 1 | 0.034 | 54 | 11.0 | 1 | 0.53 | 48 | 11.9 | 1 | 0.36 | 57 | 16.8 | 1 | 0.0014 |
| | High | 158 | 11.5 | 1.28 (1.02–1.61) | | 47 | 11.8 | 1.14 (0.76–1.70) | | 60 | 12.8 | 1.21 (0.81–1.81) | | 51 | 11.1 | 1.89 (1.27–2.80) | |
| DPD | Low | 154 | 11.9 | 1 | 0.64 | 57 | 11.5 | 1 | 0.65 | 50 | 11.5 | 1 | 0.44 | 47 | 15.5 | 1 | 0.22 |
| | High | 150 | 13.1 | 0.95 (0.75–1.20) | | 37 | 11.8 | 0.91 (0.59–1.39) | | 52 | 12.1 | 0.85 (0.56–1.29) | | 61 | 14.1 | 1.28 (0.86–1.90) | |

High ERCC1 and High TS were poor prognostic markers in advanced gastric cancer. ERCC1 and DPD were the predictive factors of tumor shrinkage in irinotecan plus cisplatin.

ERCC1, Excision repair cross-complementation group 1; TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; RR, response rate; mPFS, median progression-free survival time; MST, median overall survival time; HR, hazard ratio.



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ERCC1 who received first-line chemotherapy, the risk of death was increased by more than 30% compared with that in low ERCC1 patients.

In colorectal cancer, since many studies have examined the molecular predictors of outcomes over the past two decades, TS and DPD were newly listed in 'ASCO 2006 Tumor Marker Guidelines in Gastrointestinal Cancer' [20]. However, due to a lack of sufficient supporting evidence, the guidelines recommend that these biomarkers should not yet be used clinically to predict the prognosis or treatment response. With regard to TS in this study, while patients with high TS showed slightly lower RRs than those with low TS in both 5-FU and S-1, there was no difference in the RR regardless of the expression level of TS in IP. However, PFS and OS in patients with high TS in IP were similar to those in S-1. As a result, TS could not be a predictive marker for choosing IP over S-1. Two previous prospective trials with pharmacogenetic-tailored therapy against colorectal cancer failed to confirm the predictive values of TS and DPD [21, 22]. TS and DPD were not predictive markers for selecting 5-FU/leucovorin or irinotecan/oxaliplatin, since the group of patients who had low TS and low DPD not only had a high RR to 5-FU/leucovorin when compared with irinotecan/oxaliplatin, but they also had a longer OS [21].

As for DPD, S-1 showed a higher RR in patients with high DPD than in those with low DPD, while the reverse association between the DPD level and RRs was observed in 5-FU. However, since S-1 showed better efficacy than 5-FU regardless of the level of DPD, DPD could not be a predictive marker for choosing between S-1 and 5-FU. While IP showed a higher response, PFS and OS in patients with low DPD were slightly longer than those in patients with high DPD, and the efficacy of S-1 was slightly worse in low DPD than in high DPD, the hazard ratios of IP compared S-1 in low DPD for PFS and OS were marginal (0.87 and 0.84) and those in high DPD were 1.13 and 1.21. It is speculated that a low DPD might have some potential as a predictive marker for selecting IP rather than S-1. Similar results were observed in the CAIRO study which compared capecitabine plus irinotecan to capecitabine monotherapy for patients with metastatic colorectal cancer; the irinotecan combined regimen was more efficacious in a low DPD group. Based on our current knowledge, this association between DPD and irinotecan is difficult to explain logically, and further studies are needed to more clearly define the association between DPD and the efficacy of regimens that contain irinotecan.

In this study, patients with low ERCC1 showed a higher RR than those with high ERCC1 in IP, and RRs were similar regardless of the ERCC1 level among patients treated with S-1. On the other hand, there were no differences in PFS or OS among patients with low ERCC1 between IP and S-1. As a result, no predictive marker for selecting 5-FU or IP rather than S-1 could be found in this study. The pattern and extent of DNA damage induced by fluoropyrimidines in human cancer cell lines varies and may be affected not only by the activity of enzymes involved in DNA repair but also by downstream factors such as p53. Wild-type p53 was a strong predictor of sensitivity to 5-FU in cell lines of the National Cancer Institute's Anticancer Drug Screen panel *in vitro* [23]. Many other factors associated with chemosensitivity should be investigated in future studies to identify predictive markers of cytotoxic agents.

In conclusion, our study provides evidence that high mRNA expression of ERCC1 in primary lesions of gastric cancer is associated with significantly worse OS. We did not identify a predictive marker for choosing 5-FU or IP rather than S-1.

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# A simple prognostic scoring system for patients receiving transarterial embolisation for hepatocellular cancer

L. Kadalayil[1], R. Benini[2], L. Pallan[3], J. O'Beirne[4], L. Marelli[4], D. Yu[5], A. Hackshaw[1], R. Fox[6], P. Johnson[3], A. K. Burroughs[4], D. H. Palmer[3,†] & T. Meyer[2,7,†]

*[1]Cancer Research UK & UCL Cancer Trials Centre, London; [2]Department of Oncology, UCL Medical School, Royal Free Campus, London; [3]Cancer Research UK Institute for Cancer Studies, University of Birmingham; [4]The Royal Free Sheila Sherlock Liver Centre, Royal Free Hospital, London; [5]Department of Radiology, Royal Free Hospital, London; [6]Cancer Research UK Clinical Trials Unit, University of Birmingham, Birmingham; [7]UCL Cancer Institute, London, UK*

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**Background:** The prognosis for patients with hepatocellular cancer (HCC) undergoing transarterial therapy (TACE/TAE) is variable.

**Methods:** We carried out Cox regression analysis of prognostic factors using a training dataset of 114 patients treated with TACE/TAE. A simple prognostic score (PS) was developed, validated using an independent dataset of 167 patients and compared with Child–Pugh, CLIP, Okuda, Barcelona Clinic Liver Cancer (BCLC) and MELD.

**Results:** Low albumin, high bilirubin or α-fetoprotein (AFP) and large tumour size were associated with a two- to threefold increase in the risk of death. Patients were assigned one point if albumin <36 g/dl, bilirubin >17 μmol/l, AFP >400 ng/ml or size of dominant tumour >7 cm. The Hepatoma arterial-embolisation prognostic (HAP) score was calculated by summing these points. Patients were divided into four risk groups based on their HAP scores; HAP A, B, C

*Correspondence to: Dr Tim Meyer, UCL Cancer Institute, University College London, 72 Huntley Street, London WC1E 6BT, UK. Tel: +44-207-679-6731; Fax: +44-207-794-3341; E-mail: t.meyer@ucl.ac.uk

†Both these authors contributed equally.

