Post-hoc biomarker analyses of T4a/T4b gastric cancer from patients recruited into SAMIT, a phase III randomized controlled trial

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Abstract

Biomarkers for selecting gastric cancer (GC) patients likely to benefit from sequential paclitaxel treatment followed by fluorinated-pyrimidine-based adjuvant chemotherapy were searched for using samples of patients recruited into SAMIT, a phase III randomized controlled trial. Total RNA was extracted from 556 GC patients and the expression of 105 genes were quantified using real-time PCR. Genes predicting positive effects of sequential paclitaxel on overall survival (OS), disease-free survival (DFS), or cumulative incidence of relapse were identified based on p-values associated with the interaction between the biomarker and sequential paclitaxel or monotherapy group. Low *VSNL1* and *CD44v* expression predicted the positive effects of sequential paclitaxel in all above three endpoints. In the patient subgroup with combined low expression of both genes, sequential paclitaxel therapy was associated with a significantly improved OS (hazard ratio [HR] = 0.48 [95% confidence interval (CI), 0.30–0.78]; $p < 0.01$; interaction p-value < 0.01), particularly in patients with stage IIIB GC (HR = 0.39 [95% CI, 0.20–0.75]; $p < 0.01$; interaction p-value < 0.01). In this study, two biomarkers were identified. Our findings might open up the way for clinical trials on biomarker-oriented postoperative adjuvant chemotherapy for locally advanced cancer.

Introduction

In Japan, 134,650 patients were diagnosed with gastric cancer (GC) in 2019, of which 25,850 had stage II/III disease according to UICC TNM 8th edition[1,2]. The standard treatment for patients with stage II/III GC in Japan is curative D2 gastrectomy followed by fluorinated-pyrimidine-based chemotherapy[3] based on the results from the Japanese ACTS-GC and Korean CLASSIC randomized phase III trials[4,5,6,7]. However, despite improved overall survival (OS) with adjuvant chemotherapy, the five-year OS rate of patients with stage III GC remains unsatisfactory. While RNA expression studies from the ACTS-GC trial identified several novel GC biomarkers, none of them were significantly associated with the S-1 treatment effect[8,9,10,11]. In a post-hoc analysis of resection specimens from the CLASSIC trial, the RNA expression levels of four genes (*GZMB*, *WARS*, *SFRP4*, and *CDX1*) were able to stratify patients by risk of recurrence and to predict the benefit of adjuvant chemotherapy[12].

In addition to fluorinated-pyrimidine and platinum-based anticancer drugs, fluorinated-pyrimidine combined with taxanes such as paclitaxel or docetaxel has been considered for GC treatment[13]. Taxane-based anticancer drugs have a lower incidence of nephrotoxicity or neuropathy than platinum-based compounds, such as cisplatin or oxaliplatin, and can be administered safely in an outpatient setting. Recently, a randomized phase III study in patients with curatively resected pathological stage (pStage) III GC (JACCRO GC-07 trial) reported significantly longer three-year recurrence-free survival in patients treated with adjuvant S-1 plus docetaxel than with adjuvant S-1 alone[14]. Based on these results, chemotherapy with S-1 plus docetaxel after D2 gastrectomy was recommended as the new standard of care for patients with pStage III GC in Japan.

The Stomach Cancer Adjuvant Multi-Institutional Group Trial (SAMIT) was a randomized phase III trial that investigated whether (1) sequential treatment (paclitaxel treatment followed by tegafur, uracil (UFT), or S-1) was superior to monotherapy (UFT or S-1) as adjuvant chemotherapy, (2) S-1 was non-inferior compared to UFT as an adjuvant chemotherapeutic agent in patients with T4a/T4b GC, and (3) sequential treatment with paclitaxel followed by fluorinated-pyrimidine was superior to fluorinated-pyrimidine monotherapy as adjuvant chemotherapy for T4a/T4b GC. The results showed that S-1 was non-inferior compared to UFT and that sequential treatment improved disease-free survival (DFS) only in patients with stage IIIB GC[15].

The JACCRO GC-07 trial and SAMIT both demonstrated improved outcomes in patients with stage III GC treated with adjuvant chemotherapy using fluorinated-pyrimidine and taxane-based anticancer drugs. However, the recurrence rate within 2 years after surgery was 75.3% in the JACCRO GC-07 study (pStage III GC) and 40.0% in the SAMIT (pT4a/pT4b GC). Therefore, we hypothesized that adjuvant chemotherapy using fluorinated-pyrimidine plus taxane-based anticancer drugs may only be effective in a subset of patients. If these patients can be identified using biomarker assessment performed in the gastrectomy specimens, adjuvant treatment regimens can then be personalized to improve patient outcomes.

