An ancillary biomarker study in the SAMIT randomized trial: Sequential paclitaxel followed by UFT or S-1 versus UFT or S-1 alone as adjuvant chemotherapy for T4a/b gastric cancer

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INTRODUCTION

Stomach cancer Adjuvant Multi-Institutional group Trial (SAMIT), was a large randomized trial in order to assess superiority of sequential treatment (paclitaxel then UFT or paclitaxel then S-1) compared with monotherapy (UFT or S-1) and together with evaluation of non-inferiority of UFT compared with S-1. Sequential paclitaxel did not improve disease-free survival and UFT was not non-inferior to S-1, while S-1 was superior to UFT as adjuvant treatment for T4a or T4b gastric cancer. While in subset analysis, sequential treatment was effective for patients with stage IIIB disease¹⁾. S-1 is still a standard adjuvant treatment in Japan^{2, 3)} but the efficacy for those patients with poor prognosis is limited. Effective and individual regimens for those patients are awaited. This study aims prospective-retrospective evaluation of prognostic and predictive classifier for patients with stomach cancer after curative surgery.

PROTOCOL DIGEST OF THE STUDY

Objective

To identify molecular biomarkers for sequential pa-

clitaxel, we have corrected and analyzed mRNA from the resected gastric cancer specimens in SAMIT. The primary endpoint is overall survival, and the secondary endpoints are disease-free survival, relapse-free survival, and clinicopathological factors.

Material

Criteria for accrual were 1) cases in the full analysis set of 1433 of SAMIT trial, 2) resected specimens' paraffin-embedded blocks or slides were available for sending, 3) the study was approved by the Institutional Review Board (IRB). The study office assigned study-specific numbers for each sample, then sent the sample numbers, labels, and the Standard Operating Procedure (SOP) to the attending institutes. The attending institutes labeled paraffin-embedded blocks or slides with this study-specific numbers, and sent the samples to Kanagawa Cancer Center, Data Center. The study period is 2 years after IRB approval.

Laboratory Methods

Collected samples at Kanagawa Cancer were sent to Yokohama City University, and mRNA is extracted. Selected 104 genes and internal reference genes (beta-actin and 18S) (Table 1) were quantified with a fluorescence-based real-time PCR method (CFX Connect™ Real-Time PCR Detection System Bio-Rad Laboratories, Inc., Hercules, CA, USA). Parts of extracted RNA were sent to Singapore National Cancer Center, where mRNA

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Table 1. Measured Genes (106 genes)

1. Genes of metabolism and activation of anticancer agents							
TYMS	DPYD	OPRT	ERCC1	TOP2A	UPP1	TYMP	MTHFR
RRM1	RRM2	GGH	FPGS	DHFR	TOPI		
2. Genes (including families) of targets of molecularly targeted agents							
EGFR	ERBB2	VEGFA	VEGFR2	IGF1R	HGF	FGFR2	MUC13
PDGFRB	EZH2	EGF	MET	IGF2	AREG	EREG	ANGPT2
HDAC1	PIK3CA	PIK3CA	JAK2	PDL1	PDL2	ERBB3	
3. Cancer related genes							
CDH17	LGALS4	PTGS2	THBS1	HPSE	ITGB3	TGFA	VCAM1
MUC2	CDX2	SPARC	DUT	PLAU			
4. Tumor suppressor genes							
PTEN	RUNX3	MLH1	APC	DAPK1	MGMT	CDKN2A	SEMA3B
5. Genes related to apoptosis							
E2F1	BCL2	GADD45	FAS	BIRC5	BCL2L11	BAX	CCND1
6. Genes related to cancer stem cells							
LGR5	CD133	CD44	NANOG	MSI1			
7. Genes related to anticancer drug resistance							
ABCG2	ABCB1	ABCC1	CAV1				
8. Genes of MMP family							
MMP2	MMP7	MMP9	MMP10	MMP11	MMP14	TIMP1	
9. Genes of claudin family							
CLDN3	CLDN4	CLDN7	CLDN18				
10. Genes of chemokine receptor							
CCR7	CXCR4						
11. Genes identified by SAGE and CAST methods							
APOE	REG4	MIA	OLFM4	SEC11A	TSPAN8	TM9SF3	ZDHHC14
12. Others							
INHBA	LDHA	PTGS2	VSNL1	PLA2G2A	DSG2	SIRT1	GZMA
ESR1	ACTB	18S					

expression patterns related to histological subtype and main pathways of gastric cancer using the NanoString expression platform. DNA SNIPs of genes were analyzed by selective DNA sequencing.

Statistical analysis

The mRNA levels of each gene were categorized into low and high using the median as a cut-off point. Associations between mRNA levels and clinicopathological factors were explored using descriptive statistics, t-tests and Fisher exact tests. Survival curves for overall survival, disease-free survival, and relapse-free survival according to mRNA levels or subgroups identified through subsequent analyses were estimated by Kaplan-Meier method.

