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Research article

Modified FOLFOX-6 chemotherapy in advanced gastric cancer: Results of phase II study and comprehensive analysis of polymorphisms as a predictive and prognostic marker

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Abstract

Background: The objective of this study was to evaluate the efficacy and toxicity of infusional 5-fluorouracil (5-FU), folinic acid and oxaliplatin (modified FOLFOX-6) in patients with advanced gastric cancer (AGC), as first-line palliative combination chemotherapy. We also analyzed the predictive or prognostic value of germline polymorphisms of candidate genes associated with 5-FU and oxaliplatin.

Methods: Seventy-three patients were administered a 2 hour infusion of oxaliplatin (100 mg/m^2) and folinic acid (100 mg/m^2) followed by a 46 hour continuous infusion of 5-FU (2,400 mg/m^2). Genomic DNA from the patients' peripheral blood mononuclear cells was extracted. Ten polymorphisms within five genes were investigated including TS, GSTP, ERCC, XPD and XRCC.

Results: The overall response rate (RR) was 43.8%. Median time to progression (TTP) and overall survival (OS) were 6.0 months and 12.6 months, respectively. Toxicities were generally tolerable and manageable. The RR was significantly higher in patients with a 6-bp deletion homozygote (-6 bp/-6 bp) in TS-3'UTR (55.0% *vs.* 30.3% in +6 bp/+6 bp or +6 bp/-6 bp, $p = 0.034$), and C/A or A/A in XPD156 (52.0% *vs.* 26.1% in C/C, $p = 0.038$). The -6 bp/-6 bp in TS-3'UTR was significantly associated with a prolonged TTP and OS. In a multivariate analysis, the 6-bp deletion in TS-3'UTR was identified as an independent prognostic marker of TTP (hazard ratio = 0.561, $p = 0.032$).

Conclusion: Modified FOLFOX-6 chemotherapy appears to be active and well tolerated as first line chemotherapy in AGC patients. The 6-bp deletion in TS-3'UTR might be a candidate to select patients who are likely to benefit from 5-FU based modified FOLFOX-6 in future large scale trial.

Background

Despite improvements in the early detection of gastric cancer, a significant proportion of patients present with inoperable stages where chemotherapy is required. 5-fluorouracil (5-FU) remains the main chemotherapeutic agent for the treatment of gastric cancer, and combination chemotherapy with 5-FU has shown an improved clinical outcomes [1]. 5-FU with cisplatin showed an effective clinical outcome [2], however, toxicities were considerable [1]. Oxaliplatin, another platinum based agent, has a more favorable tolerability profile than cisplatin. Hence, a combination chemotherapy of 5-FU with oxaliplatin has been investigated in numerous phase II studies, using different doses and schedules [3-7]. However it remains to be clarified which is the best combination, with the highest efficacy and lowest toxicity. Thus, we conducted a phase II trial of 5-FU, folinic acid and oxaliplatin (a modified FOLFOX-6 regimen) in advanced gastric cancer (AGC) patients as a first line palliative chemotherapy.

Another problem in chemotherapy of AGC is the selection of patients who might benefit from specific chemotherapy. One promising therapeutic challenge is to identify genetic markers based on pharmacogenomics. Genomic polymorphism can influence drug transport, metabolism and cellular response, and lead to individual variations in terms of the response and toxicity and even to overall survival [8,9]. A number of studies have investigated the relationships between treatment outcomes and individual genetic polymorphisms which will determine the efficacies and toxicities of chemotherapeutic agents, especially of 5-FU and platinum agents.

The antitumor effect of 5-FU has ascribed to the competitive inhibition of thymidylate synthase (TS) [10]. A high intratumoral TS expression has been correlated with resistance to 5-FU and a poor clinical outcome in colorectal cancer [11-14]. Several polymorphisms in TS may influence TS mRNA transcription, stability, or protein expression. Polymorphisms with double or triple repeats of a 28-base pair (bp) sequence in the enhancer region (ER) are known to be associated with the efficacy and toxicity of 5-FU [15-17]. The -6 bp/-6 bp deletion polymorphism in the 3'UTR of TS is associated with decreased mRNA stability in vitro and lower intratumoral TS expression *in vivo*. Further, the 6 bp polymorphism varies greatly within different ethnic populations and is in linkage disequilibrium with the TS 5' tandem repeat enhancer polymorphism [18]. A functional G/C single nucleotide polymorphism (SNP) within a second repeat of triple repeat (3R) allele was found to determine two additional alleles (3G or 3C) at this locus [19]. *In vitro*, the 3G containing genotype showed a higher TS mRNA expression [19,20].

Oxaliplatin has antitumor activity by virtue of its ability to form platinum-DNA adducts. Bulky platinum-DNA adducts are mainly repaired by the nucleotide excision repair pathway, in which proteins of the excision repair cross-complementation 1 (ERCC1), xeroderma pigmentosum group D (XPD, also known as ERCC2) and X-ray repair cross-complementing group (XRCC), have important roles [13,21]. ERCC, XPD and XRCC contain SNPs that may confer different activities to platinum agents, thus modifying the clinical outcome [22-24]. Glutathione S- transferase π 1 (GSTP1), which is involved in platinum detoxification, also has a polymorphism that is associated with prolonged survival in cisplatin-treated gastric cancer [25,26].

The primary endpoint of this study was to evaluate the efficacy in terms of response rate and the secondary endpoints of this study were to evaluate the efficacy in terms of time to progression, overall survival and toxicity of modified FOLFOX-6 chemotherapy in AGC patients. Exploratory pharmacogenomic collateral study was performed to identify the predictive or prognostic value of germline polymorphisms of candidate genes associated with 5-FU and oxaliplatin.

Methods

Patients

Patients with metastatic or relapsed AGC were enrolled in this prospective phase II clinical trial. Patients were administered a modified FOLFOX-6 regimen composed of a 2 hours infusion of oxaliplatin (100 mg/m^2) and folinic acid (100 mg/m^2), followed by a 46 hour continuous infusion of 5-FU (2,400 mg/m^2), as a first-line palliative chemotherapy. Treatment was repeated every 2 weeks until disease progression, patient refusal or unacceptable adverse reactions.