In this study, we performed a post-hoc analysis of the tissue samples collected from patients recruited in the SAMIT and analyzed a comprehensive panel of RNA expression-based biomarkers to identify genes that might be suitable for selecting patients who are likely to benefit more from sequential paclitaxel and fluorinated-pyrimidine adjuvant chemotherapy than from adjuvant fluorinated-pyrimidine monotherapy.

Results

Patients and sample collection

FFPE samples were obtained from 556 patients who participated in the SAMIT. Twenty-nine patients were subsequently excluded because of insufficient RNA. Therefore, biomarker analysis was eventually performed in 527 patients (94.7%; Fig. 1). The characteristics of the patients included in the current study were representative of those of the entire SAMIT population (Table 1). Except for sex (more males in the sequential paclitaxel treatment group (p=0.04)), the clinical and pathological characteristics were well balanced between the sequential paclitaxel treatment and monotherapy subgroups (Supplementary Table S1, Online Resource 1). The median follow-up time from randomization was 56.8 (interquartile range (IQR)=45.3–69.8 months) and 59.1 months (IQR=46.2–72.8 months) for patients in the monotherapy and sequential paclitaxel arms, respectively.

Predictive biomarkers

We conducted multivariable Cox regression analysis to assess the potential relationship between gene expression level and OS, DFS, or cumulative incidence of relapse after sequential paclitaxel therapy; the genes were ranked based on the interaction-related *p*-values (Table 2: top 10 genes and Supplementary Table S2, Online Resource 1: all genes). *VSNL1* and *CD44v* were the only genes with mRNA expression

levels that were statistically significant as predictive biomarkers of sequential paclitaxel treatment for all three endpoints (Table 3).

Patients with low expression of *VSNL1*, *CD44v*, or both had significantly longer OS and DFS after sequential paclitaxel treatment than after monotherapy (Fig. 2a, b). A total of 191 (36.2%) patients showed combined low expression of both genes, which was related to the most significant benefit from sequential paclitaxel treatment compared to monotherapy (Table 4). No such effect was observed in the cumulative incidence of relapse (Fig. 2c).

The OS improvement in patients with low *VSNL1* and *CD44v* expression treated with sequential paclitaxel remained significant in multivariable analysis after adjustment for clinical and pathological factors in the treatment group (Table 4).

Patient stratification based on the pTNM stage showed that OS improvement in response to sequential paclitaxel treatment in patients with low *VSNL1* and/or *CD44v* expression was greatest in patients with stage IIIB GC (Table 4, Fig. 3).

Internal validation

The overall performance of the different statistical models, including the interactions between *VSNL1* expression and the treatment group, as well as the clinical and pathological factors for OS prediction with C statistics using the bootstrap 0.632+ estimator (0.7111) and apparent estimator (0.7266), was evaluated. The accuracy of OS prediction based on *CD44v* and *VSNL1* expression levels was comparable when the apparent estimator was used (0.7252), whereas it was not sufficiently accurate when the bootstrap 0.632+ estimator was used (0.7083) (Supplementary Table S3, Online Resource 1).

Discussion

This study is the third large-scale biomarker study in patients with gastric cancer treated with adjuvant chemotherapy in a randomized trial setting. Although previous studies using clinical samples from the ACTS-GC trial revealed several novel molecular GC biomarkers, significant interactions between S-1 treatment and the RNA expression level of a set of 63 genes could not be identified[8,9,10,11]. In a study of clinical samples from the CLASSIC trial, the RNA expression levels of four genes were able to stratify GC patients based on high, medium, or low risk of recurrence, or predict benefit from adjuvant chemotherapy. An approximately 7% difference was observed in the 5-year survival rate when patients receiving adjuvant chemotherapy were retrospectively stratified based on the four-gene classifier[12].

Although several candidate biomarkers of resistance or sensitivity to paclitaxel have previously been suggested[16,17,18,19,20,21,22,23,24,] none have been validated in a second independent series. Hence, there remains a clinical need to validate the proposed biomarkers and/or identify new biomarkers that can be used in routine clinical practice to identify patients likely to benefit from paclitaxel therapy[25]. Moreover, multiple studies have reported an association between the expression of several genes or proteins and

benefits from paclitaxel in different tumor types[26,27,28,29,30]. For example, *CCND1* overexpression promotes paclitaxel-induced apoptosis in breast cancer[27]. Members of the BCL-2 and P-glycoprotein families, such as ABCB1, have been reported to be involved in paclitaxel resistance in esophageal cancer[28]. SPARC expression in tumor stromal cells has been suggested as a potential negative predictor of paclitaxel treatment in patients with lung cancer[29,30]. However, the expression levels of these genes were not significantly associated with patient outcomes in the current study. This may be related to the cancer type, sample size, case mix, ethnic differences, or methodological differences.