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ORIGINAL ARTICLE

# High false-negative proportion of intraoperative histological examination as a serious problem for clinical application of sentinel node biopsy for early gastric cancer: final results of the Japan Clinical Oncology Group multicenter trial JCOG0302

**Isao Miyashiro · Masahiro Hiratsuka · Mitsuru Sasako · Takeshi Sano · Junki Mizusawa · Kenichi Nakamura · Atsushi Nashimoto · Akira Tsuburaya · Norimasa Fukushima · The Gastric Cancer Surgical Study Group (GCSSG) in the Japan Clinical Oncology Group (JCOG)**

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**Abstract**
*Background* To evaluate the feasibility and accuracy of diagnosis using sentinel node (SN) biopsy in T1 gastric cancer, a multicenter trial was conducted by the Japan Clinical Oncology Group (JCOG).
*Methods* Sentinel node biopsy with indocyanine green (ICG) was performed in patients with T1 gastric cancer. Green-stained nodes (GNs), representing SNs, were removed first, and gastrectomy with lymphadenectomy was then performed. GNs in one plane (with the largest dimension) were histologically examined intraoperatively by frozen section with hematoxylin and eosin (H&E) stain. All harvested lymph nodes (GNs and non-GNs) were histologically examined by paraffin section after surgery. The primary endpoint was to determine the proportion of false negatives, which was defined as the number of patients with negative GNs by frozen section divided by those with positive GNs and/or positive non-GNs by paraffin section. The sample size was set at 1,550, based on the expected and threshold value as 5 and 10 % in the proportion of false negatives.
*Results* Accrual was suspended when 440 patients were enrolled because the proportion of false negatives was high. In the primary analysis, the proportion of false negatives was 46 % (13/28) after a learning period with 5 patients for each institution. Seven of 13 patients had nodal metastases outside the lymphatic basin. False negatives remained at 14 % (4/28) even by examining additional sections of GNs by paraffin section.
*Conclusions* The proportion of false negatives was much higher than expected. Intraoperative histological

I. Miyashiro (✉)
Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari-ku, Osaka 537-8511, Japan
e-mail: miyashir@biken.osaka-u.ac.jp

M. Hiratsuka
Department of Surgery, Itami City Hospital, Hyogo, Japan

M. Sasako
Department of Surgery, Hyogo College of Medicine, Nishinomiya, Japan

T. Sano
Department of Surgery, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan

J. Mizusawa
JCOG Data Center, Multi-institutional Clinical Trial Support Center, National Cancer Center, Tokyo, Japan

K. Nakamura
JCOG Operations Office, Multi-institutional Clinical Trial Support Center, National Cancer Center, Tokyo, Japan

A. Nashimoto
Department of Surgery, Niigata Cancer Center Hospital, Niigata, Japan

A. Tsuburaya
Department of Surgery, Kanagawa Cancer Center, Yokohama, Japan

N. Fukushima
Department of Surgery, Yamagata Prefectural Central Hospital, Yamagata, Japan

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examination using only one plane is not an appropriate method for clinical application of SN biopsy in gastric cancer surgery.

**Keywords** Sentinel node · Gastric cancer · Indocyanine green · Multicenter clinical trial

## Introduction

Early gastric cancer is almost completely curable, although nodal metastasis is found in approximately 20 % pathologically. Because nodal disease cannot be identified before or during surgery, the standard treatment is gastrectomy with nodal dissection, which has been uniformly selected for early gastric cancer. Theoretically, standard surgery is unnecessary for patients without nodal metastases.

The sentinel node (SN) technique has been used in the management of various cancers to avoid unnecessary lymphadenectomy [1–3]. The technique is based on the concept that the tumor-bearing status of the SN, which is defined as a lymph node (LN) that directly drains a specific cancer, reflects the tumor status of the remaining nodes.

Regarding gastric cancer surgery, two Japanese studies were reported in the early 2000s [4, 5]. Thereafter, several studies reported the validity of the SN concept for gastric cancer [6, 7]. To apply the SN concept and partial gastrectomy without radical lymphadenectomy to patients with T1 gastric cancer, the proportion of false negatives should be sufficiently low. Because nodal metastasis was only 20 % in T1 disease, a large number of patients was necessary to confirm the SN concept. However, most reports were from single-institutional studies with a small sample size and inadequate endpoint. Moreover, the SN technique varied according to the surgeon.

As a result of these limitations, the Japan Clinical Oncology Group (JCOG) conducted a multicenter clinical trial, JCOG0302 (GCSSG-SNB, UMIN-CTR ID: C000000059), to evaluate the feasibility and accuracy of diagnosis using SN biopsy in T1 gastric cancer.

## Patients and methods

### Patients

Patients had to fulfill the following preoperative eligibility criteria: T1 gastric cancer without indication for endoscopic resection, i.e., clinically T1a (cT1a) undifferentiated adenocarcinoma, cT1a differentiated adenocarcinoma with ulceration or with maximal diameter more than 2 cm, or cT1b adenocarcinoma; no existence of multiple foci; maximum diameter of 4 cm or less; distance from esophagogastric (EG) junction or pyloric ring of 2 cm or more; age 20–80 years; no prior treatment for gastric cancer; no prior surgery for gastric or duodenal ulcer. All patients provided written informed consent before surgery. Patients also had to fulfill the following intraoperative eligibility criteria: before dye injection; open surgery (laparoscopic surgery was excluded); cT1; palpable tumor or clips (four clips recommended) that were marked endoscopically for tumor location before surgery; no apparent lymph node metastasis; no severe adhesion around the stomach.

### Sentinel node biopsy

Patients were enrolled intraoperatively before injection of indocyanine green (ICG) by means of a telephone call to the JCOG Data Center (Fig. 1). Then, 4–5 ml (25 mg/5 ml) ICG (Diagnogreen; Dai-Ichi Sankyo, Tokyo, Japan) dye was injected around the primary tumor using a fine needle (26-gauge) from the serosal surface of the stomach. Five minutes after dye injection, all LNs that stained green (GN), representing SNs, were excised one by one before lymphadenectomy. Each GN was then histologically examined intraoperatively in one plane (with the largest dimension) by frozen section with H&E staining. The protocol stated that harvesting of GNs must be finished within 30 min.

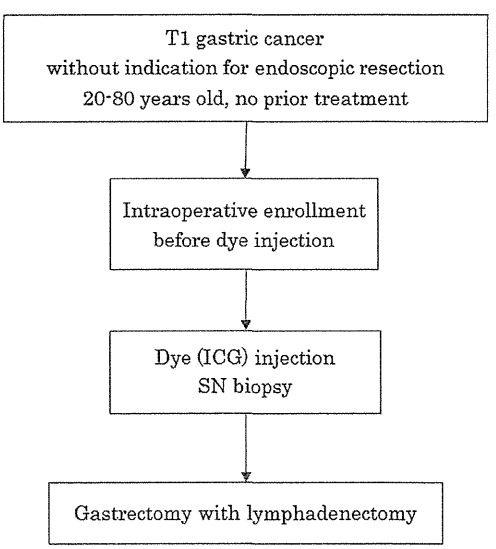


**Fig. 1** Trial scheme of the JCOG0302 trial. After confirmation of eligibility criteria by the surgeon, patients were enrolled intraoperatively by means of a telephone call to the JCOG Data Center. Gastrectomy with lymphadenectomy was performed after sentinel node (*SN*) biopsy. *JCOG* Japan Clinical Oncology Group, *ICG* indocyanine green

Gastrectomy with lymphadenectomy was performed after SN biopsy according to the gastric cancer treatment guidelines edited by the Japanese Gastric Cancer Association [8]. After completing surgery, LNs were obtained as non-GNs from the resected stomach.