Eligibility criteria included: 1) pathologically confirmed gastric adenocarcinoma with a bi-dimensionally measurable lesion; 2) no prior chemotherapy except adjuvant chemotherapy administered more than 6 months previously; 3) ECOG performance 0–2; 4) adequate bone marrow, hepatic, and renal functions. ECOG performance status [27] defined as follows: ECOG 0-fully active, able to carry on all pre-disease performance without restriction; ECOG 1-restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature; ECOG 2 – ambulatory and capable of all self care but unable to carry out any work activities; ECOG3 – capable of only limited self care, confined to bed or chair more than 50% of waking hours; ECOG 4 – completely disabled.

Patients received a blood test for toxicity every cycle and were re-evaluated every 3 cycles by abdominal computed

tomography. The tumor responses were evaluated using WHO criteria [28] and all responses were confirmed at least 4 weeks after initial assessment. Toxicity was graded according to National Cancer Institute common terminology criteria for adverse events (CTCAE version 3.0). Peripheral sensory neuropathy was also graded according to same toxicity criteria. In the event of toxicity, dose modification and treatment delays were performed according to the protocol. Dose modification of oxaliplatin to 85 mg/m^2 was planned if the patient experienced grade 2 or 3 sensory neuropathy and we strictly followed the protocol which permitted the initiation of chemotherapy after recovery from all toxicities less than grade 2.

Genotyping

To evaluate the clinical usefulness of germline genotyping, we analyzed peripheral blood samples that were obtained from patients with informed consent. Genomic DNA was extracted from peripheral blood samples using QIAmp DNA blood kits (Qiagen Inc, Valencia, CA, USA), and each polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism methods. Table 1 summarizes the primer sequences, the restriction enzymes used [15,16,19,29-34]. The polymorphisms investigated included TS (28-bp repeat in enhancer region [16], G/C SNP in the 3R allele [19], a 6-bp deletion in 3'UTR [15]), GSTP1 (Ile105Val [29]), ERCC1 (Asn118Asn [30], C8092A [31]), XPD (Arg156Arg [32], Asp312Asn [33], Lys751Gln [33]), and XRCC (Arg399Gln [34]).

The study protocol was reviewed and approved by the institutional review board of Seoul National University Hospital.

Statistical analyses

A single-stage design by A'Hern is chosen for definition of the total number of patients required for the phase II study [35]. We set response rate of 35% as the target activity level [6] and chose 20% as the lowest response rate. Our study is designed to have 90% power to accept the hypothesis and 5% significance to reject the hypothesis. In this phase II study, at least 73 evaluable patients will be required to be enrolled. Associations between response rate and polymorphism were assessed by a Chi-square test and Fisher's exact test, where appropriate. To verify allelic frequencies, a Chi-square test was used to confirm agreement with the Hardy-Weinberg equilibrium. Time to pro-

Table 1: Primer sequences and restriction enzymes

Gene	Polymorphisms	Location	Primer	Restriction enzyme	References
TS (Ch 18p11.32)	2R or 3R VNTR in ER*	ER (5'UTR)	Forward: GTGGCTCCTGCGTTTCCCCC Backward: GCTCCGAGCCGGCCACAGGCATGGCGCGG		16
	G/C SNP in 3R	5'UTR	Forward:: GTGGCTCCTGCGTTTCCCCC Backward: GCTCCGAGCCGGCCACAGGCATGGCGCGG	Hae III	19
	6 bp insertion(+)/ deletion(-)	3'UTR	Forward: CAAATCTGAGGGAGCTGAGT Backward: CAGATAAGTGGCAGTACAGA	Dra I	15
GSTP1 (Ch.11q13)	A/G, Ile105Val	Exon5	Forward: CTCTATGGGAAGGACCAGCA Backward: TGAGGGCACAAGAAGCCCCT	BsmA I	29
ERCC1 (Ch.19q13.2)	C/T, Asn118Asn	Exon4	Forward: TCATCCCTATTGATGGCTTCTGCCC Backward: GACCATGCCCAGAGGCTTCTCATAG	BsrD I	30
	C8092A	3'UTR	Forward: CAGAGACAGTGCCCCAAGAG Backward: GGGCACCTTCAGCTTTCTTT	Mbo II	31
XPD (Ch.19q13.3)	C/A, Arg156Arg	Exon6	Forward: CACACCTGGCTCATTTTTGTAT Backward: TCATCCAGGTTGTAGATGCCA	Tfi I	32
	G/A, Asp312Asn	Exon10	Forward: CTGTTGGTGGGTGCCCGTATCTGTTGGTCT Backward: (TAATA)TCGGGGCTCACCCTGCAGCACTTCCT	Sty I	33
	A/C, Lys751Gln	Exon23	Forward: GCCCGCTCTGGATTATACG Backward: CTATCATCTCCTGGCCCCC	Pst I	33
XRCC (Ch.19q13.2)	G/A, Arg399Gln	Exon10	Forward: TTGTGCTTTCTCTGTGTCCA Backward: TCCTCCAGCCTTTTCTGATA	Msp I	34

Ch, chromosome; VNTR, variable number tandem repeats; UTR, untranslated region; ER, enhancer region; TS, thymidylate synthase; GSTP, glutathione S- transferase π; ERCC1, excision repair cross-complementation 1; XPD, xeroderma pigmentosum group D; XRCC, X-ray repair cross-complementing group.
*VNTR polymorphism is a double repeat (2R) or triple repeat (3R) of 28-bp sequence in TS 5'UTR.

Table 2: Patient characteristics

Characteristics	No. of Pts (N = 73)	%
Sex		
Male	48	65.8
Female	25	34.2
Age, years		
median	59	
range	24–77	
Performance status		
ECOG 0–1	60	82.2
ECOG 2	13	17.8
Histopathologic type		
Intestinal type	47	64.4
Diffuse type	23	31.5
Unknown	3	4.1
Disease status		
Relapsed	19	26.0
Initial stage IV	54	74.0
Metastatic site		
Liver	30	41.1
Peritoneum	29	39.7
LN (distant M1 node)	23	31.5
Others	18	24.7

ECOG, eastern cooperative oncology group; LN, lymph node.

gression (TTP) was defined as the interval between the initiation of treatment and the date when disease progression was first documented, or the date of death from any cause. Overall survival (OS) was measured from the date of treatment initiation to the date of death. Median TTP and OS according to prognostic factors were calculated using the Kaplan-Meier method. Multivariate analyses were carried out using Cox proportional hazard regression models for TTP and OS. All values were two sided and statistical significance was accepted at the $p < 0.05$ level. SPSS version 12.0 software (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

Cell culture and 5-FU sensitivity

Seven human gastric cancer cell lines (SNU 1, 5, 16, 484, 601, 620 and 638, obtained from the Korean Cell Line Bank, Seoul, Korea) were examined. Genotypes of each cell line were determined by same methods as described above. Equal numbers of cells harvested during the exponential growth phase were plated in 100 μl per well with various concentrations (0.1 nM to 50 μM, diluted in semilog fashion) for 72 hours. The growth inhibition was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT test; Sigma, St Louis, MO, USA) and the concentrations required to inhibit cell growth by 50% (IC_{50}) were calculated.