In the current study, we identified low expression levels of *VSNL1* and/or *CD44v* as potential novel predictive biomarkers of benefit from paclitaxel chemotherapy after curative D2 gastrectomy. *VSNL1* encodes visinin-like protein 1 (VILIP-1)[31]. It has been suggested that the absence or reduced expression of VILIP-1 results in increased cancer cell motility, suggesting a potential tumor suppressor function of this protein[32]. Additionally, VILIP-1 was shown to prevent epithelial-mesenchymal transition of cancer cells by regulating the expression of the transcription factor SNAIL1 in a cAMP-dependent manner[33]. The mechanisms underlying the relationship between *VSNL1* expression and the benefits of paclitaxel chemotherapy remain to be elucidated. CD44v is a cell-surface molecule that senses, integrates, and relays cellular microenvironmental signals to membrane-associated cytoskeletal proteins or to the nucleus and regulates the expression of numerous genes encoding cell behavior-related proteins[34,35,36]. CD44v has been identified as a prognostic and cancer stem cell marker in several different types of cancer, including GC, and has been reported to regulate cancer stemness-related properties, including self-renewal, tumor initiation, aggressiveness, relapse, chemoradiotherapy resistance, and metastasis[37, 38]. However, the biological mechanisms underlying the survival benefit of sequential paclitaxel and fluorinated-pyrimidine adjuvant chemotherapy in patients with GC with low CD44v and/or VSNL1 expression are yet to be clarified.

To our knowledge, this is the first and most comprehensive study to explore and identify potential RNA expression-based biomarkers for the prediction of survival benefit from sequential paclitaxel and fluorinated-pyrimidine adjuvant chemotherapy in patients with GC. However, this study has certain limitations. Although we demonstrated that the study cohort was representative of the entire SAMIT patient cohort with respect to clinicopathological characteristics, including survival, we were only able to retrieve material from approximately one-third of the original SAMIT population. Furthermore, we only analyzed RNA samples from a single tissue block. Therefore, intratumoral heterogeneity at the gene expression level was not assessed.

In conclusion, our study, to the best of our knowledge, is the first to identify biomarkers for selecting patients with locally advanced GC who most likely benefit from adjuvant chemotherapy with sequential paclitaxel and fluorinated-pyrimidine treatment after curative D2 gastrectomy. The validation of our findings in a second independent series followed by a prospective trial is necessary before we can consider using these biomarkers in routine clinical practice. Nevertheless, personalized adjuvant chemotherapy using these biomarkers may improve treatment outcomes in patients with locally

advanced GC. Our results may further help patient selection in clinical trials for biomarker-oriented adjuvant chemotherapy.

Methods

Patients and sample collection

This study was approved by the Institutional Review Board (IRB) of Kanagawa Cancer Center, the central institute for this study (approval number: 26 - 42), as well as the IRBs of all institutions that participated in this study. Representative blocks from formalin-fixed, paraffin-embedded (FFPE) gastrectomy specimens were collected retrospectively from participating institutions according to the following inclusion criteria: (1) patients were participants in the SAMIT, (2) FFPE blocks or unstained cut sections were available, and (3) the translational study protocol was approved by the IRB of the respective institution. Samples were collected by the data center of Kanagawa Cancer Center and shipped to Yokohama City University for RNA extraction and analysis. Sections (each 10-μm thick) were cut from the FFPE blocks and stored at 4°C until microdissection.

RNA extraction and complementary DNA (cDNA) synthesis

Hematoxylin and eosin-stained slides were reviewed, and the area with the highest tumor content was outlined manually. After manual microdissection, total RNA was isolated using NucleoSpin® FFPE RNA XS (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). For RNA quality control, the OD_{260}/OD_{280} ratio was measured using a NanoDrop 2000 (Thermo Fisher Scientific Inc., MA, USA; RRID:SCR_018042). The total RNA integrity number was measured using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Waldbronn, Germany, RRID:SCR_018043). To confirm that the total RNA samples were not contaminated with DNA, *RNA18S1* expression was evaluated by quantitative real-time PCR (qRT-PCR) in each sample before cDNA preparation. cDNA was prepared from samples that passed all the quality control checks. cDNA was synthesized from 0.4 μg of total RNA using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., CA, USA), diluted to 0.2 μg/μL with distilled water, and stored at −20°C until use.

qRT-PCR

qRT-PCR was performed using the QuantiFast™ Probe Assay (QIAGEN, Venlo, Netherlands) and QuantiFast™ Probe PCR (QIAGEN) according to the manufacturer's instructions. The expression of each gene was quantified in triplicate. A standard curve was plotted for each run using three fixed concentrations of human control cDNA synthesized using Xpress Ref Universal Total RNA (QIAGEN) with an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.) to measure the mRNA expression levels in all samples. The concentration of each sample was determined based on the point of intersection of the sample value with the standard curve. *β-actin* and *RNA18S1* were used as the internal controls.

