Copy number changes can be a predictor for hemoglobin reduction after S-1 monotherapy in gastric cancer

HEI CHEUL JEUNG[1,3], SUN YOUNG RHA[1,2,3], CHAN HEE PARK[1,2], CHONG-KUN IM[1,2,3], SANG JOON SHIN[1,3], JOONG BAE AHN[1,3], SUNG HOON NOH[1,2,4], JAE KYUNG ROH[1,2,3] and HYUN CHEOL CHUNG[1,2,3]

[1]Cancer Metastasis Research Center, Yonsei Cancer Center, [2]Brain Korea 21 Project for Medical Science, Departments of [3]Internal Medicine and [4]Surgery, Yonsei University College of Medicine, Seoul, Korea

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Abstract. Anemia is a unique side effect in Korean gastric cancer patients after S-1 monotherapy. We studied gastric cancer patients from a phase II trial of S-1 monotherapy with a 2-week treatment and 1-week rest schedule. Patients from a phase II trial of S-1 monotherapy with a 4-week treatment and 2-week rest were used as a reference group. The patients were categorized into two groups based on the degree of hemoglobin reduction per cycle of S-1: the mild reduction group (MRG ΔHb/cycle ≤1.0) or severe reduction group (SRG ΔHb/cycle >1.0). ΔHb/cycle was calculated from maximum reduction of hemoglobin per one cycle of the treatment. Microarray-CGH was performed using a 17K cDNA microarray containing 15,723 unique genes. We selected genes with copy number variation defined as amplification ($\log_2$R/G >0.68) or deletion ($\log_2$R/G <-0.68), and a genetic aberration frequency difference of ≥30% between the MRG and the SRG. There were no differences in clinical factors, S-1 treatment-related factors (dose, dose intensity), toxicity, S-1 metabolism-related gene copy numbers (*CYP2A6*, *DPD*), or progression-free survival between the MRG and the SRG. Three genes were selected from microarray-CGH and logistic regression model: logit LN(Z) = (1.321) + (1.038 x PTX1) + (0.211 x MYO5A) + (0.516 x ZNF664). In the SRG, all 3 genes showed a trend of higher copy numbers than the MRG. There were no common anemia-related genes identified from different chemotherapy schedule of S-1 monotherapy. The logistics obtained from 3 genes predicted the hemoglobin reduction with an accuracy of 78%. The AUC was 0.744 for the final regression model. The combined copy number changes of the 3 genes can be developed into a biomarker in predicting S-1 treatment-related anemia.

Correspondence to: Dr Hyun Cheol Chung, Yonsei Cancer Center, Yonsei University College of Medicine, 134, Shinchon-Dong, Seodaemun-Ku, Seoul 120-752, Korea
E-mail: unchung8@yuhs.ac

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Introduction

Fluoropyrimidine is still the main drug used for the treatment of gastric cancer. The target enzyme of 5-fluoropyrimidine (5-FU), thymidylate synthase (TS), can be inhibited continuously by protracted infusion of 5-FU (1). A continuous exposure to 5-FU can be also achieved by frequent administration of oral 5-FU or a 5-FU prodrug. S-1 is a new drug consisting of the 5-FU prodrug [ftorafur (FT)], the dihydropyrimidine dehydrogenase (DPD) inhibitor [5-chloro-2,4-dihydroxypyrimidine (CDHP)] and the phosphoribosyl transferase inhibitor (oxonic acid) (2). Conversion of FT to 5-FU is catalyzed by cytochrome P450 2A6 and occurs predominantly in the liver and tumor (3). Thymidine phosphorylase has been postulated to play a role in this conversion. Eighty percent of 5-FU is eliminated by degradation catalyzed by DPD (4), which is competitively inhibited by CDHP (5). Thus, co-administration of CDHP with FT results in a 5-10 times higher 5-FU concentration in the tumor compared to that in tumors treated by FT or 5-FU alone. Oxonic acid inhibits the conversion of 5-FU to 5-fluorouridine-5'-monophosphate, a precursor of 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP), which inhibits the target enzyme of TS without affecting the pharmacokinetics of 5-FU.

In phase I trials, the most common dose limiting toxicity (DLT) in Caucasians was diarrhea regardless of the treatment schedule, duration, or dosage of S-1 monotherapy. However, with a modified schedule of a 2-week treatment and a 1-week rest schedule (2-1 week schedule), there were no grade IV toxicities, even with diarrhea as the DLT (6). In a small Japanese retrospective study, the overall toxicity was lower for a 2-1 week schedule compared to that of a 4-week treatment and a 2-week rest schedule (4-2 week schedule) (7). The major toxicity in Asian patients was granulocytopenia. Interestingly, in Korean patients, anemia was the major grade III-IV toxicity with the 4-2 week schedule, which was not that frequently found in Japanese or European patients (8). With the 2-1 week schedule, anemia was still the major toxicity in Korean

patients (9). This kind of ethnic difference in S-1 toxicity may come from genomic differences associated with S-1 metabolism.

The PK profiles for Japanese patients were quite similar to those of Korean patients. The AUC of 5-FU in Korean patients increased only marginally at a higher dosage (80 mg/m^2 vs. 70 mg/m^2), suggesting that the conversion of FT to 5-FU is almost saturated at the 70 mg/m^2 dosage. This conversion level was similar to that seen in the Japanese population (10). Because the PK study could not explain the discrepancy in the incidences of anemia observed between Korean and Japanese or Caucasian patients, we reported a pharmacogenomic study using genomic DNA (8). A pharmacogenomic study can be done using either genomic DNA from peripheral blood mononuclear cells or tumor DNA from the cancer. Since genomic DNA is considered to represent the genetic information from the normal tissue, and not from the tumor tissue (11), we used genomic DNA from peripheral blood mononuclear cells.