Western blot

TS protein expressions were determined by Western immunoblotting, as previously described [36]. Equivalent amounts of protein were processed by 12% SDS-PAGE, and electroblotted on to Hybond ECL nitrocellulose membranes, which were blocked overnight using blocking buffer, and then incubated with primary antibody against human TS (monoclonal mouse anti-TS antibody 1:1000 dilution in blocking buffer; TS Ab-1, clone TS106, Neomarkers). Proteins were detected by enhanced chemiluminescence. For the semiquantitative analysis of Western blots, subsaturated autoradiograms were scanned and signals were analyzed by densitometry (TINA 2.09 software; Raytest, Straubenhardt, Germany).

Results

Patient characteristics and clinical outcomes

Eighty patients were included in this trial and 7 patients were lost to follow up. A total of 73 patients, composed of 48 men and 25 women, with a median age of 59 years (range: 24–77) were finally evaluated in the study which ran from March 2003 to March 2005. The clinical characteristics of patients are summarized in Table 2. During a median follow-up of 21.2 months, 42 death events and 68 progression events occurred. A total of 470 cycles of modified FOLFOX-6 were delivered with a median number of 6 cycles per patient (range, one to 12). Dose intensity level was 83.2%. The median cumulative dose for oxaliplatin was 570 mg/m^2. The overall response rate (RR) was 43.8% (Table 3). The median TTP and OS were 6.0 months (95% CI, 4.8–7.2 months) and 12.6 months (95% CI, 8.7–16.5 months), respectively. Only performance status was found to be a statistically significant prognostic factor for TTP (6.3 months in ECOG 0–1 *vs*. 3.7 months in ECOG 2, $p = 0.037$). RR, TTP and OS were not different according to histotype (diffuse *vs*. intestinal type). Otherwise no significant association was observed between patient characteristics and clinical outcomes.

Toxicities

Severe hematologic toxicities were uncommon (Table 4). The main grade 3 and 4 toxicity (per patients) was neutropenia (11.0%). Non-hematologic toxicities were mild and 1.4% experienced grade 3 peripheral neuropathy. No febrile neutropenia was observed. Toxicities were generally brief, reversible and manageable.

Correlation between genotypes and response rates

We analyzed 10 germline polymorphisms within 5 genes. All observed genotype frequencies were in agreement with the Hardy-Weinberg equilibrium. The genotype distributions of -6 bp/-6 bp, +6 bp/-6 bp and +6 bp/+6 bp were observed in 40 (54.8%), 30 (41.1%) and 3 (4.1%), respectively. The frequencies of 3G containing genotype and non-3G containing genotype were 69.9% and 28.8%, respectively. Table 5 compares RR and polymorphisms. RR was significantly higher in patients with a -6 bp/-6 bp homozygote in TS-3'UTR (55.0% *vs*. 30.3% in +6 bp/+6 bp or +6 bp/-6 bp, $p = 0.034$). There was no difference in

Table 3: Results of modified FOLFOX-6 chemotherapy

Response	No. of Pts (%)
Complete response	0 (0.0)
Partial response	32 (43.8)
Stable disease	21 (28.8)
Progressive disease	20 (27.4)

RR according to TS enhancer region polymorphism (47.1% in 3G containing genotype *vs*. 33.3% in non-3G containing genotype, $p = 0.285$). In XPD Arg156Arg, a higher RR was observed in C/A or A/A genotypes compared with the C/C genotype (52.0% *vs*. 26.1%, $p = 0.038$). The other polymorphisms were not found to be significantly associated with RR. No association was observed between polymorphisms and toxicities (data not shown).

Time to progression, overall survival, and the correlation with genotype

Among the polymorphisms investigated, only the 6-bp deletion homozygote (-6 bp/-6 bp) in TS-3'UTR was found to be significantly associated with a favorable TTP and OS. The TTP of patients with -6 bp/-6 bp in TS-3'UTR was 6.3 months, whereas that of patients with +6 bp/+6 bp or +6 bp/-6 bp was 4.7 months ($p = 0.014$, *p*-value based on log rank test), and a significant difference was also observed between these genotypes in terms of OS (17.8 months in -6 bp/-6 bp *vs*. 10.3 months in +6 bp/+6 bp or +6 bp/-6 bp, $p = 0.032$, *p*-value based on log rank test). In Figure 1 Kaplan-Meier plots are shown and reveal the relationship between TTP and OS according to the TS-3'UTR polymorphism. In addition, a prolonged TTP was observed in the C/A or A/A genotype of XPD Arg156Arg (6.2 months *vs*. 4.1 months in C/C, $p = 0.022$). However, no association was observed for the other polymorphisms and TTP or OS (Table 6). Variables showing association with TTP in univariate analysis with $p < 0.10$ (6-bp deletion in TS-3'UTR, XPD- Arg156Arg, XPD-Asp312Asn, performance status) were included for multivariate analysis using Cox proportional hazard regression models. In multivariate analysis, TS-3'UTR was identified as an independent prognostic marker of survival (Table 7). The 6-bp deletion in TS-3'UTR was independently associated with a prolonged TTP. (Hazard Ratio [HR] = 0.561).

