Phase II study of a triplet regimen of S-1 combined with irinotecan and oxaliplatin in patients with metastatic gastric cancer: clinical and pharmacogenetic results

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Received 15 April 2010; revised 23 June 2010; accepted 5 July 2010

Background: The aim of this study was to investigate the efficacy and safety of S-1/irinotecan/oxaliplatin (TIROX) in metastatic gastric cancer (MGC) and the association between treatment outcome and uridine diphosphate-glucuronosyltransferase (UGT) 1A polymorphisms.

Patients and methods: Patients with previously untreated MGC received S-1 40 mg/m² b.i.d. on days 1–14 and irinotecan 150 mg/m² plus oxaliplatin 85 mg/m² on day 1 every 3 weeks.

Results: Forty-four patients were enrolled. In intent-to-treat analysis, the objective response rate was 75%, including the complete response (CR) rate of 14%. The median time to progression and overall survival was 10.2 and 17.6 months, respectively. Ten (26%) of the 39 patients with primary gastric tumor showed biopsy-confirmed gastric CR. Grade 3/4 neutropenia developed in 66% of patients and grade 3 febrile neutropenia in 16%. The most common grade 3 nonhematologic toxic effects were abdominal pain (18%), anorexia (16%), and diarrhea (14%). UGT1A polymorphisms were associated with significantly higher incidence of grade 4 leukopenia (UGT1A1*6), neutropenia (UGT1A1*6, UGT1A6*2, and UGT1A7*3), grade 3/4 febrile neutropenia (UGT1A1*6), and grade 3 abdominal pain (UGT1A1*6).

Conclusions: The TIROX regimen induced marked tumor reduction and promising survival with a manageable toxicity profile in MGC patients. UGT1A genotype may be predictive of TIROX toxicity.

Key words: gastric cancer, irinotecan, oxaliplatin, S-1, uridine diphosphate-glucuronosyltransferase
a phase II study of TIROX as first-line chemotherapy in patients with MGC and we examine the association between treatment outcome and polymorphisms of uridine diphosphate-glucuronosyltransferase 1A (UGT1A1), a key irinotecan metabolizer.

patients and methods

eligibility

Patients were eligible for this study if they were older than 18 years and had histologically confirmed gastric adenocarcinoma, metastatic disease, measurable disease by the RECIST 1.0 [14], Eastern Cooperative Oncology Group (ECOG) performance status of zero to two, and adequate baseline bone marrow, hepatic, and renal function. In addition, they had to be previously untreated for recurrent or metastatic disease, but prior adjuvant therapy except for oxaliplatin or irinotecan was allowed if at least 12 months had elapsed between its completion and enrollment into this study.

All patients provided written informed consent, and this study was approved by the Institutional Review Board at the National Cancer Center, Republic of Korea.

treatment schedule

According to the recommended dose determined in the TIROX phase I study, patients received oral S-1 40 mg/m² twice daily (within the hour following the morning and evening meals) on days 1–14, a 90-min intravenous infusion of irinotecan 150 mg/m², and a 120-min intravenous infusion of oxaliplatin 85 mg/m² in a 3-week cycle. Prophylactic administration of granulocyte-colony stimulating factor was not allowed. Treatment was continued in the absence of disease progression or unacceptable toxicity for a maximum of 12 cycles.

dose modifications

The next chemotherapy cycle was delayed if patients had absolute neutrophil count <1500/mm³, platelet count <100 000/mm³, or any nonhematologic toxicity over grade 1, excluding alopecia. The doses of S-1, irinotecan, and oxaliplatin were reduced by 20% in subsequent cycles if a patient experienced grade 3 or 4 neutropenia associated with infection or fever, grade 4 thrombocytopenia, or grade 3 nonhematologic toxicity. The oxaliplatin dose was reduced to 75 mg/m² in case of persisting grade 2 peripheral neuropathy between cycles or grade 3 peripheral neuropathy lasting >7 days but not persisting between cycles. Oxaliplatin was discontinued permanently in case of persisting grade 3 neuropathy between cycles or grade 4 neuropathy. In case of grade 4 nonhematologic toxicity, treatment was discontinued or continued at half doses if it was the best interest of the patient.