With the patients who had been treated by the 2-1 week schedule of S-1 monotherapy, we compared the clinical factors and the copy number changes of S-1 metabolism-related genes, such as cytochrome p450 2A6 (CYP 2A6) and DPD, between patients with severe and mild anemia. We also performed an additional pharmacogenomic study with microarray-CGH and compared the results to the study from patients treated by the 4-2 week schedule of S-1 monotherapy.

Materials and methods

Patients. We studied the data of 27 patients from the phase II trial (9) who had been treated by the 2-1 week schedule (group A). From these patients we obtained genomic DNA from lymphocytes. We also used data of 36 patients from a phase II trial (8) who had been treated by the 4-2 week schedule as a reference group (group B). Patients were required to have histologically confirmed gastric adenocarcinoma with inoperable or metastatic disease, age ≥18 years, performance status ≤3 according to the criteria of Eastern Cooperative Oncology Group (ECOG), a life expectancy of ≥3 months, no prior chemotherapy for advanced disease (adjuvant chemotherapy should have been completed at least 6 months before enrollment), bidimensionally measurable lesions and adequate organ functions [WBC ≥4,000/μl, platelets ≥100,000/μl, serum creatinine ≤1.5 x upper limit of normal (ULN), total bilirubin ≤1.25 x ULN and serum aminotransferase ≤2.5 x ULN]. Patients with other active malignancies, brain metastasis or severe comorbid conditions were excluded.

Evaluation of patients. Tumor response was measured bidimensionally according to the World Health Organization (WHO) criteria. Patients were included in the evaluation if they had received a minimum of one cycle of treatment with at least one follow-up tumor measurement. All patients were evaluated for toxicity from the time of their first cycle. Adverse events were recorded every week and graded according to the NCI-Common Toxicity Criteria (NCI-CTC, Version 2.0). Patients were categorized into two groups based on the degree of hemoglobin (Hb) reduction (ΔHb; the lowest hemoglobin - initial hemoglobin) per cycle (mg/dl) of S-1: mild reduction group (MRG, ΔHb/cycle ≤1.0) or severe reduction group (SRG, ΔHb/cycle >1.0).

cDNA-based microarray-CGH. We used a 17K cDNA microarray-CGH containing 15,723 unique genes. The entire gender-matched study was performed according to an approved protocol of the Cancer Metastasis Research Center (CMRC), Yonsei University College of Medicine, Korea (11,12). Briefly, 8 μg of genomic DNA was isolated from patients' peripheral mononuclear cells and labeled with Cy3- or Cy5-dUTP, using a Bioprime labeling kit (Invitrogen, Carlsbad, CA, USA). The labeled probes were then mixed with human Cot-1 DNA (Gibco-BRL, Gaithersburg, MD, USA), yeast t-RNA (Gibco-BRL), and poly-A RNA (Sigma, St. Louis, MO, USA). After concentration and denaturation, the probe mixture was applied to a microarray and hybridized in a chamber at 65˚C for 16 h. After hydridization, the slides were scanned using a GenePix 4000B scanner (Axon Instruments, Foster City, CA, USA) and the TIFF images were analyzed. The intensity signal of each spot was transformed as a $\log_2$ red to green (R/G) ratio. Whole microarray spots were mapped for their chromosomal location, using the software SOURCE (http://genome-www5.stanford.edu/cgi-bin/source/sourceSearch) and DAVID (http://apps1.niaid.nih.gov/david/). A within-slide global normalization was applied, which subtracted the median intensity ratio of the $\log_2$(R/G) from the $\log_2$-transformed data.

Anemia-related gene selection. To identify anemia-related genes that can differentiate the MRG from the SRG, we selected genes showing; i) copy number changes defined as amplification ($\log_2$R/G >0.68) or deletion ($\log_2$R/G <-0.68), and ii) genetic aberration frequency difference of ≥30% between the MRG and the SRG with a statistical significance of p-value <0.05 (13). The selected genes were clustered and visualized using CLUSTER and TREEVIEW (12).

Logistic regression model. By univariate analysis, clinical factors and genes that correlate with hemoglobin reduction were selected. Then, using the binary outcomes of the MRG and SRG as a dependent variable, the best logistic regression model was identified by performing stepwise selection among factors selected from univariate analysis as follows; logit LN(Z) = (constant) + (A x PTX1) + (B x MYO5A) + (C x ZNF664). The diagnostic accuracy of this model with regard to the severity of hemoglobin reduction was quantified via prediction accuracy and receiver operating characteristic (ROC) analysis (8). In prediction accuracy analysis, we determined that the SRG was 50 to 100% of the predictive value and the MRG was 0 to 50% of the predictive value (14).

Statistical analysis. Statistical analysis was performed using the SPSS program (version 12.0, SPSS, Inc., Chicago, IL). Progression-free survival (PFS) was defined as the time interval from the start of treatment until disease progression or death by any cause and overall survival (OS) was defined as interval from the start of treatment to death. Time-dependent

Table I. Comparison of patient characteristics in group A and B.

	Group A			Group B		
	MRG (n=16)	SRG (n=11)	P-value	MRG (n=22)	SRG (n=14)	P-value
Median age (range)	56 (40-74)	57 (36-76)		54 (34-69)	57 (28-73)	
Gender						
Male	11 (69)	8 (73)		15 (68)	12 (86)	
Female	5 (31)	3 (27)	P=1.000	7 (32)	2 (14)	P=0.432
Performance status						
0-1	0 (0)	0 (0)	P=1.000	20 (91)	14 (100)	P=0.512
2-3	16 (100)	11 (100)		2 (9)	0 (0)	
Histology						
Well/moderately differentiated	3 (19)	4 (36)		12 (55)	4 (29)	
Poorly differentiated	11 (69)	3 (27)		9 (41)	7 (50)	
Signet ring cell	1 (6)	3 (27)		-	3 (21)	
Others	1 (6)	1 (10)	P=0.161	1 (4)	-	P=0.131
Prior gastrectomy						
Yes	13 (81)	7 (64)		4 (18)	7 (50)	
No	2 (19)	4 (36)	P=0.391	18 (82)	7 (50)	P=0.067

Table II. Comparison of hemoglobin change and physical status.