Correlations between in vitro data and the TS polymorphism

The above clinical result led to further investigation for theoretical confirmation in *in vitro* condition. Using seven human gastric cancer cell lines and Western blotting, we investigate for correlations between genotypes, protein expressions, and 5-FU sensitivities. Two out of seven cell lines (SNU 601, 620) had -6 bp/-6 bp homozygotes polymorphism in TS 3'UTR. Six bp deletion homozygotes tended to show lower TS protein expression (TS/tubulin expression ratio 0.88 in -6 bp/-6 bp *vs*. 1.15 in +6 bp/+6 bp or +6 bp/-6 bp, $p = 0.095$ by Mann-Whitney U test). Subsequent Western blot analysis showed lower TS expression was associated with higher sensitivity to 5-FU in our *in vitro* data [36]. Linear regression analysis revealed a statistically significant correlation between TS protein expression and the IC_{50} of 5-FU ($p = 0.002$, $r^2 = 0.887$), and the 6-bp deletion polymorphism in TS-3'UTR was associated with lower TS expression and more sensitivity to 5-FU in gastric cancer cell lines *in vitro*.

Table 4: Toxicities according to National Cancer Institute CTCAE (per patient)

Toxicities	Grade 1–2		Grade 3–4	
	No. of Pts	%	No. of Pts	%
Hematologic				
Leucopenia	13	17.8	0	0.0
Neutropenia	10	13.7	8	11.0
Anemia	12	16.4	0	0.0
Thrombocytopenia	5	6.8	1	1.4
Non-hematologic				
Neuropathy	12	16.4	1	1.4
AST/ALT abnormality	3	4.1	0	0.0
Nausea	19	26.0	0	0.0
Vomiting	9	12.4	0	0.0
Mucositis	1	1.4	1	1.4

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase.

Discussion

In this work we evaluated the efficacy of modified FOLFOX-6 chemotherapy in AGC patients, and examined the relevance of the relationship between germline genetic polymorphisms and the clinical outcome. The results indicate that a modified FOLFOX-6 chemotherapy appears to be active and well tolerated.

With an overall RR of 43.8%, our results compare favorably with other phase II studies of FOLFOX chemotherapy, which range from 38% to 56% [3-7]. By contrast to the FOLFOX-6 regimen for AGC [3], the regimen here omitted the 5-FU bolus injection in order to reduce myelosuppression. In terms of toxicities, the modified FOLFOX-6 regimen showed an 11.0% occurrence of grade 3 or 4 neutropenia, which is lower than the 38% level shown with the FOLFOX-6 regimen [3]. Grade 3 or 4 peripheral sensory neuropathy occurred in only 1.4% of the patients. This was lower than original FOLFOX-6 using oxaliplatin of 100 mg/m^2 with a median cumulative dose of 901 mg/m^2 for oxaliplatin. In our study the median cumulative dose of 570 mg/m^2 for oxaliplatin which is lower than original FOLFOX-6. With considering median cumulative dose of oxaliplatin, this was comparable to other lower

Table 5: Response rate according to the clinical factors and the genotypes

		Overall frequency		Responder		
		No. of Pts	%	No. of Pts	RR (%)	*p*-value*
Age	≤ 55	31	42.5	15	48.4	0.501
	> 55	42	57.5	17	40.5	
Performance	ECOG 0–1	60	82.2	28	46.7	0.295
	ECOG 2	13	17.8	4	30.8	
Histotype	Intestinal	47	67.1	19	40.4	0.557
	Diffuse	23	32.9	11	47.8	
	unknown	3				
Disease status	Initial stage IV	54	74.0	27	50.0	0.107
	Relapsed	19	26.0	5	26.3	
TS in 5'UTR#	2R/2R, 2R/3C, 3C/3C	21	28.8	7	33.3	0.285
	2R/3G, 3C/3G, 3G/3G	51	69.9	24	47.1	
	unknown	1	1.4			
TS 6-bp deletion in 3'UTR	-6/-6	40	54.8	22	55.0	0.034
	+6/+6 or +6/-6	33	45.2	10	30.3	
GSTP1-Ile105Val (A105G)	A/A	44	60.3	22	50.0	0.191
	A/G or G/G	29	39.7	10	34.5	
ERCC-Asn118Asn	C/C	40	54.8	17	42.5	0.800
	C/T or T/T	33	45.2	15	57.5	
ERCC-C8092A	C/C	44	60.3	18	40.9	0.535
	C/A or A/A	29	39.7	14	48.3	
XPD- Arg156Arg	C/C	23	31.5	6	26.1	0.038
	C/A or A/A	50	54.0	26	52.0	
XPD-Asp312Asn	G/G	8	11.0	1	12.5	0.072
	G/A	65	89.0	31	47.7	
XPD-Lys751Gln	A/A	62	84.9	28	45.2	0.746
	A/C or C/C	11	15.1	4	36.4	
XRCC1-Arg399Gln	G/G	48	65.8	21	43.8	0.984
	G/A or A/A	25	34.2	11	44.0	

RR, response rate.
*p-value for the comparison between response rate and genotypes, based on Pearson's χ 2 test (using Fisher's exact test, if N ≤ 5).
#Analysis of the TS-5'UTR VNTR polymorphism with G/C SNP change in 3R allele carriers.

dose oxaliplatin-based regimen or omitting the 5-FU bolus [4,7]. In our study, dose modification of oxaliplatin to 85 mg/m^2 was performed if the patient experienced grade 2 peripheral neuropathy and we strictly followed the protocol which permitted the initiation of chemotherapy after recovery from all toxicities less than grade 2.

In the present study, TS and XPD polymorphisms were found to be associated with RR, and these polymorphisms could be used as predictive markers. TS, the target of 5-FU, has been investigated repeatedly in various aspects to predict the response to 5-FU, in terms of polymorphism, mRNA and protein expressions [10,16]. In our study, the 6-bp deletion polymorphism in TS-3'UTR was found to be correlated with favorable clinical outcome. That means that the patients with -6 bp/-6 bp were found to have a higher RR and prolonged time to progression. TS-3'UTR plays a role as a post transcriptional regulator, mainly by controlling mRNA stability and/or translational efficiency. The 6-bp deletion in TS-3'UTR reduces mRNA stability and lowers intratumoral TS mRNA levels [18]. A reduced level of TS mRNA might also lead to a lower TS protein expression, and make tumors more sensitive to 5-FU based chemotherapy [13,36,37]. Previous studies in colorectal cancer provide strong evidence that a lower TS expression appears to be associated with a higher RR and prolonged survival with 5-FU based chemotherapy [11,13,14]. These findings are supported by *in vitro* data that -6 bp/-6 bp in TS-3'UTR affect to lower TS expression and higher sensitivity to 5-FU in gastric cancer cell lines. In gastric cancer, we confirmed that a 6-bp deletion is associated with better clinical outcome in homogenous patients treated with a first line modified FOLFOX-6 regimen.