Gene selection

The 105 selected genes included 63 genes analyzed in an exploratory biomarker study of ACTS-GC trial participants[10]. Of these, 57 genes have been previously reported as biomarkers of paclitaxel resistance or sensitivity. The functional annotation of each gene using DAVID 6.7, is outlined in Supplementary Table S4 (Online Resource 1).

Defining the predictive value of the biomarkers

The mRNA expression level of each gene was classified as low versus high using the median mRNA expression level as a cut-off point, as described previously[40]. If the mRNA expression level of a particular gene was below 1.0×10^{-8} ng/μL, the expression level was set to '0.00'. The value of a biomarker in predicting the benefit of sequential paclitaxel based on the OS, DFS, and cumulative incidence of relapse was determined by examining the *p*-values of the interaction between the dichotomized gene expression level and the treatment group (sequential paclitaxel versus monotherapy) after adjusting for clinical and pathological factors using Cox regression or Fine-Gray models[41,42]. The genes were ranked according to treatment interaction-related *p*-values. Values were considered significant at $p<0.05$. Additionally, we combined the expression levels of selected genes to identify sensitive and non-sensitive patient subsets.

Internal validation

We adopted an internal validation strategy, as proposed by Wahl et al.[43], to address the potential overestimation of the standard error owing to multiple imputations and optimism in the predictive performance. We used Harrell's C statistics to analyze the predictive performance of the survival data and addressed the optimistic bias by Harrell's C statistics using the bootstrap 0.632+ method with 20 bootstrap samples from the original dataset with replacement, followed by multiple imputations.

Statistical analysis

The pre-defined statistical analysis plan for this study has been reported previously[40]. The primary and secondary endpoints were the OS and DFS, respectively. The OS and DFS curves were constructed using the Kaplan-Meier method, and the cumulative incidence curves of relapse were constructed using the Aalen-Johansen method[44] to compare sequential paclitaxel and monotherapy, considering the expression levels of the selected genes either individually or in combination. The adjusted hazard ratios (HRs), 95% confidence intervals (CIs), and *p*-values of the major treatment effects and interactions were estimated for the entire patient population and subgroups according to the UICC TNM 8^{th} ed stage[2]. We used multiple imputations to handle missing clinical and pathological factor data and generated 20 multiply imputed datasets for parameter estimates. The reported *p*-values were two-tailed, and the major effects and interactions were considered statistically significant at $p<0.05$. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Declarations

Ethical statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for inclusion in the study.

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Author Contributions

Conceptualization and study design were undertaken by A.T., K.Y., and J.S. Tissue specimens were collected using the T.Y. Y.R. The experiments were performed using the T.O. and Y.M. Statistical analysis and interpretation were performed by J.G. and S.T. Data were interpreted by all investigators. The article and figures were drafted by T.O., A.T, J.G., S.T., P.T., and H.I.G. This article was revised and approved by all investigators, and all authors actively participated in this study.

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Competing Interests

The authors declare no competing interest.

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Tables

Table 1. Clinical and pathological characteristics of patients included in the biomarker analysis compared to the entire SAMIT patient cohort

		Included (*n*=527)		Entire SAMIT cohort (*n*=1433)		
		No. of patients	%	No. of patients	%	*p*-value
Arms	S-1 only	128	24.3	359	25.1	0.98
	UFT only	134	25.4	364	25.4	
	Paclitaxel then UFT	130	24.7	355	24.8	
	Paclitaxel then S-1	135	25.6	355	24.8	
Age	<65 years	246	46.7	670	46.8	1.00
	≥65 years	281	53.3	763	53.2	
Sex	male	361	68.5	980	68.4	1.00
	female	166	31.5	453	31.6	
Histological subtype						
	Differentiated	203	39.2	558	38.9	0.83
	Undifferentiated	315	60.8	844	58.9	
pT (depth of invasion)						
	T1/T2	168	32.0	378	26.4	**0.02**
	T3/T4	357	68.0	1046	73.0	
pN (lymph node status)						
	N0	109	20.8	268	18.7	0.12
	N1	207	39.5	634	44.2	
	N2/3	208	39.7	518	36.1	
pTNM stage						
	IA to II	192	36.6	449	31.3	0.06
	IIIA	144	27.5	458	32.0	
	IIIB	188	35.9	514	35.9	

UFT, tegafur/uracil; T, tumor; N, node; M, metastasis.

Table 2. Top ten genes ranked by interaction *p*-values for benefits from sequential paclitaxel in separate Cox or Fine-Gray regression models adjusted for clinical and pathological characteristics for overall survival, disease-free survival, and cumulative incidence of relapse

Gene	OS		DFS		Cumulative incidence of relapse	
	Rank	Interaction *p*-value	Rank	Interaction *p*	Rank	Interaction *p*-value
VSNL1	1	**<0.01**	1	**0.01**	2	**0.03**
CD44v	2	**0.01**	2	**0.01**	1	**0.02**
MTHFR	3	**0.01**	19	0.27	30	**0.36**
CDH17	4	**0.03**	8	0.08	6	0.06
AREG	5	**0.03**	7	0.08	7	0.07
MSI1	6	0.07	28	0.33	62	0.67
CXCR4	7	0.07	43	0.45	46	0.46
IGF2	8	0.08	4	0.04	13	0.14
CDKN2A	9	0.09	34	0.36	22	0.22
MMP14	10	0.13	36	0.38	34	0.38

OS, overall survival; DFS, disease-free survival.