	Group A		Group B	
	MRG (n=16)	SRG (n=11)	MRG (n=22)	SRG (n=14)
Mean initial Hb (mg/dl)	11.0 (9.2-14.2)	12.2 (9.7-14.3)	11.3 (9.2-14.2)	12.3 (9.7-15.3)
Mean nadir Hb (mg/dl)	1.02 (8.7-12.2)	9.2 (7.4-11.0)	9.9 (8.9-13.2)	9.4 (7.0-13.2)
Mean Hb reduction/cycle	0.4 (0-0.8)	1.8 (1.1-3.9)	0.5 (0-0.9)	2.2 (1.2-4.1)
Mean body weight (kg)	55	57	62	59
Mean height (m)	1.59	1.62	1.62	1.65
Body mass index (BMI, kg/m^2)	23 (11-34)	22 (13-27)	24 (19-33)	22 (14-27)
Body surface area (BSA, m^2)	1.56 (1.35-1.78)	1.59 (1.32-1.86)	1.67 (1.41-2.09)	1.63 (1.27-1.87)

variables were estimated using the Kaplan-Meier methods. Comparison between the two groups was done by Chi-square test or t-test.

Results

Patient characteristics. We obtained genomic DNA from 27 patients in group A. Thirty-six patients in group B were the same patients who were studied previously for the selection of 18 anemia-related genes by microarray-CGH study (8). The 2-1 week schedule was designed for the poor performance status patients (group A). Therefore, performance status was poorer (p<0.001) and body surface area was smaller (p=0.04) in group A than in group B, while body weight (p=0.065), body mass index (p=0.855) and hemoglobin reduction per cycle (p=0.455) showed no differences between the group A and B. On the contrary, totally treated dose of S-1 (p<0.001), totally administered dose of S-1 to the hemoglobin nadir (p<0.001), absolute dose intensity of S-1 (p=0.03) were higher in group B than in group A. The absolute dose intensity of S-1 to the hemoglobin nadir was similar between group A and B (p=0.054). There were no differences in toxicity patterns and grades between the group A and B. From these patients, we compared clinical and genomic factors between the MRG and the SRG. In both group A and B, there were no differences in clinical characteristics between the MRG and the SRG (Table I).

Comparison of the velocity of hemoglobin reduction. There was no difference in initial hemoglobin level before treatment or nadir hemoglobin level during treatment between the MRG and the SRG in both group A and B. However, the velocity of hemoglobin reduction was 4 times higher in the SRG than in the MRG in group A, as well as in group B. We found no difference in body mass index or body surface area between the MRG and the SRG in both group A and B (Table II).

Comparison of administered S-1 dose and dose intensity. There were no differences between the MRG and the SRG in absolute dose intensity (ADI), in relative dose intensity (RDI),

Table III. Comparison of administered S-1 dose and dose intensity.

	Group A		Group B	
	MRG (n=16)	SRG (n=11)	MRG (n=22)	SRG (n=14)
Median cycle number (range)	1.5 (1-6)	1 (1-3)	4 (1-9)	2 (1-10)
Median total treated dose of S-1 (mg)	4,200 (2,100-17,360)	3,360 (1,400-7,560)	12,150 (2,800-24,950)	5,240 (960-36,260)
Median totally administered dose of S-1 to Hb nadir (mg)	2,800 (1,400-11,760)	1,820 (1,400-3,640)	10,080 (2,530-19,600)	3,430 (840-11,670)
Median ADI of S-1 (mg/m^2/week)	310 (192-339)	302 (250-338)	326 (251-372)	327 (249-373)
Median relative dose intensity (RDI) of S-1	0.95 (0.59-1.00)	0.92 (0.76-1.00)	0.98 (0.61-1.00)	0.99 (0.67-1.00)
Median absolute dose intensity (ADI) of S-1 to Hb nadir	313 (142-327)	309 (174-327)	327 (112-373)	335 (133-373)
Median RDI of S-1 to Hb nadir	0.96 (0.44-1.00)	0.95 (0.53-1.00)	1.00 (0.34-1.00)	0.99 (0.36-1.00)

Table IV. Comparison of treatment response and toxicity.

	Group A			Group B		
	MRG (n=16)	SRG (n=11)	P-value	MRG (n=22)	SRG (n=14)	P-value
Response evaluation						
CR/PR	1 (6)	-		9 (40)	1 (7)	
SD	7 (44)	2 (18)		11 (50)	11 (79)	
PD	8 (50)	9 (82)		1 (5)	2 (14)	
NA	-	-	P=1.000	1 (5)	-	P=0.054
Hematological toxicity						
Grade 0-2	15 (94)	10 (91)		17 (77)	11 (79)	
Grade 3-4	1 (6)	1 (9)	P=1.000	5 (23)	3 (21)	P=1.000
Non-hematological toxicity						
Grade 0-2	11 (69)	5 (46)		18 (82)	10 (71)	
Grade 3-4	5 (31)	6 (54)	P=0.226	4 (18)	4 (29)	P=0.683
All toxicity						
Grade 0-2	10 (63)	5 (46)		13 (59)	8 (57)	
Grade 3-4	6 (37)	6 (54)	P=0.381	9 (41)	6 (43)	P=0.908

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease and NA, not assessable.

in ADI to hemoglobin nadir or in RDI to hemoglobin nadir in group A. In group B, the number of treatment cycles was higher in the MRG than in the SRG (4 vs. 2), and thus, both of the total administered dose and administered dose to the nadir of hemoglobin were higher in the MRG. Otherwise, the patterns of S-1 dose intensity were the same for the MRG and the SRG in group B (Table III).