Associations between gene expression and overall survival, disease-free survival, and relapse-free survival as prognostic markers were explored by Cox regression. Each gene expression was initially screened through a p-value of univariate Cox regression with the critical value of $p = 0.2$. Subsequently, baseline covariates and gene expression with p values lower than 0.2 in the univariate analysis were included in multivariate Cox regression. In the multivariate model, gene expression was further screened through a backward variable selection with the critical value of $p = 0.1$.

Predictive markers of sequential paclitaxel for overall survival, disease-free survival, and relapse-free sur-

vival were identified by examining interaction p-values between a predictive marker and the treatment group (sequential paclitaxel or monotherapy) in Cox regression. Each gene expression was screened through an interaction p-value with the critical value of $p = 0.2$. The hazard ratios, 95% confidence intervals, p values of main treatment effects and interactions were estimated in each subgroup identified. The final prognostic and predictive models were validated through 10-fold cross-validation. For the validity of cutoff points of mRNA level, two subgroups by altering cutoff points of each gene expression were created and the minimum interaction p-value among different cutoff points was compared with the p-value based on the median as a cut-off point.

All reported p values are two-tailed and $p < 0.05$ is chosen as the threshold for statistical significance. The clinical data of SAMIT trial from ECRIN data center and molecular data were collected at the data center. Academic statisticians at Kyoto University (ST and JG) conducted statistical analysis using IBM SPSS Statistics Version 19.0 (SPSS, Inc., Chicago, IL, USA) and the SAS, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Study profile is shown in Figure 1. The clinical samples of 556 cases were collected among 1433 cases of SAMIT trial. Among them, 106 genes expressions were assessable in 531 cases. In addition, characteristics of

Fig. 1 Study profile
The clinical samples of 556 cases were collected among 1433 cases of SAMIT trial.
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Table 2 Patient characteristics

Factors		UFT only				S-1 only				Paclitaxel then UFT				Paclitaxel then S-1			
		Primary analysis (n=358)		Biomarker analysis (n=130)		Primary analysis (n=364)		Biomarker analysis (n=133)		Primary analysis (n=355)		Biomarker analysis (n=131)		Primary analysis (n=355)		Biomarker analysis (n=137)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Age	Median (range)	65 (29-80)		64 (29-80)		65 (30-80)		65 (38-80)		65 (22-80)		65 (35-80)		65 (36-80)		66 (37-80)	
Gender	Male	248	69	91	70	244	67	83	62	239	67	88	67	261	74	102	74
	Female	110	31	39	30	120	33	50	38	116	33	43	33	104	29	35	26
Performance status	0	316	88	113	87	314	86	110	83	301	85	108	82	303	86	114	83
	1	42	12	17	13	50	14	23	17	54	15	23	18	52	14	23	17
Histological type	Differentiated	141	39	45	35	147	40	58	44	134	38	51	39	140	39	58	42
	Undifferentiated	214	60	83	64	209	57	73	55	215	61	78	60	210	59	79	58
	Others	3	1	2	1	8	2	2	1	6	2	2	1	5	2	0	0
Pathological T	T1	4	1	2	2	0	0	0	0	0	0	0	0	1	0	0	0
	T2	88	25	42	32	15	4	6	16	18	5	6	5	97	27	46	34
	T3	243	68	80	67	323	89	121	75	300	85	113	86	240	68	87	64
	T4a	23	6	6	5	26	7	6	5	37	10	12	9	17	5	4	3
Pathological N	N0	68	19	18	14	65	18	25	19	73	21	37	28	65	18	30	22
	N1	65	18	25	19	68	19	22	17	84	24	27	21	72	20	16	12
	N2	78	22	25	19	102	28	36	27	88	25	30	23	85	24	30	22
	N3a	99	28	40	31	84	23	25	19	73	21	19	15	83	23	39	28
	N3b	48	13	22	17	45	12	25	19	37	10	18	14	50	14	22	16
CY	CY0	310	87	119	92	315	87	117	88	314	88	122	93	307	86	124	91
	CY1	26	7	8	6	29	8	12	9	24	7	8	6	27	8	10	7
	CYX	22	6	3	2	20	5	4	3	17	5	1	1	21	6	3	2
TNM Stage	IA	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	IB	21	6	5	4	4	1	3	2	6	2	1	1	19	5	12	9
	IIA	64	17	24	18	51	14	19	12	63	18	36	27	64	18	25	18
	IIB	62	17	21	16	75	21	24	18	80	23	24	18	79	22	23	17
	IIIA	71	20	25	19	101	28	37	28	91	26	33	25	68	19	24	18
	IIIB	76	21	31	24	70	19	20	15	62	17	16	12	68	19	28	20
	IIIC	37	10	15	12	34	9	18	14	29	8	13	10	30	8	15	11
	IV	26	7	8	6	29	8	12	9	24	7	8	6	27	8	10	7

these 556 cases in the biomarker study and the all 1433 cases of SAMIT trial did not differ substantially (Table 2). The manuscripts with the details of the analysis are in preparation and will be published elsewhere.

Conclusions

In this study, several molecular biomarkers for selection of the cases suitable to the treatment of sequential paclitaxel followed by fluorinated pyrimidine as adjuvant chemotherapy for T4a/b gastric cancer were identified. The papers with the details of the findings and the interpretation in this study will be published elsewhere.

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