The initial five patients enrolled in each institution (but not per surgeon) were defined as patients during the learning period and all measurements referring to accuracy were calculated, excluding the learning period patients. Surgeons observed the video performed by the study principal investigator at starting the trial.

The GNs and non-GNs were fixed in formalin solution and embedded in paraffin for histological examination with H&E staining. Both GNs and non-GNs were diagnosed by one plane (with the largest dimension).

Adverse events were recorded according to the National Cancer Institute-Common Toxicity Criteria version 2.0 and the JCOG Surgical Morbidity Criteria [9].

This study was designed as a multicenter prospective clinical trial. The study protocol was approved by the JCOG Clinical Trial Review Committee and the institutional review boards of all participating institutions.

## Study design and statistical analyses

The primary endpoint was to determine the proportion of false negatives, which was calculated by the number of patients with negative GNs by frozen section divided by those with positive GNs and/or non-GNs by paraffin section. The secondary endpoints were to calculate the proportion to detect GNs, the proportion of false positives, postoperative complications, the incidence of anaphylaxis reaction, and relapse-free survival of patients with nodal metastases. False positives were defined as patients with positive GNs by frozen section but negative GNs and non-GNs by paraffin section (Table 1). In cases in which GNs were diagnosed as positive in an intraoperative frozen section but negative in a paraffin section, this was regarded as a false positive and was not included in pathologically positive nodes.

The planned sample size was set at 1,550 based on the assumption that the expected and threshold proportion of false negatives was 5 and 10 % with one-sided alpha of 10 % and at least 90 % power. The proportion of nodal metastasis and the proportion to detect GNs were estimated as 20 and 95 %, respectively. If the proportion of false negatives was less than 10 %, the threshold, the risk to perform partial gastrectomy without lymphadenectomy erroneously for those with nodal metastasis, was supposed to be less than 2 %, which was considered sufficiently low.

The planned duration of accrual was 3.5 years with 5 years of follow-up. The protocol stated that accrual was suspended if 12 or more patients were diagnosed as false-negative at a semiannual monitoring.

**Table 1** Definition of endpoints according to the JCOG0302 protocol

| | Confirmed diagnosis of GNs and/or non-GNs by paraffin section | |
|---|---|---|
| | Positive | Negative |
| Intraoperative frozen section diagnosis of GNs | | |
| Positive | A | B |
| Negative | C | D |
| GN undetectable | E | F |

Proportion of false negatives = C/(A + C)

Proportion of false positives = B/(B + D)

Proportion to detect GNs = (A + B + C + D)/(A + B + C + D + E + F). Confirmed diagnosis of GN did not include intraoperative histological examinations of GNs by frozen section. Patients with only intraoperative histological positive GNs using frozen section were defined as false-positive and were not included in pathologically positive nodal metastases

Relapse-free survival was calculated from the date of enrollment to the date of relapse or the date of death from any cause. If patients remained alive without recurrence, they were regarded as censored cases at the date when no relapse was confirmed. Relapse-free survival was estimated by the Kaplan–Meier method.

All statistical analyses were conducted with SAS software (version 9.2; SAS Institute, Cary, NC, USA).

## Results

The trial started in May 2004. However, accrual was suspended in September 2005 when 440 patients had been enrolled because false negatives were found in 13 patients by analysis excluding the learning period. According to the recommendation by the Data and Safety Monitoring Committee of JCOG, we decided to terminate the trial in November 2008. At that time, enrollment in each hospital ranged from 2 to 37 patients, with a median of 13 patients. The number of patients was more than 30 in 4 institutions, 21–30 in 4, 11–20 in 11, and 10 or less in 8.

Background factors, perioperative outcomes, and pathological findings are shown in Table 2. After the learning period, the number of GNs ranged from 0 to 19, with a median of 4 nodes (Fig. 2), and time to harvest all GNs after ICG injection ranged from 7 to 30 min, with a median of 18 min. Within the learning period, the time required for harvesting was less than 5 min in one patient and more than 30 min in another. Although the protocol specified that more than 4 ml ICG should be injected around a tumor, the result was that sufficient ICG was successfully

**Table 2** Distribution of background, surgical, and pathological factors

| | Learning period | | Total ($n = 440$) |
|---|---|---|---|
| | Within ($n = 127$) | After ($n = 313$) | |
| Background factors | | | |
| Age (years) | | | |
| Median | 63 | 61 | 62 |
| Range | 33–79 | 26–80 | 26–80 |
| Sex | | | |
| Male | 80 (63.0 %) | 205 (65.5 %) | 285 (64.8 %) |
| Female | 47 (37.0 %) | 108 (34.5 %) | 155 (35.2 %) |
| Marking clips | | | |
| Yes | 107 (84.3 %) | 259 (82.7 %) | 366 (83.2 %) |
| No | 20 (15.7 %) | 54 (17.3 %) | 74 (16.8 %) |
| Surgical findings | | | |
| Operation time (min) | | | |
| Median | 200 | 202 | 200 |
| Range | 107–410 | 113–410 | 107–410 |
| Estimated blood loss (ml) | | | |
| Median | 230 | 200 | 205 |
| Range | 21–1,010 | 10–1,360 | 10–1,360 |
| Type of gastrectomy | | | |
| Proximal | 8 (6.3 %) | 13 (4.2 %) | 21 (4.8 %) |
| Pylorus-preserving | 29 (22.8 %) | 82 (26.2 %) | 111 (25.2 %) |
| Distal | 87 (68.5 %) | 205 (65.5 %) | 292 (66.4 %) |
| Total | 3 (2.4 %) | 13 (4.2 %) | 16 (3.6 %) |
| Tumor location (portion) | | | |
| Upper | 11 (8.7 %) | 18 (5.8 %) | 29 (6.6 %) |
| Middle | 75 (59.1 %) | 174 (55.6 %) | 249 (56.6 %) |
| Lower | 41 (32.3 %) | 121 (38.7 %) | 162 (36.8 %) |
| Time required for harvest (min) | | | |
| Median | 15 | 18 | 17 |
| Range | 3–31 | 7–30 | 3–31 |
| Number of GNs | | | |
| Median | 4 | 4 | 4 |
| Range | 0–12 | 0–19 | 0–19 |
| Coloring around tumor | | | |
| Circumferential | 89 (70.1 %) | 240 (76.7 %) | 329 (74.8 %) |
| Non-circumferential | 38 (29.9 %) | 73 (23.3 %) | 111 (25.2 %) |
| Pathological findings | | | |
| Number of tumor foci | | | |
| Single | 121 (95.3 %) | 308 (98.4 %) | 429 (97.5 %) |
| Two or more | 6 (4.7 %) | 5 (1.6 %) | 11 (2.5 %) |
| Tumor histology | | | |
| Papillary | 2 (1.6 %) | 6 (1.9 %) | 8 (1.8 %) |
| Well differentiated | 26 (20.5 %) | 75 (24.0 %) | 101 (23.0 %) |
| Moderately differentiated | 41 (32.3 %) | 76 (24.3 %) | 117 (26.6 %) |
| Poorly differentiated | 27 (21.3 %) | 73 (23.3 %) | 100 (22.7 %) |
| Signet ring cell | 29 (22.8 %) | 82 (26.2 %) | 111 (25.2 %) |
| Mucinous | 2 (1.6 %) | 1 (0.3 %) | 3 (0.7 %) |