Comparison of treatment response and toxicity. No difference in response was found between the MRG and the SRG in group A (Table IV). In group B, there was a trend of higher response rate toward MRG than SRG (40 vs. 7%, p=0.054), while no difference was found in disease control rate between the two groups (90 vs. 86%). There were also no differences in toxicities between the MRG and the SRG in both group A and B (Table IV).

Comparison of gene copy number of CYP2A6 and DPD. When we compared the $\log_2$R/G ratio in group A, there were no differences in the *CYP2A6* and *DPD* gene copy numbers between the MRG and the SRG. Similar trends were observed in group B (data not shown). In *CYP2A6*, 12 out of 23 patients in group A and 17 out of 36 patients in group B showed a higher $\log_2$R/G ratio above the median $\log_2$R/G ratio for each group. For *DPD*, 14 out of 27 patients in group A and 18 out of 36 patients in group B showed higher $\log_2$R/G ratios above

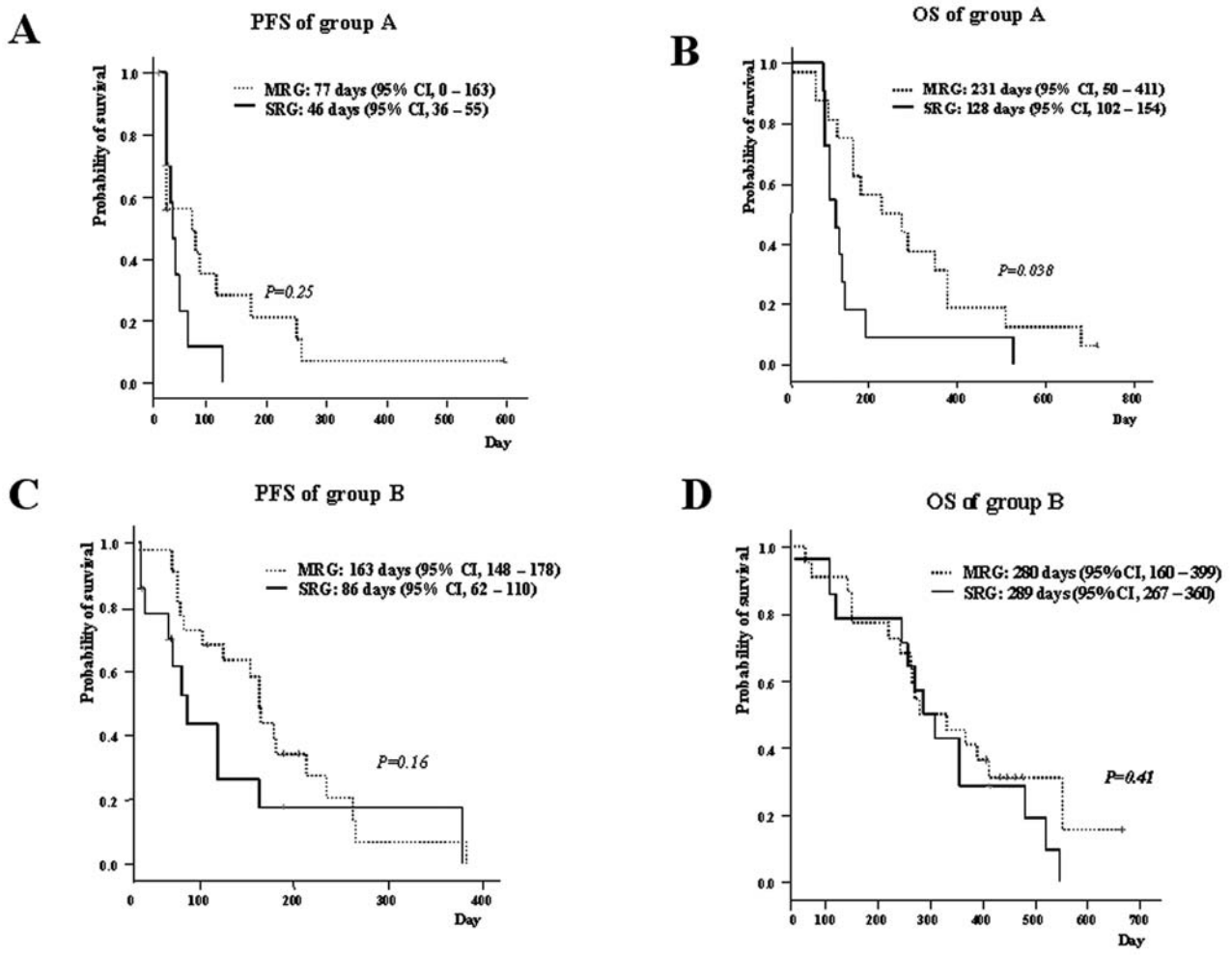

Figure 1. Comparison of progression-free survival and overall survival between the MRG and the SRG: (A) comparison of PFS in group A; (B) comparison of OS in group A; (C) comparison of PFS in group B and (D) comparison of OS in group B.

the median $\log_2$R/G ratio for each group. There were no differences in treatment response or toxicity between the higher (above median) and lower (below median) $\log_2$R/G ratio groups for either gene (data not shown).