Table 3. Effects of sequential paclitaxel followed by UFT or S-1 on overall survival, disease-free survival, and cumulative incidence of relapse, based on gene expression levels

	Subgroups	Comparison of sequential paclitaxel and monotherapy over time				
		HR	95% CI		Main effect *p*	Interaction *p*-value
Overall survival						
Total	($n = 527$)	0.76	0.57	1.01	0.05	
VSNL1	Low expression ($n = 375$)	0.61	0.44	0.84	< 0.01	**< 0.01**
	High expression ($n = 152$)	1.55	0.88	2.74	0.13	
CD44v	Low expression ($n = 261$)	0.52	0.34	0.78	< 0.01	**0.01**
	High expression ($n = 266$)	1.09	0.73	1.61	0.67	
Combined	low expression of both genes ($n = 191$)	0.48	0.3	0.78	< 0.01	**0.02**
	high expression of either gene ($n = 336$)	0.98	0.69	1.38	0.89	
Disease-free survival						
Total	(n=527)	0.91	0.7	1.17	0.44	
VSNL1	Low expression ($n = 375$)	0.74	0.55	0.99	0.04	**0.01**
	High expression ($n = 152$)	1.67	1.01	2.77	0.05	
CD44v	Low expression ($n = 261$)	0.64	0.45	0.93	0.02	**0.01**
	High expression ($n = 266$)	1.26	0.88	1.81	0.21	
Combined	low expression of both genes ($n = 191$)	0.57	0.37	0.89	0.01	**0.01**
	high expression of either gene ($n = 336$)	1.16	0.85	1.6	0.35	
Cumulative incidence of relapse						
Total	($n = 527$)	0.98	0.75	1.28	0.87	
VSNL1	Low expression ($n = 375$)	0.82	0.6	1.12	0.21	**0.03**
	High expression ($n = 152$)	1.67	0.96	2.89	0.07	
CD44v	Low expression ($n = 261$)	0.7	0.46	1.05	0.08	**0.02**
	High expression ($n = 266$)	1.36	0.94	1.96	0.1	
Combined	low expression of both genes ($n = 191$)	0.64	0.39	1.03	0.07	**0.03**
	high expression of either gene ($n = 336$)	1.23	0.88	1.71	0.22	

HR, hazard ratio; CI, confidence interval; UFT, tegafur/uracil.

Table 4. Effects of sequential paclitaxel followed by UFT or S-1 on overall survival adjusted for clinical and pathological characteristics stratified by TNM stage and gene expression levels

	Subgroups	Comparison of sequential paclitaxel and monotherapy over time				
		HR	95% CI		Main effect *p*-value	Interaction *p*-value
Stage IA to II						
Total (n = 192)		0.62	0.31	1.24	0.17	
VSNL1	Low expression (n = 128)	0.51	0.21	1.19	0.12	0.41
	High expression (n = 64)	0.94	0.28	3.13	0.92	
CD44v	Low expression (n = 101)	0.66	0.27	1.58	0.35	0.82
	High expression (n = 91)	0.56	0.17	1.80	0.33	
Combined	low expression of both genes (n = 72)	0.53	0.18	1.52	0.24	0.69
	high expression of either gene (n=120)	0.70	0.28	1.77	0.46	
Stage IIIA						
Total (n = 144)		0.76	0.41	1.39	0.37	
VSNL1	Low expression (n = 97)	0.75	0.36	1.54	0.43	0.95
	High expression (n = 47)	0.78	0.25	2.40	0.67	
CD44v	Low expression (n = 76)	0.50	0.22	1.16	0.11	0.09
	High expression (n = 68)	1.57	0.56	4.44	0.39	
Combined	low expression of both genes (n = 52)	0.80	0.31	2.06	0.64	0.93
	high expression of either gene (n = 92)	0.75	0.33	1.69	0.49	
Stage IIIB						
Total (n = 188)		0.78	0.54	1.12	0.17	
VSNL1	Low expression (n = 149)	0.58	0.38	0.87	0.01	**0.01**
	High expression (n = 39)	2.65	1.17	6.02	0.02	
CD44v	Low expression (n = 82)	0.49	0.28	0.86	0.01	**0.03**
	High expression (n = 106)	1.10	0.68	1.78	0.68	
Combined	low expression of both genes (n = 66)	0.39	0.20	0.75	< 0.01	**0.01**
	high expression of either gene (n = 122)	1.11	0.71	1.72	0.66	

HR, hazard ratio; CI, confidence interval; UTF, tegafur/uracil.