assessment of efficacy and toxicity

Complete blood cell counts with differential and serum chemistry and electrolytes were carried out weekly during the first two chemotherapy cycles and every 3 weeks thereafter. A computed tomography (CT) scan was carried out every two cycles or when required for evaluation of the response to treatment, which was assessed according to RECIST 1.0 [14]. In patients who were in objective response or stable disease at the time of discontinuation of study therapy, CT scans were carried out every 6–8 weeks until documented disease progression became evident. Toxic effects were graded using the National Cancer Institute Common Toxicity Criteria (version 3.0).

genotyping assay

Genomic DNA was extracted from whole blood with a QIAamp Blood Kit (Qiagen, Hilden, Germany). Appropriate primers were designed for UGT1A1, UGT1A6, and UGT1A7. The included polymorphic sites were the following: UGT1A1 [211G>A (UGT1A1*6, rs4148323)], −53[T>A] (UGT1A1*28, rs8175347), and −3279T>G (UGT1A1*50, rs4124874); UGT1A6 [197T>G (rs6759892), 315A>G (rs1103880), 541A>G (rs2070939), and 552A>C (rs1103879)]; UGT1A7 [387T>G (rs17868323), 391G>A (rs17863778), 392G>A (rs17683324), and 622T>C (rs11692021)]. The polymerase chain reaction (PCR) reaction was carried out with a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA). Sequencing was carried out with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.0, on an automated ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Primer sequences and genotyping details are available on request.

statistical analysis

The primary end point was the overall response rate, and the secondary end points were TTP, OS, and the association between UGT1A genotype and treatment outcome. We used a two-stage minimax design proposed by Simon et al. [15] to determine the sample size. Assuming P0 = 0.3, P1 = 0.5, with α-error = 0.05 and β-error = 0.2, the first stage required at least 7 of the 19 patients to have a response before proceeding to the second stage. In the second stage, an additional 20 assessable patients were to be enrolled and if 17 or more of the 39 assessable patients had a response, the treatment would be considered sufficiently effective. On the assumption that 10% of patients would not be evaluable, the planned sample size was 44 patients.

We defined TTP as the time between the date of the initiation of treatment and the date of documented disease progression and OS as the time between the initiation of treatment and death from any cause or the last follow-up visit. We used the Kaplan–Meier method in all survival analyses and the Pearson χ² test or Fisher’s exact test to compare proportions of patients who had treatment response or toxicity according to the genotype factors. We considered a two-sided P value < 0.05 as significant. We used the χ² test to observe Hardy–Weinberg equilibrium, PHASE software v2.1 to analyze haplotypes [16, 17], and Haploview program, v3.2, to determine linkage disequilibrium between gene polymorphisms [18].

results

patient characteristics

From June 2007 to December 2007, 44 patients were enrolled. Their median age was 54 years (range, 27–66); Table 1 shows their clinical characteristics. Most of the patients (95%) had an ECOG performance status of one, and all had metastatic disease. The majority (68%) had multiple metastases involving two or more organ systems, with five having recurrent metastatic disease after prior curative gastrectomy.

treatment administration

A total of 393 cycles were administered, with a median of 11 cycles per patient (range, 1–12). The median dose intensities were 333.6 mg/m²/week (89.4% of planned) for S-1, 47.4 mg/m²/week (94.8% of planned) for irinotecan, and 26.9 mg/m²/week (94.9% of planned) for oxaliplatin. Treatments were discontinued for disease progression (n = 18, 41%), death unrelated to treatment [n = 2 (one in a motorcycle accident and one from tumor bleeding), 5%), toxicity [n = 1 (oxaliplatin-induced hepatotoxicity with ascites), 2%], and loss to follow-up (n = 2, 5%). The remaining 21 patients (48%) finished the planned maximum of 12 cycles and are being followed.
**efficacy**

Two of the patients were lost to follow-up after the first chemotherapy cycle. Six (14%) achieved a complete response (CR) and 27 (61%) achieved a partial response (PR), for an overall response rate of 75% [95% confidence interval (CI) 62% to 88%] in intent-to-treat analysis. All objective responses were confirmed by follow-up CT at least 4 weeks after the initial response documentation. Four patients (9%) had an unconfirmed PR, two (5%) had stable disease, and three (7%) had progressive disease. Figure 1 depicts a waterfall plot of the maximum percent reduction in tumor size in metastatic target lesions per RECIST [14]. All patients but one showed a reduction of tumor burden in target lesions. The median tumor burden change from baseline was −75.3% (range, −100% to 3.5%). The median duration of response was 9.9 months (range, 2.8–23.5+).