Comparison of survival. We did not observe any differences in the progression-free survival between the MRG and the SRG in both groups A and B. In overall survival rates, in group A, the MRG group had a better overall survival compared to that of the SRG (p=0.038). But there was no difference between the MRG and the SRG in group B (Fig. 1).

Selection of anemia-related genes. From group A, we could select 12 genes, which differentiated the SRG from the MRG (Fig. 2) (Table V). Among these genes and clinical factors, the best logistic regression model (Z) was determined as follows: logit LN(Z) = (1.321) + (1.038 x PTX1) + (0.211 x MYO5A) + (0.516 x ZNF664). The prediction accuracy was 78% (MRG 75%, SRG 82%) (Fig. 3A). The AUC was 0.744 for the final regression model (Fig. 3B). There were no common genes found in both groups A and B. When we predicted the hemoglobin reduction from group B using genes from group A or *vice versa*, the accuracy was quite low (data now shown).

Correlations between copy number change and hemoglobin reduction in 3 genes. When we evaluated the association between copy number and hemoglobin reduction in 3 anemia-related genes (*PTX1*, *MYO5A*, *ZNF644*) from group A, weak positive correlations were observed (Fig. 4). When we also evaluated the association between copy number and hemoglobin reduction in 3 anemia-related genes from group B, a trend of positive correlation was found in *C10orf127* gene (r^2=0.11). A trend of negative correlation was found in *XPNPEP2* (r^2=0.14) and *HIST1H2BL* (r^2=0.20) genes (Fig. 4).

When we compared the copy numbers of the three selected genes between the MRG and the SRG, we observed trends of higher copy numbers in the SRG than in the MRG from group A. From group B, *C10orf127* showed a trend of higher copy number in the SRG than MRG, while *HIST1H2BL* and *XPNPEP* showed a trend of lower copy numbers in the SRG than MRG (Fig. 5).

Discussion

Anemia is a unique side effect found in Korean gastric and colorectal cancer patients after S-1 monotherapy (8,9,15). Factors contributing to the ethnic differences in toxicity are clinical status of the patient, pharmacokinetics, pharmaco-

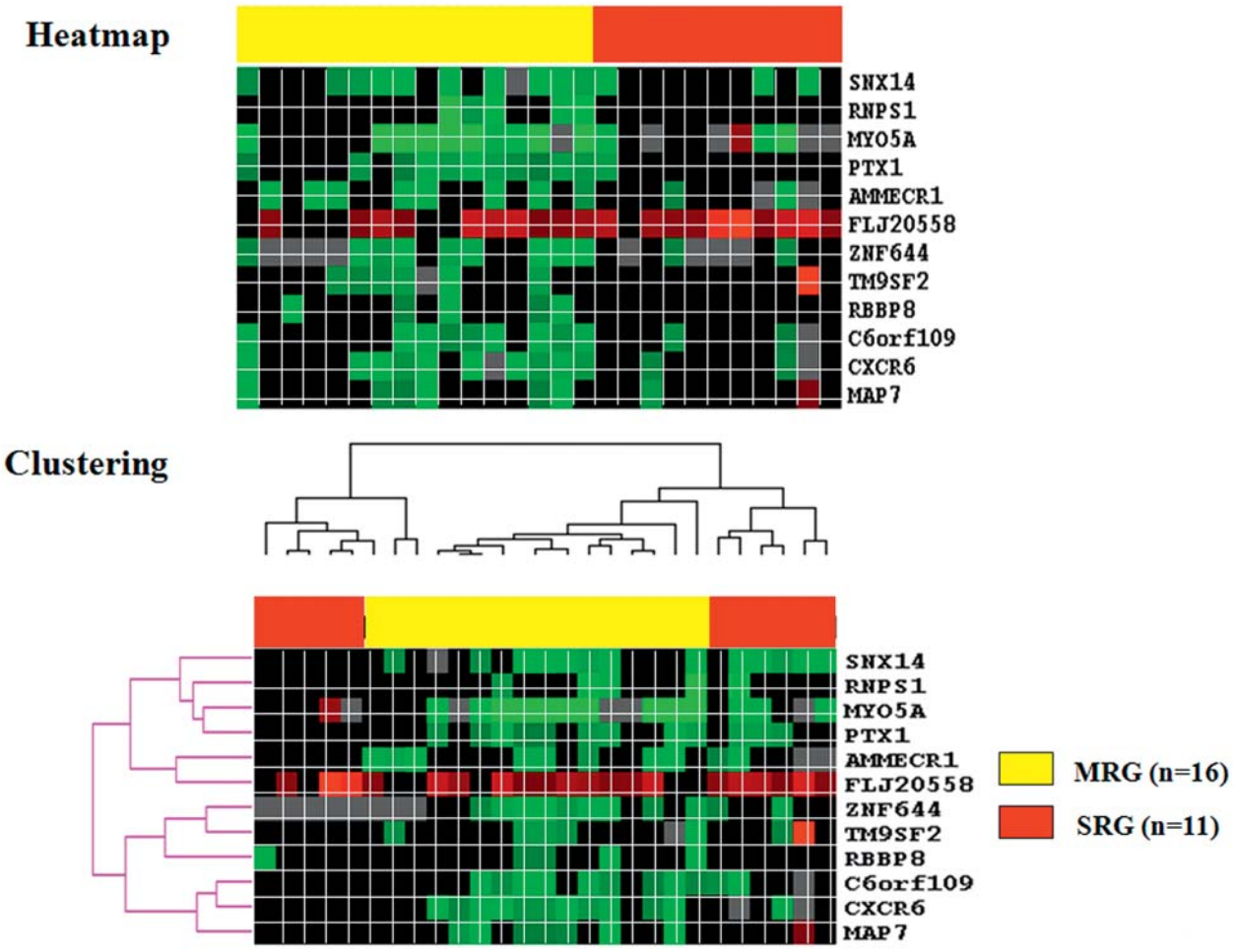

Figure 2. Supervised clustering of the patients with selected 12 genes. Copy number change pattern was expressed as red (amplification), black (normal copy number) or green (deletion).