Table 5. Genes investigated (n=105)

1. Genes encoding proteins related to the metabolism or activation of anticancer agents							
TYMS	*DPYD*	*UMPS*	*UPP1*	*TYMP*	*GGH*	*DUT*	*MTHFR*
RRM1	*RRM2*	*FPGS*	*DHFR*	*TOP1*	*ERCC1*	*TOP2A*	*MAPT*
2. Genes encoding growth factors and receptor tyrosine kinases							
EGF	*AREG*	*EREG*	*VEGFA*	*IGF2*	*HGF*	*MET*	*FGFR2*
EGFR	*ERBB2*	*ERBB3*	*KDR*	*IGF1R*	*PDGFRB*		
3. Genes encoding proteins related to the p13K-AKT, RAS, and RAP1 signaling pathways							
PIK3CA	*JAK2*	*PTEN*	*ITGB3*	*PLA2G2A*	*THBS1*		
4. Tumor suppressor protein-encoding genes							
SEMA3B	*RUNX3*	*MLH1*	*APC*	*DAPK1*	*MGMT*	*CDKN2A*	
5. Genes encoding apoptosis-related proteins							
E2F1	*BCL2*	*GADD45*	*FAS*	*BIRC5*	*BCL2L11*	*BAX*	*CCND1*
6. Genes related to cancer stem cells							
LGR5	*PROM1*	*CD44*	*NANOG*	*MSI1*			
7. Genes related to anticancer drug resistance							
ABCG2	*ABCB1*	*ABCC1*	*CAV1*				
8. Genes encoding members of the MMP family							
MMP2	*MMP7*	*MMP9*	*MMP10*	*MMP11*	*MMP14*	*TIMP1*	
9. Genes encoding cell adhesion factor and ECM							
CDH17	*LGALS4*	*VCAM1*	*HPSE*	*DSG2*	*CDX2*		
10. Genes encoding members of the claudin family							
CLDN3	*CLDN4*	*CLDN7*	*CLDN18.2*				
11. Genes encoding chemokine receptors							
CCR7	*CXCR4*						
12. Genes related to immune checkpoint regulation							
PDL1	*PDL2*						
13. Epigenetic repression genes							
HDAC1	*EZH2*						
14. Genes identified by SAGE and CAST methods [17]							
APOE	*REG4*	*MIA*	*OLFM4*	*SEC11A*	*TSPAN8*	*TM9SF3*	*ZDHHC14*
15. Other genes							
INHBA	*LDHA*	*PTGS2*	*VSNL1*	*TGFA*	*MUC13*	*SIRT1*	*GZMA*
ESR1	*MUC2*	*SPARC*	*ANGPT2*	*PLAU*	*PECAM1*		