The median follow-up time was 23.8 months (range, 9.9–26.4). The median TTP was 10.2 months (95% CI 7.7–12.7 months) and the median OS was 17.6 months (95% CI 9.0–26.2 months) (Figure 2). The 2-year survival rate was 47.7% (95% CI 33.0% to 62.4%), and 19 patients (43%) remain alive at this writing. Patients who achieved CR compared with those who did not had a better TTP [median, 17.2 (95% CI not reached) vs 9.1 months (95% CI 5.4–12.8); \( P = 0.004 \)] (Figure 3A) and OS [median, 24.5 (95% CI not reached) vs 15.8 months (95% CI 12.5–19.1); \( P = 0.039 \)] (Figure 3B).

Ten (26%) of the 39 patients who had primary gastric tumor lesions showed a biopsy-confirmed complete regression of the lesion (gastric CR). Patients who achieved gastric CR compared with those who did not had a better TTP [median, 14.4 (95% CI 11.8–17.0) vs 7.4 months (95% CI 5.3–9.5); \( P = 0.006 \)] (Figure 3C) and OS [median, 24.5 (95% CI 2.5–46.5) vs 12.9 months (95% CI 9.5–16.3); \( P = 0.004 \)] (Figure 3D).

**toxicity**

All 44 patients were assessable for toxicity. Table 2 summarizes the chemotherapy toxic effects encountered. The most common grade 3 or 4 hematologic toxicity was neutropenia (66%). The seven patients (16%) who had grade 3 febrile neutropenia experienced just 1 or 2 days of fever and were successfully treated with antibiotics and granulocyte-colony stimulating factor. Nonhematologic toxic effects were generally mild to moderate and manageable. No patient experienced grade 4 nonhematologic toxicity and the most common grade 3 nonhematologic toxicity was abdominal pain (8, 18%). No patient experienced grade 3 or 4 peripheral neuropathy, while grade 1 neuropathy occurred in 32 patients (73%) and grade 2 occurred in 5 patients (11%). Grade 1 hand–foot syndrome developed in eight patients (18%) while no higher grade occurred.

**UGT1A genotype and allele frequencies**

The UGT1A1, UGT1A6, and UGT1A7 variants typed in this study and their genotype frequencies were as follows:

![Waterfall plot depicting the maximum percentage change in tumor measurements for target lesions from baseline per RECIST.](https://academic.oup.com/annonc/article-abstract/22/4/890/214486)
Figure 2. Kaplan–Meier plot for (A) time to progression and (B) overall survival.

Figure 3. Kaplan–Meier plot according to overall complete response (CR) for (A) time to progression and (B) overall survival and according to gastric CR for (C) time to progression and (D) overall survival.
We have shown here that a new triplet combination—TIROX, consisting of S-1 plus irinotecan and oxaliplatin—was a highly active first-line chemotherapy regimen for MGC. The overall response rate of 75% (14% CR, 61% PR), median TTP of 10.2 months, and median OS of 17.6 months are encouraging, even with the phase II study limitation of a small number of patients. Most patients had a marked reduction of tumor burden from baseline, with a median reduction of 73.3% in target lesions. Moreover, this decrease occurred early in treatment; most of responders (88%) who had at least a PR achieved it after the second chemotherapy cycle.

It is noteworthy that the TIROX regimen was as active at the primary lesion as at the metastatic sites. All six patients who had a CR had it for both types of lesions, and an additional four patients had CR of the primary gastric tumor. Although endoscopic biopsy-confirmed CR cannot exclude the possibility of residual disease, considering the low CR rate in most palliative and neoadjuvant chemotherapy settings, a 26% gastric CR rate and a 14% overall CR rate indicates that the TIROX regimen showed high antitumor activity. Interestingly, patients who achieved overall and/or gastric CR had a superior TTP and OS compared with those who did not (Figure 3), which is similar to the finding that patients with pathological CR after neoadjuvant chemoradiotherapy have a better survival rate than those who have less than a pathological CR [19, 20]. Similarly, a survival advantage associated with CR after systemic treatment either alone or with a multimodal approach has been demonstrated in locally advanced or metastatic colorectal cancer [21]. Thus, the introduction of therapy that is able to induce CR more frequently may improve survival in MGC.