Table V. Gene annotation of the 12 genes selected from group A.

GeneBank ID	Name	Symbol	Chromosome	Cytoband
AL834128	ERGIC and golgi 2	ERGIC2	12	12p11.22
NM_004800	Transmembrane 9 superfamily member 2	TM9SF2	13	13q32.3
U90942	Myosin VA (heavy chain 12, myoxin)	MYO5A	15	15q21
U73531	Chemokine (C-X-C motif) receptor 6	CXCR6	3	3p21
AK000565	TIA1 cytotoxic granule-associated RNA binding protein	TIA1	2	2p13
NM_201269	Zinc finger protein 644	ZNF644	1	1p22.2
AK021433	Yip1 domain family, member 3	YIPF3	6	6p21.1
NM_003980	Microtubule-associated protein 7	MAP7	6	6q23.3
AK095380	Sorting nexin 14	SNX14	6	6q14.3
BX107845	Transcribed locus	ESTs	X	-
AK092131	RNA binding protein S1, serine-rich domain	RNPS1	16	16p13.3
NM_002894	Retinoblastoma binding protein 8	RBBP8	18	18q11

dynamics, pharamacogenetics especially in genes with drug metabolism, and pharmacogenomics. Recently, we identified three anemia-related genes (*HIST1H2BL*, *C10orf127*, *XPXPEP2*) and used a logistic regression model to predict their association with hemoglobin reduction from a phase II trial group that received a 4-2 week schedule (group B) (8). In this study, we also identified 3 anemia-related genes in patients treated with a 2-1 week schedule of S-1 monotherapy (group A) (9). We used this group as an independent group from group B because, even though we observed S-1 treatment-related anemia in this patient group, the treatment schedule and patient population were different from those of

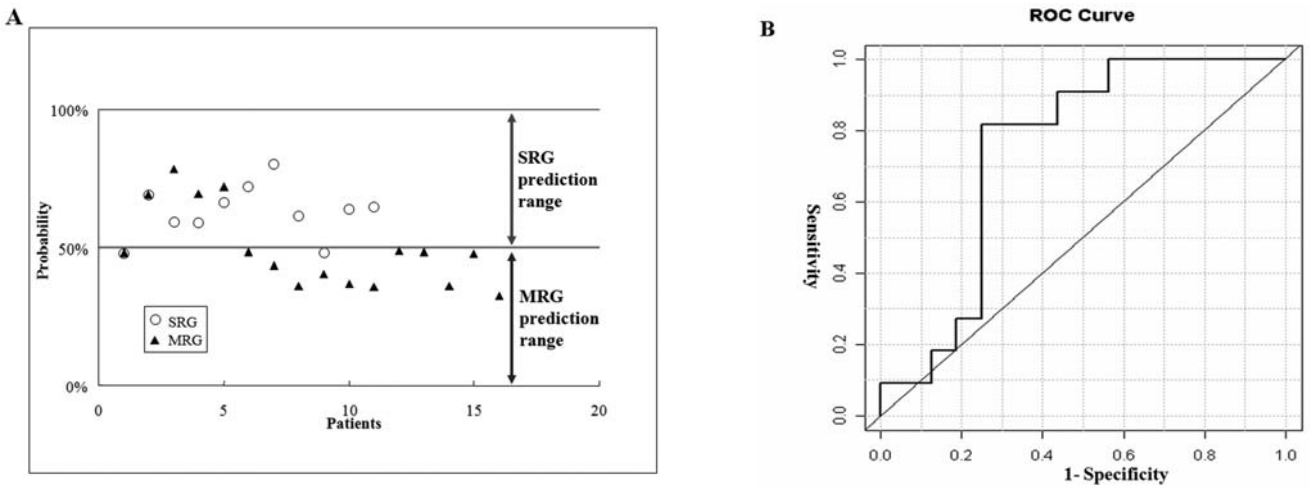

Figure 3. Accuracy of anemia prediction by logistic regression model with the selected 3 genes: logit LN (Z) = (1.321) + (1.038 x PTX1) + (0.211 x MYO5A) + (0.516 x ZNF644). (A) Total accuracy was 77.8% (21/27), accuracy for SRG was 81.8% (9/11), accuracy for MRG was 75.0% (12/16). (B) AUC curve of the final regression model. AUC was 0.744 and the significance was 0.034.

