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 Japan2,3) 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 paclitaxel, 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
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 survival 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
### Table 2  Patient characteristics

| Factors                  | UFT only (n=358) | S-1 only (n=364) | Paclitaxel then UFT (n=355) | Paclitaxel then S-1 (n=137) |
|--------------------------|------------------|------------------|-----------------------------|-----------------------------|
|                          | No. %            | No. %            | No. %                       | No. %                       |
| Age                      |                  |                  |                             |                             |
| Median (range)           | 65 (29-80)       | 65 (30-80)       | 65 (35-80)                  | 65 (36-80)                  |
| Gender                   |                  |                  |                             |                             |
| Male                     | 248 (69)         | 244 (67)         | 239 (67)                    | 261 (74)                    |
| Female                   | 110 (31)         | 120 (33)         | 116 (33)                    | 104 (29)                    |
| Performance status       |                  |                  |                             |                             |
| 0                        | 316 (88)         | 314 (86)         | 301 (85)                    | 303 (86)                    |
| 1                        | 42 (12)          | 50 (14)          | 54 (15)                     | 52 (14)                     |
| Histological type        |                  |                  |                             |                             |
| Differentiated           | 141 (39)         | 147 (40)         | 134 (38)                    | 140 (39)                    |
| Unidifferentiated        | 214 (60)         | 209 (57)         | 215 (61)                    | 210 (59)                    |
| Others                   | 3 (1)            | 8 (2)            | 6 (2)                       | 5 (2)                       |
| Pathological T           |                  |                  |                             |                             |
| T1                       | 4 (1)            | 0 (0)            | 0 (0)                       | 1 (0)                       |
| T2                       | 88 (25)          | 15 (4)           | 18 (5)                      | 97 (27)                     |
| T3                       | 243 (68)         | 323 (89)         | 300 (85)                    | 240 (68)                    |
| T4a                      | 23 (6)           | 26 (7)           | 37 (10)                     | 17 (5)                      |
| Pathological N           |                  |                  |                             |                             |
| N0                       | 68 (19)          | 65 (18)          | 73 (21)                     | 65 (18)                     |
| N1                       | 65 (18)          | 68 (19)          | 84 (24)                     | 72 (20)                     |
| N2                       | 78 (22)          | 102 (28)         | 88 (25)                     | 85 (24)                     |
| N3a                      | 99 (28)          | 84 (23)          | 73 (21)                     | 83 (23)                     |
| N3b                      | 48 (13)          | 45 (12)          | 37 (10)                     | 50 (14)                     |
| CY                       |                  |                  |                             |                             |
| CY0                      | 310 (87)         | 315 (87)         | 314 (88)                    | 307 (86)                    |
| CY1                      | 26 (7)           | 29 (8)           | 24 (7)                      | 27 (8)                      |
| CYX                      | 22 (6)           | 20 (5)           | 17 (5)                      | 21 (6)                      |
| TNM Stage                |                  |                  |                             |                             |
| IA                       | 1 (0)            | 0 (0)            | 0 (0)                       | 0 (0)                       |
| HB                       | 21 (6)           | 4 (1)            | 2 (6)                       | 1 (1)                       |
| HA                       | 64 (17)          | 51 (14)          | 63 (18)                     | 64 (18)                     |
| HB                       | 62 (17)          | 75 (21)          | 80 (23)                     | 79 (22)                     |
| HA                       | 71 (20)          | 101 (28)         | 91 (26)                     | 68 (19)                     |
| HB                       | 76 (21)          | 70 (19)          | 62 (17)                     | 68 (19)                     |
| HNC                     | 37 (10)          | 34 (9)           | 29 (8)                      | 30 (8)                      |
| JV                       | 26 (7)           | 29 (8)           | 24 (7)                      | 27 (8)                      |

Fig. 1  Study profile
The clinical samples of 556 cases were collected among 1433 cases of SAMIT trial. Among them, 106 genes expressions were assessable in 531 cases.
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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References

1) Tsuburaya A, Yoshida K, Kobayashi M, Yoshino S, Takahashi M, Takiguchi N, Tanabe K, Takahashi N, Imamura H, Tatsumoto N, Hara A, Nishikawa K, Fukushima R, Nozaki I, Kojima H, Miyashita Y, Oba K, Buyse M, Morita S, Sakamoto J. (2014) Sequential paclitaxel followed by tegafur and uracil (UFT) or S-1 versus UFT or S-1 monotherapy as adjuvant chemotherapy for T4a/b gastric cancer (SAMIT): a phase 3 factorial randomised controlled trial. Lancet Oncol. 15: 886-93.

2) Sakuramoto S, Sasaki M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, Furukawa H, Nakajima T, Ohashi Y, Imamura H, Higashino M, Yamamura Y, Kurita A, Arai K; ACTS-GC Group. (2008) Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med. 2007 Nov 1; 357(18): 1810-20. Erratum in: N Engl J Med. 358: 1977.

3) Sasako M, Sakuramoto S, Katai H, Kinoshita T, Furukawa H, Yamaguchi T, Nashimoto A, Fujii M, Nakajima T, Ohashi Y. (2011) Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. J Clin Oncol. 29: 4387-93.