**Table 2** continued

| | Learning period | | Total ($n = 440$) |
|---|---|---|---|
| | Within ($n = 127$) | After ($n = 313$) | |
| Tumor diameter (cm) | | | |
| Median | 2.4 | 2.3 | 2.3 |
| Range | 0.5–8.6 | 0–18 | 0–18 |
| Depth of tumor invasion | | | |
| T1a | 74 (58.3 %) | 174 (55.6 %) | 248 (56.4 %) |
| T1b | 46 (36.2 %) | 121 (38.7 %) | 167 (38.0 %) |
| T2 | 5 (3.9 %) | 12 (3.8 %) | 17 (3.9 %) |
| T3 | 2 (1.6 %) | 6 (1.9 %) | 8 (1.8 %) |
| Number of dissected LNs | | | |
| Median | 37 | 37 | 37 |
| Range | 4–90 | 1–137 | 1–137 |
| Residual tumor | | | |
| R0 | 126 (99.2 %) | 312 (99.7 %) | 438 (99.5 %) |
| R1 | 1 (0.8 %) | 1 (0.3 %) | 2 (0.5 %) |

Depth of tumor invasion and residual tumor were classified based on the *UICC TNM Classification of Malignant Tumors*, 7th edition

Learning period with five patients for each institution was adopted in the JCOG0302 trial

injected in all patients. However, circumferential coloring was not observed in 23.3 % (73/313) after the learning period. There were no remarkable differences in operation time or estimated blood loss between the period of learning and after learning. Pathological tumor diameter ranged from 0 to 18 mm, with a median of 2.3 mm overall. The number of resected LNs ranged from 1 to 137, with a median of 37 nodes after the learning period. Two patients underwent R1 resection, one because of positive peritoneal lavage cytology and the other was pathologically positive for proximal margin.

Two patients were judged as ineligible after the learning period because the presence of palpable tumor or clips was not confirmed before registration in one patient and registration was completed before all eligibility criteria were confirmed in the other (Fig. 3). No GN was detected in 7 of 311 patients enrolled after the learning period. Nodal metastasis, diagnosed by paraffin section, was found in 28 of these 311 patients.

The proportion of false negatives after the learning period, which was the primary endpoint, was 46.4 % (13/28; 80 % CI, 33.1–60.1 %, 95 % CI, 27.5–66.1 %) (Tables 3, 4). Seven of 13 false-negative patients had nodal metastases outside the lymphatic basin.

The proportion to detect GNs was 97.8 % (304/311; 95 % CI, 95.4–99.1 %). The proportion of false positives was 0.7 % (2/276; 95 % CI, 0.1–2.6 %). No patient had an adverse event caused by the ICG injection or grade 4 postoperative complications. Five-year relapse-free survival of patients with nodal metastases ($n = 44$) was 90.9 % (95 % CI, 77.6–96.5 %). Five patients developed a relapse after R0 resection: sites of recurrence were bone in three patients, peritoneum in one, and LN in one.

To further clarify the reason why the proportion of false negatives was unexpectedly high, additional exploratory analyses were performed. First, we examined the number of patients in whom GNs were negative by frozen section but positive by paraffin section. Seven of 13 false-negative patients were such cases, and thus the remaining number of false negatives was 6. Next, we examined the patients in whom GNs were negative in both frozen and single-plane paraffin section but were positive by examining multiple sections of the GNs left for final diagnosis. All the node-positive patients with negative GNs in both frozen and paraffin sections were encountered in the first 19 patients in each institution. Tumor deposits were found in the GNs of 2 of these 6 patients, although the remaining GNs were not entirely whole in some cases and sometimes comprised only half a side. Thus, these additional analyses of GNs decreased the proportion of false negatives to 14.3 % (4/28; 95 % CI, 4.0–32.7 %).

## Discussion

The SN concept for gastric cancer surgery was first suggested by Japanese studies at the beginning of the 21st century. Kitagawa et al. [4] reported their preliminary data on the use of an intraoperative radiation technique with a gamma probe. Hiratsuka et al. [5] reported that SN biopsy using ICG can be performed with a high detection probability, and that SN status can predict LN status with a high degree of accuracy. Given the daily clinical setting, the JCOG0302 trial was basically designed in accordance with the study by Hiratsuka et al. [5], i.e., the ICG dye-guided method for open surgery followed by histopathological

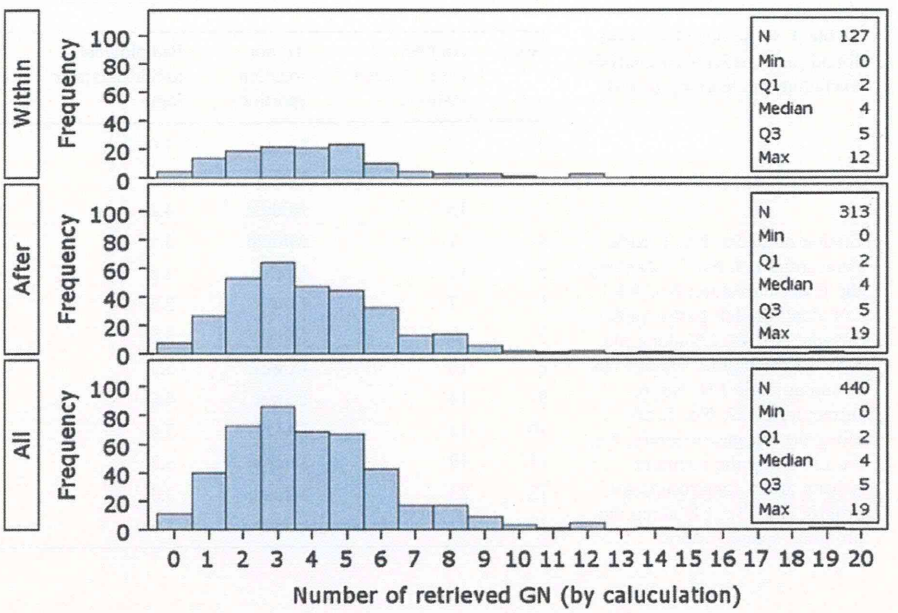


**Fig. 2** Number of retrieved green nodes (GNs) in intraoperative frozen section diagnosis. Number of GNs from each patient ranged from 0 to 19 with a median of 4 nodes (0 to 12, with a median of 4 nodes within the learning period and 0 to 19, with a median of 4 nodes after the learning period, respectively). *GN* green node