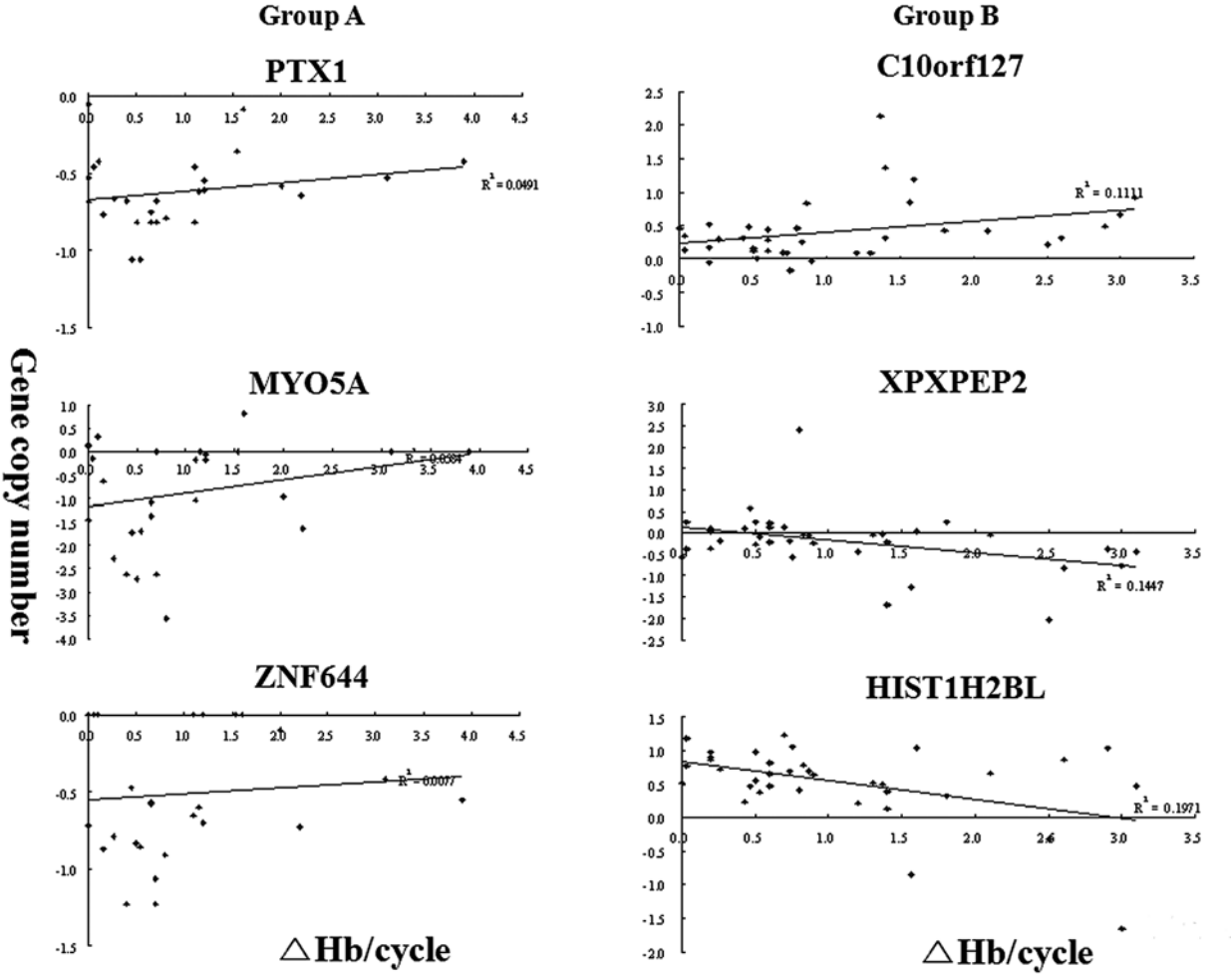

Figure 4. Correlations between copy number change and hemoglobin reduction for the 3 genes in each group A and B.

the 4-2 week schedule. The performance status was poorer and BSA was smaller in group A than B, while total administered dose of S-1 to hemoglobin nadir was higher in group B than A.

Among clinical parameters, there were no differences between the MRG and the SRG groups. Generally, patients with poor survival receive a shorter period of treatment than patients with a better survival. This situation might result in the false, biased interpretation that patients with poor survival experience less treatment-related toxicity. In our study, less-treated patients appeared in the SRG and hemoglobin reduction appeared in the early days of the S-1