Many studies have investigated whether a new active agent such as taxane, irinotecan, or oxaliplatin in a triplet regimen would increase treatment efficacy. A recent phase III trial showed that compared with a doublet of CF, a triplet of docetaxel, cisplatin, and 5-FU (DCF) had a superior response rate (37% versus 25%; P = 0.01), TTP (median, 5.3 versus 3.7 months; P = 0.001), and OS (median, 9.2 versus 8.6 months; P = 0.02) [22]. Those modest benefits, however, were accompanied by more severe toxic effects, including grade 3 or 4 neutropenia (82% versus 57%), complicated neutropenia (29% versus 12%), and grade 3 or 4 diarrhea (19% versus 8%) [22]. In recent phase II studies, a triplet regimen of biweekly 5-FU/LV/oxaliplatin/irinotecan (FOLFOXIRI) showed encouraging efficacy with a 33%–66.7% response rate, higher OS (median, 11.9–14.8 months) [10–12]. While FOLFOXIRI showed a moderate hematologic toxicity profile with 49%–56% of patients showing grade 3 or 4 neutropenia and 42.8%–73% showing febrile neutropenia, nonhematologic toxicity such as grade 3 emesis occurred in 20%–43.8% of patients despite prophylactic antiemetic treatment. In patients with metastatic colorectal cancer, FOLFOXIRI was also associated with high nonhematologic toxicity (16%–27.2% of treated patients had grade 3/4 diarrhea and 19%–37% had grade 2/3 peripheral neurotoxicity) and moderate hematologic toxicity (35%–59% had grade 3/4 neutropenia and 5%–12% had febrile

<table>
<thead>
<tr>
<th>Toxicity (NCI–CTC)</th>
<th>No. of patients (%)</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
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<tr>
<td>Leukopenia</td>
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<td>19 (43)</td>
<td>5 (11)</td>
<td>4 (9)</td>
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<tr>
<td>Neutropenia</td>
<td>7 (16)</td>
<td>7 (16)</td>
<td>21 (48)</td>
<td>8 (18)</td>
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<tr>
<td>Anemia</td>
<td>30 (68)</td>
<td>6 (14)</td>
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<tr>
<td>Thrombocytopenia</td>
<td>15 (34)</td>
<td>4 (9)</td>
<td>3 (7)</td>
<td>3 (7)</td>
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<tr>
<td>Nausea</td>
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<td>—</td>
<td>7 (16)</td>
<td>0 (0)</td>
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<tr>
<td>Vomiting</td>
<td>19 (43)</td>
<td>14 (32)</td>
<td>8 (18)</td>
<td>0 (0)</td>
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<tr>
<td>Diarrhea</td>
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<td>15 (34)</td>
<td>6 (14)</td>
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<td>Stomatitis</td>
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<td>Anorexia</td>
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<td>21 (48)</td>
<td>7 (16)</td>
<td>0 (0)</td>
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<tr>
<td>Nausea</td>
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<td>14 (32)</td>
<td>2 (5)</td>
<td>0 (0)</td>
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<tr>
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<td>23 (52)</td>
<td>2 (5)</td>
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<td>Alopecia</td>
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<td>Hand–foot syndrome</td>
<td>8 (18)</td>
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<td>0 (0)</td>
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<tr>
<td>Peripheral neuropathy</td>
<td>32 (73)</td>
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<tr>
<td>AST/ALT elevation</td>
<td>17 (39)</td>
<td>4 (9)</td>
<td>0 (0)</td>
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<tr>
<td>Hyperbilirubinemia</td>
<td>7 (16)</td>
<td>3 (7)</td>
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<td>Infection without neutropenia</td>
<td>0 (0)</td>
<td>3 (7)</td>
<td>2 (5)</td>
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</table>