However, some studies conducted in Western countries [25,26] failed to find a correlation between the 6-bp deletion polymorphism and 5-FU sensitivity in gastric cancer, while our results are concordant with those of another

Table 6: Comparison of TTP and OS according to the clinical factors and genotypes

		Time to progression				Overall survival			
		Median TTP (Mo)*	HR#	95% CI	*p*-value#	Median OS (Mo)*	HR#	95% CI	*p*-value#
Age	≤ 55	5.6	1.000		0.485	11.9	1.000		0.146
	> 55	5.0	0.840	0.514–1.371		10.8	1.610	0.847–3.060	
Performance	ECOG 0–1	6.0	1.000		0.042	12.6	1.000		0.124
	ECOG 2	3.7	1.892	1.022–3.501		10.0	1.722	0.862–3.441	
Histotype	Intestinal	6.1	1.000		0.495	14.5	1.000		0.392
	Diffuse	5.6	1.197	0.714–2.009		10.8	1.337	0.687–2.602	
Disease status	Initial stage IV	5.6	1.000		0.362	12.6	1.000		0.903
	Relapsed	6.0	1.288	0.748–2.219		10.8	0.959	0.489–1.881	
TS 5'UTR	2R/2R, 2R/3C, 3C/3C	5.6	1.000		0.781	10.8	1.000		0.244
	2R/3G, 3C/3G, 3G/3G	6.1	0.781	0.415–1.470		17.4	0.669	0.340–1.316	
TS 6-bp deletion in 3'UTR	+6/+6 or +6/-6	4.7	1.000		0.033	10.3	1.000		0.046
	-6/-6	6.3	0.572	0.342–0.956		17.8	0.536	0.291–0.988	
GSTP1-Ile105Val	A/A	6.0	1.000		0.398	11.9	1.000		0.621
	A/G or G/G	6.3	1.243	0.750–2.061		14.5	0.621	0.452–1.606	
ERCC-Asn118Asn	C/C	6.2	1.000		0.433	14.5	1.000		0.472
	C/T or T/T	5.0	0.822	0.503–1.342		11.9	1.251	0.680–2.302	
ERCC-C8092A	C/C	6.1	1.000		0.826	17.8	1.000		0.326
	C/A or A/A	5.6	0.945	0.569–1.568		11.4	1.356	0.739–2.490	
XPD- Arg156Arg	C/C	4.1	1.000		0.022	10.5	1.000		0.355
	C/A or A/A	6.2	0.533	0.311–0.913		14.5	0.738	0.367–1.405	
XPD-Asp312Asn	G/G	6.1	1.000		0.084	14.0	1.000		0.673
	G/A	3.5	1.942	0.914–4.129		10.8	0.800	0.283–2.256	
XPD-Lys751Gln	A/A	6.1	1.000		0.693	12.6	1.000		0.173
	A/C or C/C	4.6	0.872	0.440–1.725		Not reached	0.488	0.174–1.369	
XRCC1-Arg399Gln	G/G	6.1	1.000		0.744	15.3	1.000		0.160
	G/A or A/A	5.9	1.090	0.649–1.832		10.3	1.575	0.835–2.971	

Mo, months; HR, hazard ratio; CI, confidence interval.
* By Kaplan-Meier analysis
#By Cox proportional hazard regression model, adjusted for performance
Note: If the hazard ratio is greater than 1, the hazard ratio can be thought of as the average increased risk of progression, or death at any point in time, compared with the reference group (described above line).

Table 7: Clinical factors and genotypes influencing survival in multivariate analyses

	TTP		OS	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
TS 6-bp deletion in 3'UTR	0.561 (0.331–0.950)	0.032	0.553 (0.285–1.072)	0.079
XPD-Arg156Arg	0.705 (0.407–1.220)	0.210	0.923 (0.456–1.867)	0.823
XPD-Asp312Asn	1.956 (0.902–4.240)	0.089	0.802 (0.281–2.286)	0.679
Performance (ECOG 0–1 *vs.* 2)	1.973 (1.034–3.762)	0.073	1.638 (0.816–3.288)	0.165

study conducted in an Asian [38]. One possible explanation for this contradictory result is ethnic difference.

Caucasian patients had higher proportion of favorable 2R/2R genotype (16.0% to 26.4%) [26,39,40], lower proportion of unfavorable 3R/3R or 3G containing genotypes in 5'UTR (28.8% to 39.2%) [26,34,40] and lower proportion of favorable -6 bp/-6 bp in 3'UTR (7.8% to 22.0%) [18,26,34,40]. In contrast, Asian patients showed lower proportion of favorable 2R/2R (0.0% to 6.0%) [34,41] higher proportion of unfavorable 3R/3R (66.4% to 72.3%) [34,42] and higher proportion of favorable -6 bp/