Figures

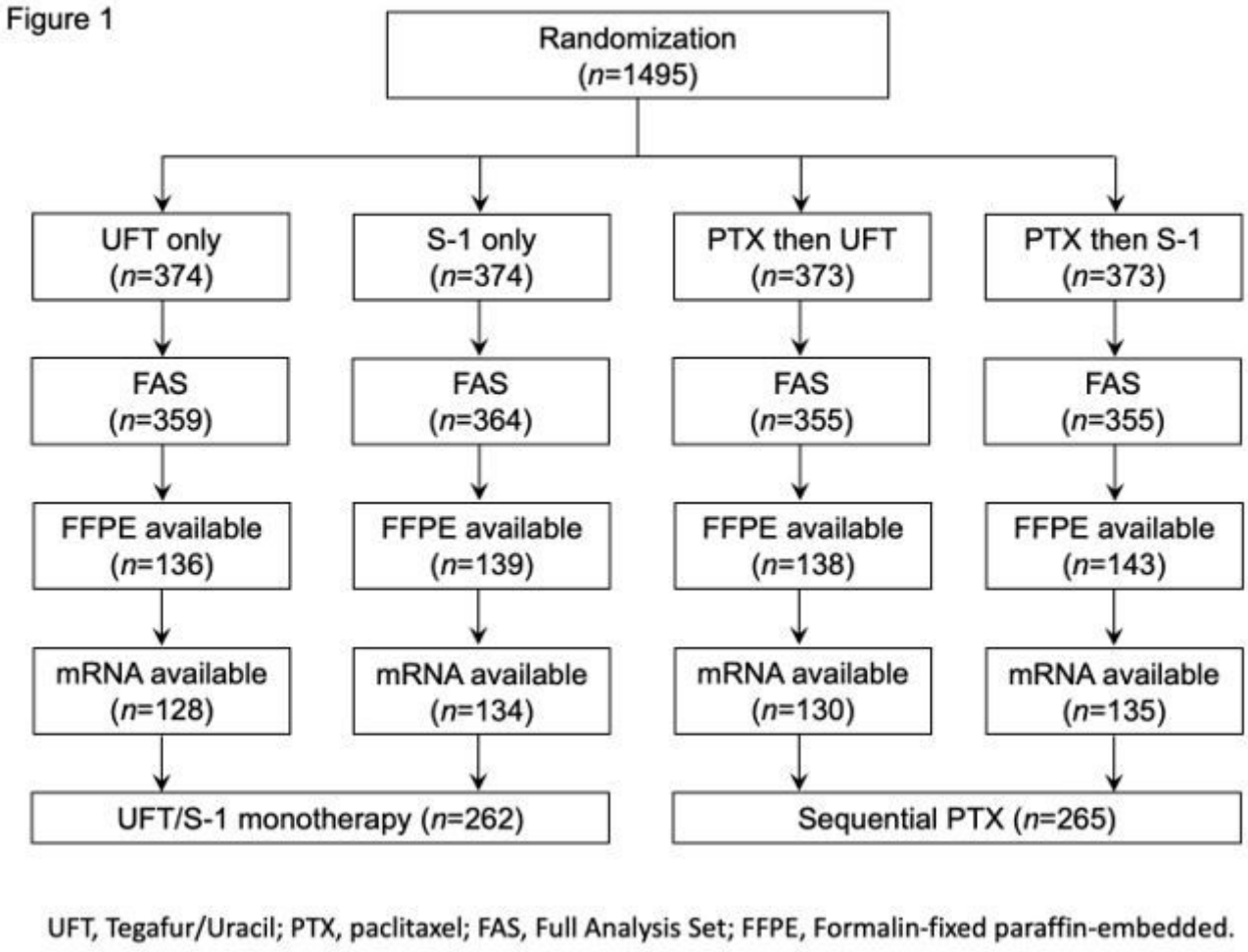

Figure 1

Flowchart of SAMIT patients available for primary analysis and subsequent biomarker analysis. Formalin-fixed, paraffin-embedded (FFPE) samples were available from 556 SAMIT patients. Twenty-nine patients had to be excluded owing to insufficient RNA.

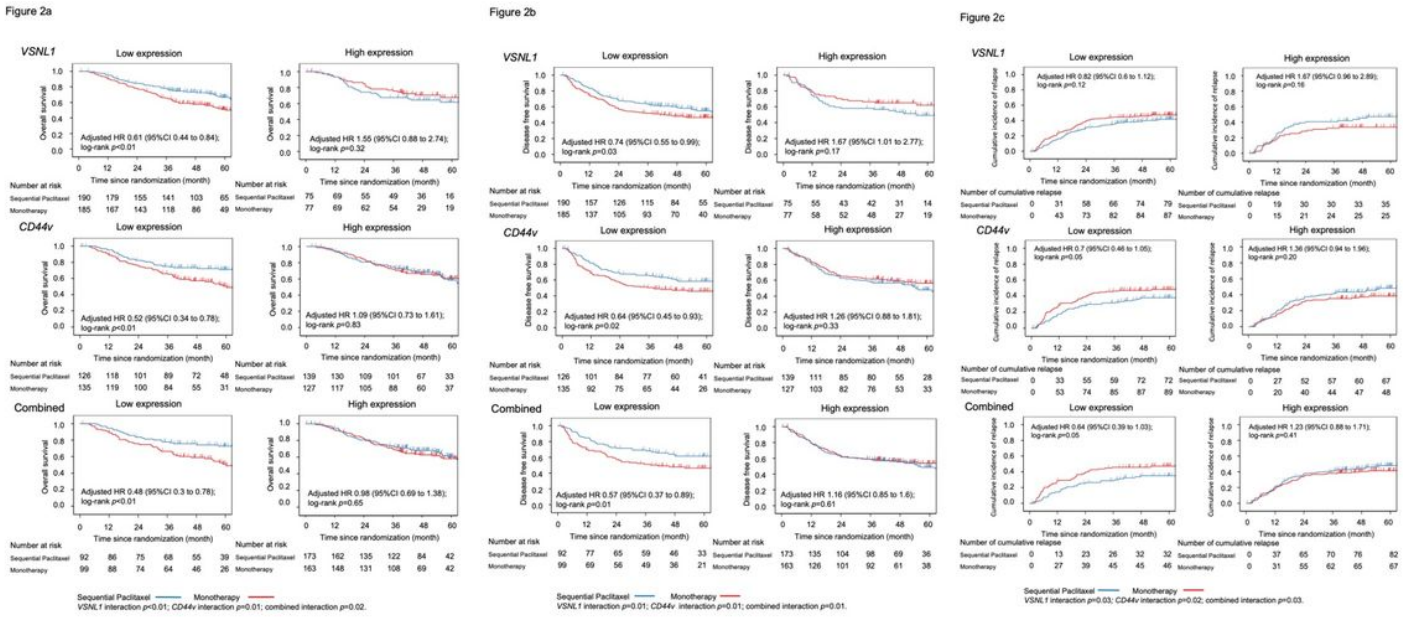

Figure 2

Kaplan-Meier curves by gene expression level in the sequential paclitaxel and monotherapy arms. Patients with low RNA expression levels of VSNL1, CD44v, or both had significantly longer overall survival (a), longer disease-free survival (b), and lower cumulative incidence of relapse (c) after sequential paclitaxel treatment than after monotherapy.