**association of UGT1A genotype with treatment toxicity and efficacy**

We examined the association of UGT1A genotype with the toxicity profile of the first cycle for all 44 patients (Table 3). Patients with UGT1A1*6 showed a significantly higher incidence of grade 4 leu kopenia, grade 4 neutropenia, grade 3/4 febrile neutropenia, grade 2/3 anoxemia, grade 2/3 nausea, and grade 3 abdominal pain. Patients with UGT1A6*2 or UGT1A7*3 showed a significantly higher incidence of grade 4 neutropenia and a trend for a high incidence of grade 2/3 nausea. Neither UGT1A1*28 or UGT1A1*60, however, was significantly associated with toxicity. Regarding treatment efficacy, no significant difference was observed between genotypes (data not shown).
neutropenia) [23–25]. Given that the current study included weekly blood tests for the first two cycles and a long treatment duration (median 3-week cycles, 11; median treatment duration, 9.5 months), TIROX had a generally tolerable toxicity profile, with the high relative dose intensities (89.4% for S-1, 94.8% for irinotecan, 94.9% for oxaliplatin). Grade 3/4 neutropenia was seen in 66% of patients, and although 16% had grade 3 febrile neutropenia, the fever was generally of short duration (median, 1 day), easily managed, and not accompanied by significant bleeding. The most common nonhematologic toxic effects—grade 3 abdominal pain (18%) and diarrhea (14%)—were also manageable. While most oxaliplatin-containing regimens cause considerable cumulative neurotoxicity, which is the major dose-limiting toxicity (grade 2/3 incidence in gastric cancer, 19.5%–37%; grade 3/4, 0%–21%) [8, 25–27], TIROX did not cause grade 3 neurotoxicity and only 11% of patients experienced grade 2 neurotoxicity despite a long treatment duration. This may be attributable to the relatively low oxaliplatin dose (85 mg/m² every 3 weeks, 28.3 mg/m²/week) compared with the usual biweekly dose (42.5 mg/m²/week).

Identifying patients at high risk for developing severe toxicity after intensive triplet regimens such as TIROX would help improve the therapeutic index. Our finding of an association between UGT1A genotype and TIROX toxicity is in keeping with other studies that show a similar genotype association with irinotecan, a major component of TIROX [28, 29]. During the first cycle, patients with UGT1A1*6 genotype had a significantly higher incidence of several hematologic and nonhematologic toxic effects, while patients with UGT1A6*2 or UGT1A7*3 genotype had a significantly higher incidence of only one, and those with UGT1A1*28 showed no significant association with any toxicity. These results are consistent with those of previous studies in Asians. Although UGT1A1*28 genotype is highly prevalent in Caucasians (frequency, 0.3–0.4) and associated with severe irinotecan toxicity, it has much lower frequency in Asians (0.07–0.14) and is consequently less important clinically [29–32]. UGT1A1*6, on the other hand, has a higher frequency than UGT1A1*28 in Asians (0.13–0.24 versus 0.07–0.14) and therefore is more relevant to the outcome of irinotecan-containing treatments in Asian patients [31–34]. Although the UGT1A6*2 and UGT1A7*3 allele were also associated with a higher incidence of grade 4 neutropenia, we observed high linkage disequilibrium across UGT1A6*2, UGT1A7*3, and UGT1A1*6 ($D’ = 1.0$), to which we attributed to the specific phenotype of these polymorphisms in this study. These data have important implications for selecting those patients who would most benefit from TIROX.

In summary, the new triplet TIROX outpatient regimen markedly reduced tumor burden in both primary and metastatic sites and showed promise of improved survival, with a manageable toxicity profile for MGC patients. Given that achievement of CR as either a gastric or overall tumor response is associated with a survival advantage, the high CR rate induced by TIROX suggests promise for both palliative and neoadjuvant chemotherapy in initially unresectable gastric cancer. In addition, although our results are preliminary, they also suggest that genotyping for UGT1A polymorphisms might be important for predicting severe toxic responses to TIROX. Larger studies are needed to confirm these findings.

**funding**

Research Institute and Hospital, National Cancer Center, Republic of Korea (grants 0710650, 1010180).

**acknowledgements**

S-1, irinotecan, and oxaliplatin were provided by Boryung Inc., Pfizer Korea/CJ Corp., and sanofi-aventis Korea, respectively.
disclosure

The authors declare no conflict of interest.

references