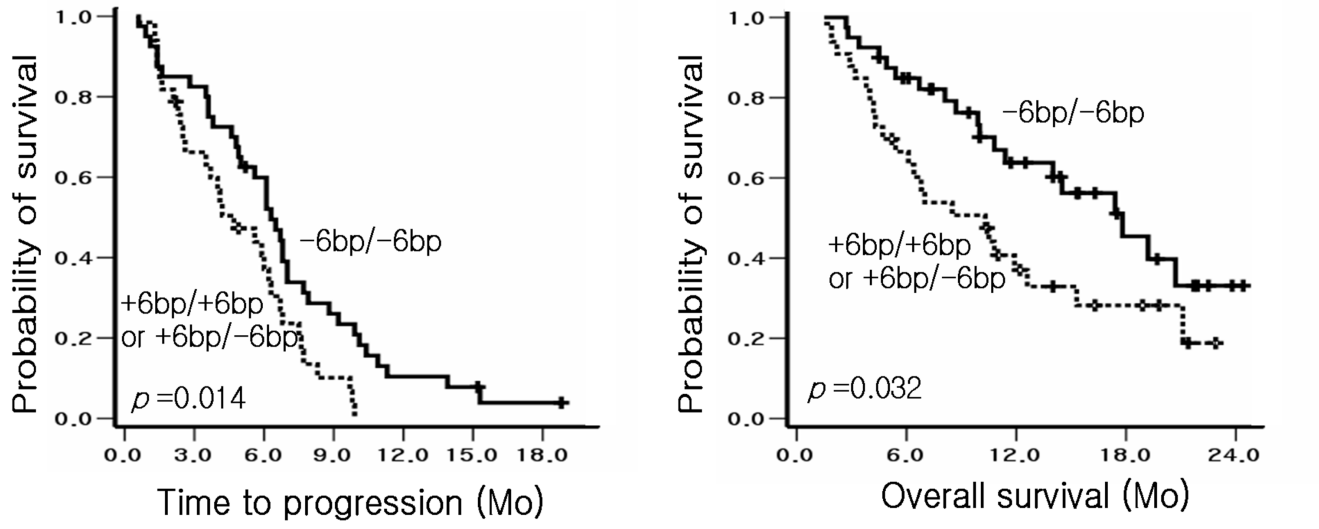

Figure 1

Kaplan-Meier plots of TTP and OS, according to 6-bp deletion polymorphism in 3'UTR of TS. The 6-bp deletion homozygote (-6 bp/-6 bp) was significantly associated with prolonged TTP (6.3 months *vs*. 4.7 months in +6 bp/+6 bp or +6 bp/-6 bp, $p = 0.014$) and OS (17.8 months vs. 10.3 months in +6 bp/+6 bp or +6 bp/-6 bp, $p = 0.032$).

-6 bp (36.4% to 56.0%) [18,34,38]. These differences between favorable and unfavorable genotype frequencies might make different results according to ethnic diversity.

The clinical influence of TS polymorphism in 5-FU based chemotherapy are often controversial because of different clinical settings (adjuvant *vs*. palliative), tumor type (colon *vs*. gastric cancer), and different 5-FU infusion times [16,25,26,39,42-45]. These experimental variables should be considered when interpreting predictive values of the TS polymorphism.

Another factor to be taken into consideration is the possible impact on outcome of other drugs combined with 5-FU. Even antitumor activity of 5-FU may be decreased by TS polymorphism, another combined drug might compensate the decreased antitumor activity and make sufficient tumor response. This would make the meaningful polymorphism in single agent protocol less significant in combination regimens [46]. Thus this could account for the differing results with prior reports. A meta-analysis could perhaps provide some clarity in this area.

XPD (also known as ERCC2) encodes DNA helicase, which is a member of the nuclear excision repair pathway and plays a role in repairing platinum-DNA adducts. The C/C genotype of XPD-Arg156Arg SNP showed lower RR than C/A or A/A genotype (26.1% *vs*. 52.0%, $p = 0.038$) and shorter TTP (4.1 months *vs*. 6.2 months, $p = 0.022$). The lower RR with a modified FOLFOX-6 chemotherapy might result from the tendency that the C/C genotype of XPD- Arg156Arg had a higher DNA repair capacity than C/A or A/A genotype [32]. However, it did not affect to prolongation of survival. The other polymorphisms examined failed to show any relation with clinical outcome.

In the presented study, we analyzed germline genotype. Hence the correlation between germline genotype from peripheral blood and somatic genotype from tumor tissues should be considered. Few studies focused on comparing both genotypes. In terms of 28-bp repeat polymorphism, tumor specific loss of heterozygosity at the TS locus has been reported [47]. In contrast, some studies have reported that there is no difference in TS ER polymorphism between genotypes of tumor and normal tissues in gastric [41] and colorectal cancer [40].

Herein, we presume that germline genotypes are nearly identical to somatic genotypes, even though it might not always reflect the tumor genotype. However, germline genotypes determined from peripheral blood mononuclear cells have many strong points. The germline genotypes offer the better clinical accessibility and applicability, compared to tumor tissue DNA or mRNA, which present difficulties in obtaining and handling samples. In our results, germline genotypes, which were obtained by simple blood test, have shown a good association with the clinical outcome and are easily interpreted. In this phase II study, the small sample size (n = 73) might remains a limitation to clarify the role of polymorphism.

However, our patient population was considerably homogeneous in terms of ethnicity and chemotherapeutic regimen, and this protocol was prospective.

Conclusion

We found that modified FOLFOX-6 chemotherapy appears to be effective. A 6-bp deletion homozygote of TS-3'UTR was found to be a predictive marker of the response and was also found to be associated with a prolonged TTP in AGC patients on modified FOLFOX-6 chemotherapy. Moreover, our results suggest that germline genetic polymorphisms of TS and XPD may be useful candidates for a pharmacogenomic prediction of the response to modified FOLFOX-6 chemotherapy in AGC. Alternative treatment other than 5-FU or platinum based therapy should be considered for the patients who have unfavorable genotype. We conclude that testing for the TS-3'UTR 6-bp deletion and XPD polymorphisms might be a candidate pharmacogenomic factors to be explored in the future larger scale study to identify the gastric cancer patients who might benefit from 5-FU based first line chemotherapy.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BK collected the data, performed the statistical analysis and drafted the manuscript. S–AI designed the concept of this study, performed the statistical analysis with interpretation and approved the final manuscript. S–WH performed the statistical analysis and critically revised the manuscript. HSH carried out the genotyping and management of the samples. D–YO, JHK, S–HL, D–WK, T–YK, DSH and Y–JB performed the chemotherapy for patients and revised the manuscript. All authors read and approved the final manuscript.

