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


1Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo; 2Department of Clinical Oncology, St. Marianna University School of Medicine, Kawasaki; 3Department of Gastrointestinal Medical Oncology, Shikoku Cancer Center, Matsuyama; 4Department of Gastroenterology, Saitama Cancer Center, Kita-adachi; 5Department of Gastroenterology, Chiba Cancer Center, Chiba; 6Department of Clinical Oncology, Kobe City Medical Center General Hospital, Kobe; 7Department of Gastroenterology, Keio University, School of Medicine, Tokyo; 8Department of Gastroenterology, Showa University, School of Medicine, Tokyo; 9Department of Clinical Oncology, Tonan Hospital, Sapporo; 10Department of Gastroenterology, Ibaraki Prefectural Central Hospital, Kassama; 11Department of Hepatobiliary and Pancreatic Oncology, Kanagawa Cancer Center, Yokohama; 12Department of Gastroenterology, Yokohama Municipal Citizen’s Hospital, Yokohama; 13Department of Gastrooncological Surgery, Hyogo Cancer Center, Akashi; 14Department of Clinical Oncology, Aichi Cancer Center Hospital, Nagoya; 15JCOG Data Center/Operations Office, National Cancer Center, Tokyo; 16National 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 \(\geq 1\) (HR 1.45; 95% CI 1.13–1.86; \(P=0.004\)), and number of metastatic sites \(\geq 2\) (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...
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 anticancer 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 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
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 ($P = 0.058$). 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

<table>
<thead>
<tr>
<th>mRNA Group</th>
<th>mRNA Group</th>
<th>All</th>
<th>5-Fluorouracil</th>
<th>S-1</th>
<th>Irinotecan plus cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n RR (%)</td>
<td>P</td>
<td>n RR (%)</td>
<td>P</td>
<td>n RR (%)</td>
</tr>
<tr>
<td>ERCC1 Low</td>
<td>120</td>
<td>34.2</td>
<td>0.064</td>
<td>40</td>
<td>17.5</td>
</tr>
<tr>
<td>High</td>
<td>123</td>
<td>22.8</td>
<td>0.058</td>
<td>40</td>
<td>32.5</td>
</tr>
<tr>
<td>TS Low</td>
<td>120</td>
<td>33.3</td>
<td>0.15</td>
<td>42</td>
<td>16.7</td>
</tr>
<tr>
<td>High</td>
<td>120</td>
<td>24.2</td>
<td>0.068</td>
<td>45</td>
<td>26.7</td>
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<tr>
<td>DPD Low</td>
<td>119</td>
<td>31.9</td>
<td>0.39</td>
<td>46</td>
<td>15.2</td>
</tr>
<tr>
<td>High</td>
<td>113</td>
<td>26.6</td>
<td>0.24</td>
<td>41</td>
<td>41.5</td>
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<tbody>
<tr>
<td>n</td>
<td>n mPFS (months)</td>
<td>HR (95% CI)</td>
<td>P</td>
<td>n mPFS (months)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>ERCC1 Low</td>
<td>162</td>
<td>4.78</td>
<td>0.31</td>
<td>53</td>
<td>3.81</td>
</tr>
<tr>
<td>High</td>
<td>160</td>
<td>3.89</td>
<td>1.12(0.90–1.40)</td>
<td>50</td>
<td>2.07</td>
</tr>
<tr>
<td>TS Low</td>
<td>159</td>
<td>5.10</td>
<td>0.015</td>
<td>54</td>
<td>3.71</td>
</tr>
<tr>
<td>High</td>
<td>158</td>
<td>3.81</td>
<td>1.32(1.06–1.65)</td>
<td>47</td>
<td>2.10</td>
</tr>
<tr>
<td>DPD Low</td>
<td>154</td>
<td>4.22</td>
<td>0.97</td>
<td>57</td>
<td>2.14</td>
</tr>
<tr>
<td>High</td>
<td>150</td>
<td>4.21</td>
<td>1.00(0.80–1.26)</td>
<td>37</td>
<td>3.58</td>
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<tr>
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<tr>
<td>n</td>
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<td>HR</td>
<td>P</td>
<td>n MST (months)</td>
<td>HR (95% CI)</td>
</tr>
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<td>ERCC1 Low</td>
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<td>14.9</td>
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</tr>
<tr>
<td>TS Low</td>
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<td>0.034</td>
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<td>11.0</td>
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<td>High</td>
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<td>11.5</td>
<td>1.28(1.02–1.61)</td>
<td>47</td>
<td>11.8</td>
</tr>
<tr>
<td>DPD Low</td>
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<td>11.9</td>
<td>0.64</td>
<td>57</td>
<td>11.5</td>
</tr>
<tr>
<td>High</td>
<td>150</td>
<td>13.1</td>
<td>0.95(0.75–1.20)</td>
<td>37</td>
<td>11.8</td>
</tr>
</tbody>
</table>

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.
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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**Disclosure**

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**References**

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 $\alpha$-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 $\mu$mol/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, D.

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


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

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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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