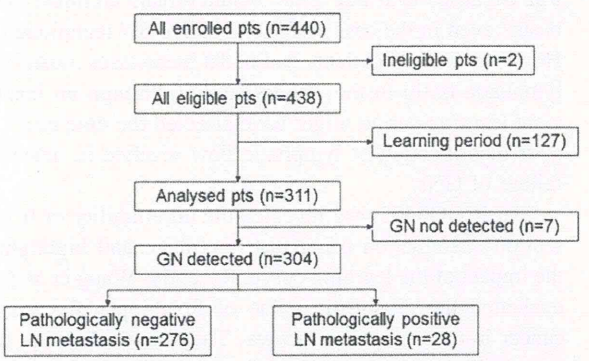


**Fig. 3** Algorithm for analyses of the JCOG0302 trial. The proportion of false negatives as the primary endpoint was defined as the number of patients with intraoperatively negative GNs divided by 28, with pathologically positive nodal metastases in paraffin section. *LN* lymph node, *pts* patients

**Table 3** Results of 311 eligible patients after learning period with 5 patients for each institution

| | Confirmed diagnosis of GN and non-GN by paraffin section | |
|---|---|---|
| | Positive | Negative |
| Intraoperative GN-positive | 15 | 2 |
| Intraoperative GN-negative | 13 | 274 |
| GNs undetectable | 3 | 4 |

examination with H&E staining. The dye-guided method is safe, convenient, inexpensive, and widely available in general hospitals, whereas the gamma probe-guided method has issues of legal restriction and the high cost of radioactive substances [10, 11]. ICG is a popular, approved, diagnostic reagent [12], and allergic reactions to ICG are fewer than those to blue dyes such as isosulfan blue (Lymphazurin) [13].

The present study evaluated the applicability of SN biopsy for T1 gastric cancer and demonstrated that the proportion of false negatives was too high to apply the current SN technique. The reasons for this are as follows.

The first critical issue is the histological examination of only one slice of GNs by frozen section, which might depend on the quality of section processing and the ability of pathologists in each institution. In the multicenter trial setting, only one plane of the largest dimension of the frozen section was adopted in the JCOG0302 trial for convenience in spite of the fact that multiple planes were adopted for detection of metastases in the study by Hiratsuka et al. [5]. The proportion of false negatives in this study was much higher than those of other studies; this is primarily because this is the only study in which GNs proven to have metastasis by final paraffin sections were not regarded as positive nodes if a frozen section failed to detect metastasis. The main purpose of the other studies was to evaluate the sensitivity of the SN concept using the final diagnosis of paraffin sections. Our primary interest was to test the intraoperative applicability of the SN concept in an ordinary clinical setting without any special tools or special staining methods such as immunohistochemistry, because there is a short timeframe during laparotomy in which to make the decision to proceed with gastrectomy. However, additional exploratory study clearly revealed that

**Table 4** False negatives were found in 13 patients by analysis excluding the learning period

| No. | Number of cases in each institute | Tumor location (portion) | Pathological tumor diameter (cm) | Number of GNs | Station number of GNs | Station number of metastatic nodes |
|---|---|---|---|---|---|---|
| 1 | 7 | Lower | 7.0 | 3 | 4d, 6 | 4d |
| 2 | 9 | Upper | 18.0 | 3 | 4d | 1, 3, 4sb, 4d, 7 |
| 3 | 13 | Middle | 4.2 | 12 | 3, 4d 6 | 4d |
| 4 | 11 | Middle | 3.1 | 3 | 3, 11p | 3 |
| 5 | 12 | Lower | 2.8 | 5 | 4d | 4d |
| 6 | 7 | Lower | 2.5 | 3 | 3, 5, 7 | 3 |
| 7 | 9 | Middle | 3.6 | 5 | 3, 4sb | 8a |
| 8 | 16 | Lower | 3.2 | 5 | 3, 7 | 4d, 7 |
| 9 | 11 | Lower | 4.6 | 1 | 8a | 1, 3 |
| 10 | 11 | Lower | 5.4 | 3 | 3, 7 | 3 |
| 11 | 19 | Middle | 3.2 | 6 | 3, 7 | 3, 6 |
| 12 | 23 | Middle | 3.0 | 5 | 4sb, 7 | 4sb, 4d, 6 |
| 13 | 32 | Lower | 3.5 | 6 | 6 | 3, 6 |

Station numbers: No. 1, right paracardial LN; No. 3, LN along the lesser curvature; No. 4sb, LN along the left gastroepiploic vessels; No. 4d, LN along the right gastroepiploic vessels; No. 5, suprapyloric LN; No. 6, infrapyloric LN; No. 7, LN along the left gastric artery; No. 8a, LN along the common hepatic artery (anterosuperior group); No. 11p, LN along the proximal splenic artery

9 of 13 patients with false negatives had metastases in GNs when the GNs were histologically examined in one slice or in serial slices by paraffin sections.

The second issue is the learning period. The planned learning period of only 5 patients in each institution (but not per surgeon) is presumed to be insufficient. A reasonable learning period is considered to be approximately 30 patients at present, as concluded from the survey conducted the Japanese Society for Sentinel Node Navigation Surgery (SNNS). Lee et al. [14] reported a learning period of 26 patients. Of 27 participating institutions in the JCOG0302 trial, only 4 institutions enrolled more than 30 patients. All the 6 node-positive patients with negative GNs in both frozen and paraffin sections were encountered in the first 19 patients in each institution. Nowadays, it is well known that surgeon inexperience was associated with detection failure [15–17]. Unfortunately, we could not find the appropriate learning period in the present trial setting because of limited sample size resulting from termination midway through the trial. It could be a critical point in the present study.

Lee et al. [18] reported that a small number of SNs ($\leq$3 nodes) was associated with false negativity. The median number of GNs in the JCOG0302 trial was 4 nodes per patients. It seems to be moderate because it is impractical for intraoperative histological diagnosis to harvest numerous SNs, which is in conflict with the SN concept, i.e., surgery without lymph node dissection.

Several investigators have argued that lymphatic basin dissection, which is a regional lymphadenectomy of one to two of five basins of the stomach, is better for harvesting SNs than node pickup biopsy adopted in the JCOG0302 trial because metastatic nodes would remain confined to the basins even in the case of false-negative SN technique [7]. However, 7 of 13 patients had nodal metastases outside the lymphatic basin in the present study, although an insufficient learning period might have affected the outcome, i.e., poor identification of lymphatic flow resulted in detection failure of GNs.

The JCOG0302 trial revealed the unreliability of frozen section examination using just one plane and highlighted the impact of the learning curve. Recently, Wang et al. [19] evaluated the diagnostic value of SN biopsy for gastric cancer in the systematic review. They concluded that further studies are needed to confirm the best procedure and standard criteria although the SN concept is technically feasible. A recent report suggested that intraoperative diagnosis using SN biopsy with ICG could be acceptable but with some additional requirements, such as multiple planes for detection, combination use of imprint cytology, and open surgery by experienced surgeons [20]. In terms of limitations of the ICG dye method, such as loss of visibility in dense fat and rapid transit, some novel ICG-based techniques such as infrared electronic endoscopy (IREE) [21, 22] and ICG fluorescence imaging [23–25] have been reported as convenient and reliable detection methods. Moreover, a prospective multicenter clinical trial of a novel semi-automated molecular-based rapid diagnostic method for LN metastases using one-step nucleic acid amplification (OSNA) indicated that the method is feasible for intraoperative detection of LN metastases in patients with gastric cancer (Kumagai et al., submitted). Such new technology could overcome the difficulties of clinical application of the SN technique [26].