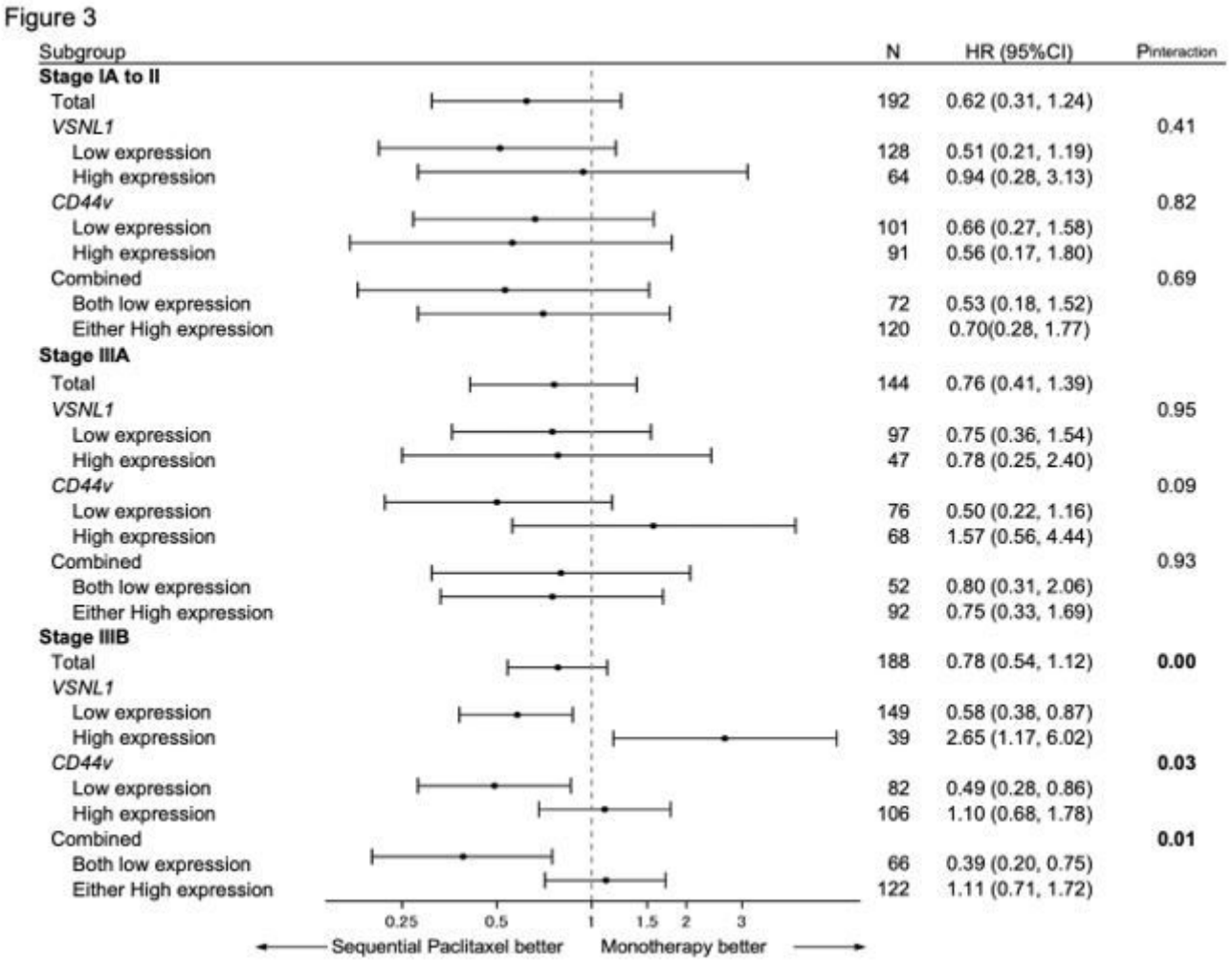

Figure 3

Forest plot of the study results. After patient stratification based on the pTNM stage, the survival benefit from sequential paclitaxel treatment was greater among patients with stage IIIB gastric cancer with low expression of either gene or combined low gene expression. The association between low expression levels of VSNL1 and CD44v and potential benefits from sequential paclitaxel treatment were significant for disease-free survival and cumulative incidence of relapse.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS13.docx
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