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References

1. Wohrer SS, Raderer M, Hejna M: **Palliative chemotherapy for advanced gastric cancer.** *Ann Oncol* 2004, **15:**1585-95.
2. Kim NK, Park YS, Heo DS, Suh C, Kim SY, Park KC, Kang YK, Shin DB, Kim HT, Kim HJ: **A phase III randomized study of 5-fluorouracil and cisplatin vs 5-fluorouracil, doxorubicin, and mitomycin C vs 5-fluorouracil alone in the treatment of advanced gastric cancer.** *Cancer* 1993, **71:**3813-8.
3. Louvet C, André T, Tigaud JM, Gamelin E, Douillard JY, Brunet R, François E, Jacob JH, Levoir D, Taamma A, Rougier P, Cvitkovic E, de Gramont A: **Phase II study of oxaliplatin, fluorouracil, and folinic acid in locally advanced or metastatic gastric cancer patients.** *J Clin Oncol* 2002, **20:**4543-8.
4. Al-Batran SE, Atmaca A, Hegewisch-Becker S, Jaeger D, Hahnfeld S, Rummel MJ, Seipelt G, Rost A, Orth J, Knuth A, Jaeger E: **Phase II trial of biweekly infusional fluorouracil, folinic acid, and oxaliplatin in patients with advanced gastric cancer.** *J Clin Oncol* 2004, **22:**658-63.
5. Chao Y, Yeh KH, Chang CJ, Chen LT, Chao TY, Wu MF, Chang CS, Chang JY, Chung CY, Kao WY, Hsieh RK, Cheng AL: **Phase II study of weekly oxaliplatin and 24-h infusion of high-dose 5-fluorouracil and folinic acid in the treatment of advanced gastric cancer.** *Br J Cancer* 2004, **91:**453-8.
6. De Vita F, Orditura M, Matano E, Bianco R, Carlomagno C, Infusino S, Damiano V, Simeone E, Diadema MR, Lieto E, Castellano P, Pepe S, De Placido S, Galizia G, Di Martino N, Ciardiello F, Catalano G, Bianco AR: **A phase II study of biweekly oxaliplatin plus infusional 5-fluorouracil and folinic acid (FOLFOX-4) as first-line treatment of advanced gastric cancer patients.** *Br J Cancer* 2005, **92:**1644-9.
7. Lordick F, Lorenzen S, Stollfuss J, Vehling-Kaiser U, Kullmann F, Hentrich M, Zumschlinge R, Dietzfelbinger H, Thoedtmann J, Hennig M, Seroneit T, Bredenkamp R, Duyster J, Peschel C: **Phase II study of weekly oxaliplatin plus infusional fluorouracil and folinic acid (FUFOX regimen) as first-line treatment in metastatic gastric cancer.** *Br J Cancer* 2005, **93:**190-4.
8. Evans WE, Relling MV: **Pharmacogenomics: translating functional genomics into rational therapeutics.** *Science* 1999, **286:**487-91.
9. Marsh S, McLeod HL: **Cancer pharmacogenetics.** *Br J Cancer* 2004, **90:**8-11.
10. Longley DB, Harkin DP, Johnston PG: **5-fluorouracil: mechanisms of action and clinical strategies.** *Nat Rev Cancer* 2003, **3:**330-8.
11. Johnston PG, Lenz HJ, Leichman CG, Danenberg KD, Allegra CJ, Danenberg PV, Leichman L: **Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors.** *Cancer Res* 1995, **55:**1407-12.
12. Leichman L, Lenz HJ, Leichman CG, Groshen S, Danenberg K, Baranda J, Spears CP, Boswell W, Silberman H, Ortega A: **Quantitation of intratumoral thymidylate synthase expression predicts for resistance to protracted infusion of 5-fluorouracil and weekly leucovorin in disseminated colorectal cancers: preliminary report from an ongoing trial.** *Eur J Cancer* 1995, **31A:**1306-10.
13. Shirota Y, Stoehlmacher J, Brabender J, Xiong YP, Uetake H, Danenberg KD, Groshen S, Tsao-Wei DD, Danenberg PV, Lenz HJ: **ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy.** *J Clin Oncol* 2001, **19:**4298-304.
14. Popat S, Matakidou A, Houlston RS: **Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis.** *J Clin Oncol* 2004, **22:**529-36.
15. Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD: **Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene.** *Cancer Epidemiol Biomarkers Prev* 2000, **9(12):**1381-5.
16. Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, Warren R, Tsao-Wei D, Groshen S, Lenz HJ: **Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy.** *Pharmacogenomics J* 2001, **1:**65-70.
17. Chen J, Hunter DJ, Stampfer MJ, Kyte C, Chan W, Wetmur JG, Mosig R, Selhub J, Ma J: **Polymorphism in the thymidylate synthase promoter enhancer region modifies the risk and survival of colorectal cancer.** *Cancer Epidemiol Biomarkers Prev* 2003, **12:**958-62.
18. Mandola MV, Stoehlmacher J, Zhang W, Groshen S, Yu MC, Iqbal S, Lenz HJ, Ladner RD: **A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels.** *Pharmacogenetics* 2004, **14:**319-27.
19. Kawakami K, Watanabe G: **Identification and functional analysis of single nucleotide polymorphism in the tandem repeat**