## Conclusions

The proportion of false negatives in the present study was unacceptably high. SN biopsy with ICG and intraoperative histological examination of a single plane is not recommended for clinical use in patients with early gastric cancer. Further improvement in the procedure should be explored to apply the SN concept to gastric cancer surgery. The JCOG0302 multicenter trial never denied the SN concept itself.

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**Conflict of interest** The authors declare no conflict of interest.

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# Randomized phase II study of gemcitabine plus S-1 versus S-1 in advanced biliary tract cancer: A Japan Clinical Oncology Group trial (JCOG 0805)

Chigusa Morizane,[1,9] Takuji Okusaka,[1] Junki Mizusawa,[2] Atsuo Takashima,[2] Makoto Ueno,[3] Masafumi Ikeda,[4] Yasuo Hamamoto,[5] Hiroshi Ishii,[6] Narikazu Boku[7] and Junji Furuse[8]

[1]Hepatobiliary and Pancreatic Medical Oncology, National Cancer Center Hospital, Tokyo; [2]Japan Clinical Oncology Group Data Center, Multi-institutional Clinical Trial Support Center, National Cancer Center, Tokyo; [3]Department of Hepatobiliary and Pancreatic Medical Oncology, Kanagawa Cancer Center, Kanagawa; [4]Hepatobiliary and Pancreatic Medical Oncology, National Cancer Center Hospital East, Chiba; [5]Department of Oncology, Tochigi Cancer Center, Tochigi; [6]Hepatobiliary and Pancreatic Division, Cancer Institute Hospital, Tokyo; [7]Department of Clinical Oncology, St. Marianna University School of Medicine, Kanagawa; [8]Department of Medical Oncology, Kyorin University School of Medicine, Tokyo, Japan

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**The oral fluoropyrimidine, S-1, combined with or without gemcitabine is considered to be a promising agent for treating advanced biliary tract cancer; gemcitabine plus cisplatin is the current standard regimen. This randomized phase II trial was designed to evaluate the safety and efficacy of two regimens: gemcitabine plus S-1 (GS) (gemcitabine: 1000 mg/m$^2$, day 1 and day 8; S-1: 60 mg/m$^2$, twice daily on days 1–14, repeated every 3 weeks); and S-1 (80 mg/m$^2$, days 1–28, given orally twice daily for 4 weeks, followed by a 2-week rest, repeated every 6 weeks). The regimen with a higher 1-year survival would be selected for a subsequent phase III trial. Between February 2009 and April 2010, 101 patients were randomized. For the GS ($n$ = 51) and S-1 ($n$ = 50) arms, the 1-year survival was 52.9% (95% confidence interval, 38.5–65.5) and 40.0% (95% confidence interval, 26.5–53.1), and the median survival times were 12.5 and 9.0 months, respectively. Grade 3/4 hematological toxicities were more frequent in the GS arm (leucocytes 29.4%, neutrophils 60.8%, hemoglobin 11.8%, platelets 11.8%) than in the S-1 arm (leucocytes 2.0%, neutrophils 4.0%, hemoglobin 4.0%, platelets 4.0%). Although two treatment-related deaths occurred in the GS arm, all other grade 3/4 non-hematological toxicities were reversible. In conclusion, GS was considered to be more promising and was selected as the test regimen for a subsequent phase III trial comparing GS with gemcitabine plus cisplatin combination therapy. This study was registered at the UMIN Clinical Trials Registry as UMIN 000001685 (http://www.umin.ac.jp/ctr/index.htm). (*Cancer Sci* 2013; 104: 1211–1216)**

Biliary tract cancer (BTC) includes carcinomas of the intrahepatic bile duct (IHBD), extrahepatic bile duct (EHBD), gallbladder (GB), and ampulla of Vater (AV). Biliary tract cancer is relatively rare, but high incidence rates have been reported in eastern Asia. Recently, a rising tendency of BTC incidence, especially in IHBD, was reported in Europe and North America.[1–4] For BTC, curative surgical resection offers the only chance for cure; however, most patients are initially diagnosed with unresectable disease. Even after curative surgery, many patients subsequently develop recurrence.[5] For unresectable or recurrent BTC, gemcitabine, platinum analogues, and fluoropyrimidine have been considered the key drugs for treatment.[5,6]

Until recently, gemcitabine alone was regarded as the standard regimen for the treatment of advanced BTC. However, gemcitabine plus cisplatin combination therapy (GC) has become the new standard regimen based on the results of the ABC-02 trial,[7] which showed superiority in overall survival of patients treated with GC versus gemcitabine alone. A randomized phase II trial (BT22 trial) was carried out in Japan to evaluate both gemcitabine alone and GC in BTC patients.[8] Its outcome was similar to that of the ABC-02 study. From the results of these two clinical studies, GC therapy has been accepted as the standard therapy in Japan.

In a phase II trial for BTC, S-1 monotherapy showed promising results with a response rate (35%) and median survival time (9.4 months) associated with mild toxicity.[9] Therefore, S-1 is considered a promising agent for the treatment of advanced BTC. Kanai *et al.* and Sasaki *et al.* reported two phase II studies of gemcitabine plus S-1 combination therapy (GS) for advanced BTC. In their reports, GS also showed promising response rates (30% and 34%, respectively) and median survival times (12.7 and 11.6 months, respectively).[10,11]

Based on these observations, we planned the present randomized phase II trial. The aim of this study was to evaluate the safety and efficacy of the two regimens and to determine which regimen would be more promising as a test arm regimen in a subsequent phase III trial.

## Materials and Methods

**Patients.** The eligibility criteria for inclusion were as follows: clinical diagnosis of BTC including carcinomas of IHBD, EHBD, GB, and AV; unresectable or recurrent disease; histologically proven adenocarcinoma or adenosquamous carcinoma for patients with EHBD, GB, or AV carcinomas, or adenocarcinoma for IHBD carcinomas; absence of central nervous system metastasis; absence of moderate or severe ascites and/or pleural effusion; no previous chemotherapy or radiotherapy for BTC or other malignancies; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; sufficient oral intake; age 20–79 years; preserved organ functions such as leucocyte count ≥3000/mm$^3$; neutrophil count ≥1500/mm$^3$; hemoglobin level ≥9.0 g/100 mL; platelet count ≥100 000/mm$^3$; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations ≤100 IU/L (≤150 IU/L in patients with biliary drainage); total bilirubin level ≤2 mg/dL (≤3 mg/dL in patients with biliary drainage); creatinine concentration ≤1.2 mg/dL; and written informed consent.

[9]To whom correspondence should be addressed.
E-mail: cmorizan@ncc.go.jp

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Patients with interstitial pneumonia, lung fibrosis, or watery diarrhea were excluded.

This study was approved by the Japan Clinical Oncology Group (JCOG) Protocol Review Committee and the institutional review board of each participating institution (see Appendix 1). The JCOG Data and Safety Monitoring Committee (DSMC), which is a standing committee, monitored patient safety, adverse events, and the progress of the trial.