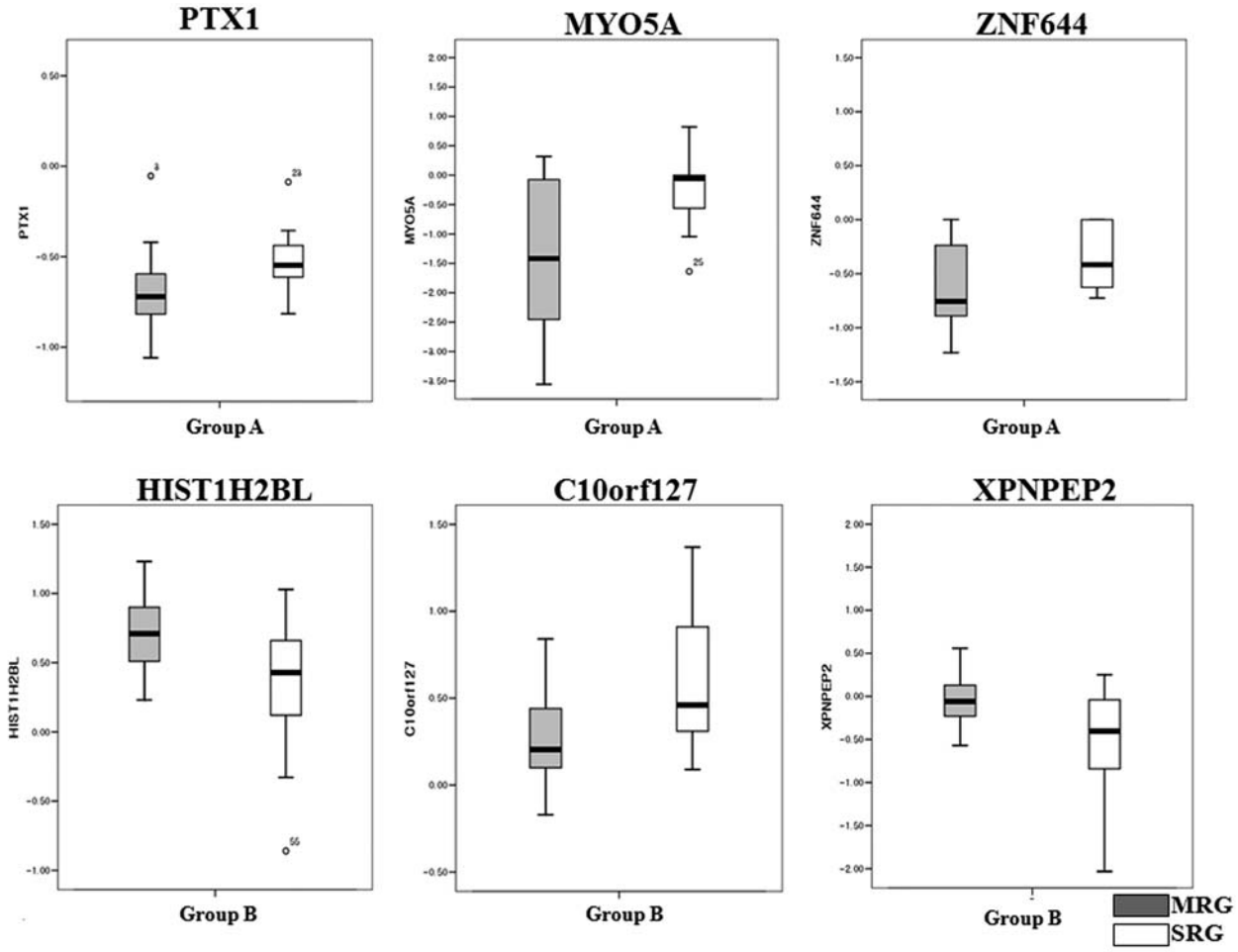

Figure 5. Comparison of copy numbers of the 3 genes between the MRG and the SRG in group A and B.

treatment (Table III) and thus we could exclude the possibility of this bias. We also confirmed that there were no associations between ADI, RDI of S-1 and anemia in either the MRG or the SRG. Some relationship between toxicity and clinical response has been suggested in other cancers, which is still controversial. Breast cancer patients with hematological toxicity have longer survival than patients without toxicity. In non-small cell lung cancer patients, a longer survival was found in patients with neutropenia. In contrast, gastric cancer patients with moderate neutropenia have a longer survival than patients with mild or severe neutropenia. In our patients, there was no difference in clinical response, hematological toxicity, and non-hematological toxicity between the MRG and the SRG. As a result, there was also no difference in PFS between the MRG and the SRG. Same trends were found in group B patients.

Because there was no difference in the pharmacokinetic profiles between Korean and Japanese subjects (8), we compared the copy numbers of the *CYP2A6* and *DPD* genes between the MRG and the SRG groups. Even though *CYP2A6* and *DPD* are the major determinants of 5-FU levels in the blood and tissue, we could not find any differences in the copy numbers of *CYP2A6* or *DPD* between the MRG and the SRG. We did not check the polymorphisms, expression, or biochemical activity of these two genes in this study. However, we are able to conclude that the gene copy numbers of these genes are not determinants of S-1-associated anemia in Korean patients. This result was confirmed in our analysis of group B patients. We are now performing an association study between DPD expression and hemoglobin reduction in Korean patients after S-1 monotherapy.

The process of molecular marker discovery and validation for diagnosis and prognosis are not as well developed as treatment (16). In microarray analysis, the list of genes in classifiers depends greatly on the selection of the patients in the training set (17). Therefore, training and test sets should be large enough to employ either cross-validation or split-sample validation. An independent validation set with sufficient homogeneous patients is a prerequisite for adoption into clinical application (18). However, from a practical point of view, it is very hard to validate the findings from genomics solely by large randomized phase III trials. For this reason, independent validation is carried out in only 10% of studies with microarray data (19). Different patient groups involved in the same clinical problem invariably produce different sets of genes identified as predictors. There can be several different, but equally good, predictor gene sets for the same clinical problem. Therefore, generally, there are very few genes in common between different gene sets that show similar prognostic values. In this situation, the predictive values are real, but highly context-dependent. The 12 selected genes from this study did not overlap with the 18 genes from

group B. When we applied the gene set from group B to this study patients, the prediction accuracy was low as expected (data not shown). Similarly, the 12 genes did not accurately predict hemoglobin reduction in group B. This finding confirms that gene sets are to be selected differently based on the patient population, treatment regimen, dosage and schedule.

As we expected from the logistic regression, all of the 3 selected genes showed a positive correlation. The grade of association was higher in the SRG than the MRG group. This finding demonstrates that these 3 genes are associated with hemoglobin reduction in S-1 treated patients, even though the degree of involvement for each gene may vary. All cross-platform analysis of published data showed diminished, but not completely lost, classification accuracies on data generated by different platforms (20,21). However, at the pathway level, there was highly significant overlapping among different platforms (22). Our findings confirm that distinct molecular mechanisms underlie the same clinical situation of anemia induced from different treatment schedules of S-1. The critical issues such as gene selection bias, error estimation, gene signature fragility, and over-optimistic performance estimation in this early study necessitate more validations with independent patient populations and prospective clinical trials. Presently, the signatures of gene expression from microarray (Mamma Print, Agendia, Amsterdam, The Netherlands) (23,24) and RT-PCR (Oncotype DX assay, Genomic Health, USA) (25,26) analysis are being applied in categorizing the specific groups of the patients for specific types of treatment.

Clinical factor-based prediction model lacks chemotherapy regimen specificity and can not be applied in the selection of one regimen over another. The copy numbers of DPD or cytochrome p450 2A6 were not major determinants of S-1-related anemia in Korean patients. The combined copy number changes of the 3 genes can be developed into a biomarker in predicting S-1 treatment-related anemia. The challenge is converting the identified predictors into diagnostic biomarkers that can guide and optimize clinical decisions through the randomized prospective trials.

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