sequence of thymidylate synthase gene. *Cancer Res* 2003, **63:**6004-7.
20. Morganti M, Ciantelli M, Giglioni B, Putignano AL, Nobili S, Papi L, Landini I, Napoli C, Valanzano R, Cianchi F, Boddi V, Tonelli F, Cortesini C, Mazzei T, Genuardi M, Mini E: **Relationships between promoter polymorphisms in the thymidylate synthase gene and mRNA levels in colorectal cancers.** *Eur J Cancer* 2005, **41:**2176-83.
21. Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L: **ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy.** *J Clin Oncol* 1998, **16:**309-16.
22. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei DD, Groshen S, Lenz HJ: **A Xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer.** *Cancer Res* 2001, **61:**8654-8.
23. Gurubhagavatula S, Liu G, Park S, Zhou W, Su L, Wain JC, Lynch TJ, Neuberg DS, Christiani DC: **XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy.** *J Clin Oncol* 2004, **22:**2594-2601.
24. Suk R, Gurubhagavatula S, Park S, Zhou W, Su L, Lynch TJ, Wain JC, Neuberg D, Liu G, Christiani DC: **Polymorphisms in ERCC1 and grade 3 or 4 toxicity in non-small cell lung cancer patients.** *Clin Cancer Res* 2005, **11:**1534-8.
25. Goekkurt E, Hoehn S, Wolschke C, Wittmer C, Stueber C, Hossfeld DK, Stoehlmacher J: **Polymorphisms of glutathione S-transferases (GST) and thymidylate synthase (TS)-novel predictors for response and survival in gastric cancer patients.** *Br J Cancer* 2006, **94:**281-6.
26. Ruzzo A, Graziano F, Kawakami K, Watanabe G, Santini D, Catalano V, Bisonni R, Canestrari E, Ficarelli R, Menichetti ET, Mari D, Testa E, Silva R, Vincenzi B, Giordani P, Cascinu S, Giustini L, Tonini G, Magnani M: **Pharmacogenetic profiling and clinical outcome of patients with advanced gastric cancer treated with palliative chemotherapy.** *J Clin Oncol* 2006, **24:**1883-91.
27. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP: **Toxicity and response criteria of the Eastern Cooperative Oncology Group.** *Am J Clin Oncol* 1982, **5:**649-55.
28. Miller AB, Hoogstraten B, Staquet M, Winkler A: **Reporting results of cancer treatment.** *Cancer* 1981, **47:**207-14.
29. Stoehlmacher J, Park DJ, Zhang W, Groshen S, Tsao-Wei DD, Yu MC, Lenz HJ: **Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer.** *J Natl Cancer Inst* 2002, **94:**936-42.
30. Mort R, Mo L, McEwan C, Melton DW: **Lack of involvement of nucleotide excision repair gene polymorphisms in colorectal cancer.** *Br J Cancer* 2003, **89:**333-7.
31. Zhou W, Gurubhagavatula S, Liu G, Park S, Neuberg DS, Wain JC, Lynch TJ, Su L, Christiani DC: **Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy.** *Clin Cancer Res* 2004, **10:**4939-43.
32. Vogel U, Hedayati M, Dybdahl M, Grossman L, Nexo BA: **Polymorphisms of the DNA repair gene XPD: correlations with risk of basal cell carcinoma revisited.** *Carcinogenesis* 2001, **22:**899-904.
33. Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, Guo Z, Lei L, Mohrenweiser H, Wei Q: **Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients.** *Cancer Res* 2001, **61:**1354-7.
34. Stoehlmacher J, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S, Lenz HJ: **A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer.** *Br J Cancer* 2004, **91:**344-54.
35. Freedman LS: **Tables of the number of patients required in clinical trials using the log rank test.** *Stat Med* 1982, **1:**121-9.
36. Kim JH, Lee KW, Jung Y, Kim TY, Ham HS, Jong HS, Jung KH, Im SA, Kim TY, Kim NK, Bang YJ: **Cytotoxic effects of pemetrexed in gastric cancer cells.** *Cancer Sci* 2005, **96:**365-71.
37. Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Garcia Y, Li J, Leichman L: **Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival.** *J Clin Oncol* 1996, **14:**176-82.
38. Lu JW, Gao CM, Wu JZ, Cao HX, Tajima K, Feng JF: **Polymorphism in the 3'-untranslated region of the thymidylate synthase gene and sensitivity of stomach cancer to fluoropyrimidine-based chemotherapy.** *J Hum Genet* 2006, **51:**155-60.
39. Jakobsen A, Nielsen JN, Gyldenkerne N, Lindeberg J: **Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphism in normal tissue as predictors of fluorouracil sensitivity.** *J Clin Oncol* 2005, **23:**1365-9.
40. Dotor E, Cuatrecases M, Martinez-Iniesta M, Navarro M, Vilardell F, Guino E, Pareja L, Figueras A, Mollevi DG, Serrano T, de Oca J, Peinado MA, Moreno V, Germa JR, Capella G, Villanueva A: **Tumor thymidylate synthase 1494del6 genotype as a prognostic factor in colorectal cancer patients receiving fluorouracil-based adjuvant treatment.** *J Clin Oncol* 2006, **24:**1603-11.
41. Kim JS, Kim MA, Kim DW, Im SA, Kim TY, Yang HK, Kim WH, Heo DS, Bang YJ, Kim NK: **Pharmacogenomic analysis to predict relapse in curatively resected gastric cancer patients treated with adjuvant 5-fluorouracil/cisplatin (FP) chemotherapy.** *Proc Am Soc Clin Oncol* 2005, **23:**322. abstr 4057
42. Tsuji T, Hidaka S, Sawai T, Nakagoe T, Yano H, Haseba M, Komatsu H, Shindou H, Fukuoka H, Yoshinaga M, Shibasaki S, Nanashima A, Yamaguchi H, Yasutake T, Tagawa Y: **Polymorphism in the thymidylate synthase promoter enhancer region is not an efficacious marker for tumor sensitivity to 5-fluorouracil-based oral adjuvant chemotherapy in colorectal cancer.** *Clin Cancer Res* 2003, **9:**3700-4.
43. Etienne MC, Chazal M, Laurent-Puig P, Magne N, Rosty C, Formento JL, Francoual M, Formento P, Renee N, Chamorey E, Bourgeon A, Seitz JF, Delpero JR, Letoublon C, Pezet D, Milano G: **Prognostic value of tumoral thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: phenotypic and genotypic analyses.** *J Clin Oncol* 2002, **20:**2832-43.
44. Yeh KH, Yeh SH, Hsu CH, Wang TM, Ma IF, Cheng AL: **Prolonged and enhanced suppression of thymidylate synthase by weekly 24-h infusion of high-dose 5-fluorouracil.** *Br J Cancer* 2000, **83:**1510-5.
45. Kawakami K, Graziano F, Watanabe G, Ruzzo A, Santini D, Catalano V, Bisonni R, Arduini F, Bearzi I, Cascinu S, Muretto P, Perrone G, Rabitti C, Giustini L, Tonini G, Pizzagalli F, Magnani M: **Prognostic role of thymidylate synthase polymorphisms in gastric cancer patients treated with surgery and adjuvant chemotherapy.** *Clin Cancer Res* 2005, **11:**3778-83.
46. Ichikawa W, Takahashi T, Suto K, Yamashita T, Nihei Z, Shirota Y, Shimizu M, Sasaki Y, Hirayama R: **Thymidylate synthase predictive power is overcome by irinotecan combination therapy with S-1 for gastric cancer.** *Br J Cancer* 2004, **91:**1245-50.
47. Uchida K, Hayashi K, Kawakami K, Schneider S, Yochim JM, Kuramochi H, Takasaki K, Danenberg KD, Danenberg PV: **Loss of heterozygosity at the thymidylate synthase (TS) locus on chromosome 18 affects tumor response and survival in individuals heterozygous for a 28-bp polymorphism in the TS gene.** *Clin Cancer Res* 2004, **10:**433-9.

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