**Randomization and masking.** We sent the information of each patient to the JCOG Data Center by fax or telephone. The data managers checked the eligibility, completed registration if appropriate, and randomly allocated the patient to either the GS or S-1 treatment group, using a minimization method with an algorithm (concealed to the investigator) to balance the following stratification factors: institution; primary site (GB/others); and clinical stage (II or III/IV or recurrent). The treatment allocation was then communicated to the investigator by fax or telephone. The treatment allocation was not masked from the investigators or the patients. All the data in the case report forms were sent to the JCOG Data Center and were checked by the central data managers.

**Treatment.** The dose and schedule of the GS arm was planned based on those adopted in the randomized phase III study of pancreatic carcinoma carried out in Japan and Taiwan.[12] For the GS arm, 1000 mg/m$^2$ gemcitabine was infused on days 1 and 8, and 30 mg/m$^2$ S-1 (60 mg/day for a body surface area [BSA] <1.25 m$^2$; 80 mg/day for 1.25 ≤ BSA < 1.50 m$^2$; and 100 mg /day for BSA ≥1.50 m$^2$) was given orally twice daily from days 1 to 14, repeated every 3 weeks. For the S-1 monotherapy arm, 40 mg/m$^2$ S-1 (80 mg/day for BSA <1.25 m$^2$, 100 mg/day for 1.25 ≤ BSA < 1.50 m$^2$, and 120 mg/day for BSA ≥1.50 m$^2$) was given orally twice daily for 4 weeks, followed by a 2-week rest, repeated every 6 weeks.

If the patients showed a leucocyte count <2500/mm$^3$, neutrophil count <1000/mm$^3$, platelet count <75 000/mm$^3$, total bilirubin level >3.0 mg/dL, AST and ALT levels >150 IU/L, creatinine level >1.2 mg/dL, diarrhea ≥ grade 2, mucositis in the oral cavity ≥ grade 2, or a rash ≥ grade 3, the initiation of the next cycle was postponed until recovery from those conditions, in both treatment arms. During the cycle, if patients in the GS arm showed a leucocyte count <2000/mm$^3$ or neutrophil count <1000/mm$^3$, platelet count <70 000/mm$^3$, creatinine level ≥1.5 mg/dL, total bilirubin level ≥3.1 mg/dL, diarrhea ≥ grade 2, mucositis in the oral cavity ≥ grade 2, or rash ≥ grade 3, gemcitabine was not given on day 8 and S-1 treatment was suspended. The dose of gemcitabine was reduced to 800 mg/m$^2$ when patients experienced: (i) grade 4 leucopenia or neutropenia; (ii) febrile neutropenia or infection with grade 3 leucopenia or neutropenia; (iii) grade 4 thrombocytopenia or grade 3 thrombocytopenia requiring transfusion; (iv) grade 3 rash; or (v) grade 3 or grade 4 non-hematological adverse reaction. If patients experienced: (vi) creatinine level ≥1.5 mg/dL; (vii) diarrhea ≥ grade 3; (viii) mucositis in the oral cavity ≥ grade 3; or (ix) rash ≥ grade 3, the dose of S-1 was reduced by 20 mg/day in the subsequent cycle. For the S-1 monotherapy arm, when patients experienced any of the above (i–ix), the dose of S-1 was reduced by 20 mg/day in the subsequent cycle. The treatment was discontinued if disease progression was diagnosed clinically or detected by imaging, if a serious adverse event occurred, if a treatment cycle was delayed because of an adverse event longer than 42 days after the final anticancer drug administration in the previous cycle, if subsequent dose reduction was required after a second reduction, if the patient refused treatment, or if the treating doctor judged to discontinue the protocol treatment for other reasons.

Physical examination and laboratory tests were repeated at least on days 1 and 8 of each cycle in the GS arm and at least once every 2 weeks in the S-1 monotherapy arm. All adverse events were evaluated according to the Common Terminology Criteria for Adverse Events, version 3.0. The JCOG DSMC reviewed the serious adverse events and assessed the attribution of adverse events to the treatment in order to judge whether the study was to be continued or to be modified. The tumor response was assessed every 6 weeks according to RECIST (version 1.0). The response rates were calculated without confirmation.

The primary end-point was 1-year survival. The secondary end-points were progression-free survival, the response rate in patients with measurable lesions, the incidence of adverse events, and that of serious adverse events. Serious adverse events were defined as death within 30 days after treatment, treatment-related death (TRD) beyond 30 days after treatment, grade 4 non-hematological toxicities. The overall survival was calculated from the date of randomization to the date of death or censored on date of last contact for surviving patients, and the progression-free survival was counted to the date on which disease progression or death was detected, or was censored on the last date when progression-free status was confirmed. The dose intensity (DI) was defined as the total amount of drug actually given per 1 week (mg/m$^2$/week) during eight cycles (GS arm) or four cycles (S-1 monotherapy arm) from the start of chemotherapy.

**Statistical analysis.** This study adopted selection design,[13] in that the regimen with a higher 1-year survival would be selected. The sample size was determined as follows using Simon's selection design. We assumed that the 1-year survival for one regimen would be 30% and that for the other regimen would be higher than 40%. In this situation, the sample size ensuring a probability of at least 85% for correct selection of the more effective regimen was 98 patients, with 49 patients per arm. Considering the likelihood of some ineligible patients being enrolled, the total number of patients was set at 100. Overall survival and progression-free survival were estimated by using the Kaplan–Meier method, and curves were compared using an unstratified log–rank test. Hazard ratios of treatment effects were estimated by using the unstratified Cox regression model. We carried out all the analyses based on an intention-to-treat using SAS version 9.2 (SAS Institute, Cary, NC, USA). Unless otherwise specified, two-sided *P*-values for superiority were used.

**Role of the funding source.** The sponsor of the study was the Ministry of Health, Labour and Welfare, Japan, which had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit the report for publication.

## Results

From February 2009 to April 2010, 101 patients with BTC (GB, *n* = 38; IHBD, *n* = 35; EHBD, *n* = 20; AV, *n* = 8) were enrolled. Fifty-one patients were allocated to the GS arm and 50 patients were allocated to the S-1 arm (Fig. 1). The baseline characteristics were well balanced between the treatment groups (Table 1). All the individuals had an ECOG PS of 0 or 1. Eighty-nine percent (90/101) of the participants had at least one measurable lesion. Fifteen patients had unresectable stage II or III (locally advanced) disease, 61 patients had stage IV (metastatic) disease, and 25 patients had recurrent disease after curative resection.

**Drug exposure and duration of treatments.** At the data cut-off point (April 2011; median follow-up time for all randomized patients, 10.6 months), four patients in the GS arm and one patient in the S-1 arm were still receiving the protocol treatment. Among the other patients, the median number of



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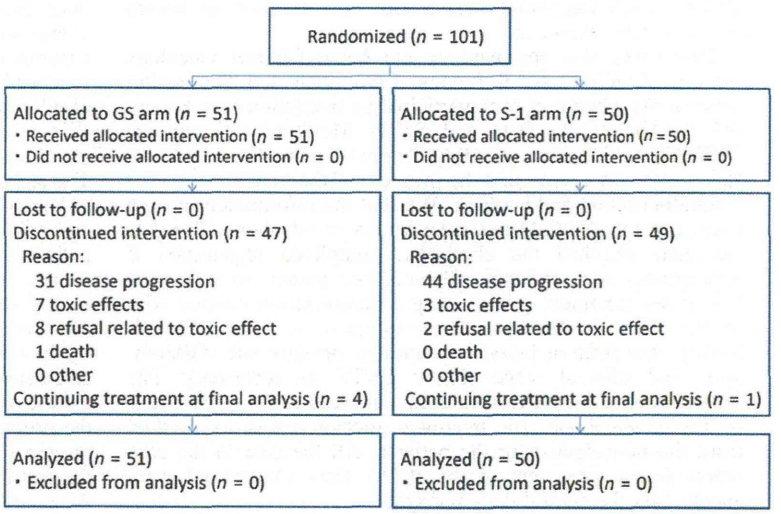


**Fig. 1.** CONSORT diagram showing progress of the randomized phase II study of gemcitabine plus S-1 combination therapy (GS) *versus* S-1 alone in patients with advanced biliary tract cancer.

**Table 1. Characteristics of patients with advanced biliary tract cancer who participated in the randomized phase II study of gemcitabine plus S-1 combination therapy (GS) versus S-1 alone**

| | GS (*n* = 51) | S-1 (*n* = 50) | Total |
|---|---|---|---|
| Age (years) | | | |
| Median (range) | 66.0 (39–78) | 62.5 (49–79) | |
| Gender, *n* (%) | | | |
| Male | 27 (52.9) | 28 (56.0) | 55 |
| Female | 24 (47.1) | 22 (44.0) | 46 |
| PS, *n* (%) | | | |
| 0 | 39 (76.5) | 37 (74.0) | 76 |
| 1 | 12 (23.5) | 13 (26.0) | 25 |
| Target lesion, *n* (%) | | | |
| Present | 44 (86.3) | 46 (92.0) | 90 |
| Absent | 7 (13.7) | 4 (8.0) | 11 |
| Primary tumor, *n* (%) | | | |
| IHBD | 20 (39.2) | 15 (30.0) | 35 |
| EHBD | 9 (17.6) | 11 (22.0) | 20 |
| GB | 19 (37.3) | 19 (38.0) | 38 |
| AV | 3 (5.9) | 5 (10.0) | 8 |
| Stage, *n* (%) | | | |
| II or III | 8 (15.7) | 7 (14.0) | 15 |
| IV | 29 (56.9) | 32 (64.0) | 61 |
| Recurrent | 14 (27.5) | 11 (22.0) | 25 |
| Biliary drainage, *n* (%) | | | |
| – | 32 (62.7) | 31 (62.0) | 63 |
| + | 19 (37.3) | 19 (38.0) | 38 |

AV, ampulla of Vater; EHBD, extrahepatic biliary duct; GB, gallbladder; IHBD, intrahepatic biliary duct; PS, performance status.

cycles of GS given was 10 (range, 1–34; interquartile range, 3–14) and that of S-1 was 3 (range, 1–9; interquartile range, 1–4). At the data cut-off point, 95% (96/101) of the patients terminated the protocol treatment. The protocol treatment was terminated because of disease progression in 61% (31/51) of the patients in the GS arm and 88% (44/50) in the S-1 arm. Termination because of adverse events was observed in 29.4% (15/51) in the GS arm and 10.0% (5/50) in the S-1 arm (Fig. 1). The median DI of gemcitabine and S-1 was 641.5 mg/m$^2$/week (96.2% of planned DI) and 258.8 mg/m$^2$/week (92.4% of planned DI) in the GS arm, and the median dose intensity of S-1 was 358.3 mg/m$^2$/week (96.0% of planned DI) in the S-1 arm.

**Table 2. Adverse events (CTCAE version 3.0) recorded in patients with advanced biliary tract cancer within 6 months after randomization of gemcitabine plus S-1 combination therapy (GS) or S-1 alone**

| | GS (*n* = 51) | | | S-1 (*n* = 50) | | |
|---|---|---|---|---|---|---|
| | G3 (%) | G4 (%) | All grades (%) | G3 (%) | G4 (%) | All grades (%) |
| Leucocytes | 29.4 | 0.0 | 90.2 | 2.0 | 0.0 | 40.0 |
| Hemoglobin | 9.8 | 2.0 | 82.4 | 4.0 | 0.0 | 66.0 |
| Platelets | 5.9 | 5.9 | 51.0 | 0.0 | 4.0 | 22.0 |
| Neutrophils | 43.1 | 17.6 | 88.2 | 4.0 | 0.0 | 40.0 |
| Bilirubin | 9.8 | 0.0 | 52.9 | 14.0 | 0.0 | 64.0 |
| ALP | 7.8 | 0.0 | 70.6 | 12.0 | 2.0 | 76.0 |
| AST | 11.8 | 0.0 | 72.5 | 12.0 | 2.0 | 70.0 |
| ALT | 13.7 | 0.0 | 64.7 | 12.0 | 0.0 | 62.0 |
| Creatinine | 0.0 | 0.0 | 29.4 | 0.0 | 0.0 | 12.0 |
| Fatigue | 7.8 | 0.0 | 56.9 | 4.0 | 0.0 | 62.0 |
| Anorexia | 7.8 | 0.0 | 51.0 | 6.0 | 0.0 | 60.0 |
| Nausea | 2.0 | 0.0 | 35.3 | 4.0 | 0.0 | 52.0 |
| Vomiting | 2.0 | 0.0 | 13.7 | 0.0 | 0.0 | 28.0 |
| Rash | 9.8 | 0.0 | 39.2 | 2.0 | 0.0 | 16.0 |
| Fever | 0.0 | 0.0 | 39.2 | 2.0 | 0.0 | 26.0 |
| Mucositis (oral cavity) | 5.9 | 0.0 | 25.5 | 0.0 | 0.0 | 18.0 |
| Cheilitis | 0.0 | – | 15.7 | 0.0 | – | 16.0 |
| Hyperpigmentation | – | – | 23.5 | – | – | 32.0 |
| Taste alteration | – | – | 15.7 | – | – | 18.0 |
| Diarrhea | 2.0 | 0.0 | 19.6 | 6.0 | 0.0 | 34.0 |
| Constipation | 0.0 | 0.0 | 31.4 | 0.0 | 0.0 | 12.0 |
| Alopesia | – | – | 13.7 | – | – | 2.0 |
| Pruritus | 0.0 | – | 11.8 | 0.0 | – | 8.0 |
| Infection with normal ANC | 7.8 | 0.0 | 19.6 | 10.0 | 2.0 | 12.0 |
| Infection with grade 3 or 4 ANC | 4.0 | 0.0 | 7.8 | 0.0 | 0.0 | 0.0 |
| Febrile neutropenia | 2.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 |

Events with a frequency of more than 10.0% or high-grade events (grades 3,4) are listed. –, not applicable; ALP, alkaline phosphatase; AST, aspartate amino transferase; ALT, alanine aminotransferase; ANC, absolute neutrophil count; CTCAE, Common Terminology Criteria for Adverse Events.

**Safety.** Table 2 shows the adverse events recorded within 6 months after randomization. For patients assigned to the GS arm, grade 3 or 4 leucopenia (29.4%) and neutropenia (60.8%)