Clinical Cancer Research

Randomized Phase II Study of Adjuvant Chemotherapy with Long-term S-1 versus Cisplatin+S-1 in Completely Resected Stage II–IIIA Non–Small Cell Lung Cancer

Yasuo Iwamoto[1], Tetsuya Mitsudomi[2], Kazuko Sakai[3], Takeharu Yamanaka[4], Hiroshige Yoshioka[5], Makoto Takahama[6], Masahiro Yoshimura[7], Ichiro Yoshino[8], Masayuki Takeda[9], Shunichi Sugawara[10], Tomoya Kawaguchi[11], Toshiaki Takahashi[12], Mitsunori Ohta[13], Yukito Ichinose[14], Shinji Atagi[15], Morihito Okada[16], Hideo Saka[17], Kazuhiko Nakagawa[9], Yoichi Nakanishi[18], and Kazuto Nishio[3]

Abstract

Purpose: The aims of this study were to evaluate the efficacy and safety of S-1 versus cisplatin (CDDP)+S-1 in patients with completely resected stage II and IIIA non–small cell lung cancer, and to identify predictive biomarkers whose expression in the tumors was significantly associated with patient outcome.

Experimental Design: A total of 200 patients were randomly assigned to receive either S-1 (40 mg/m^2 twice per day) for 2 consecutive weeks repeated every 3 weeks for 1 year (S group) or CDDP (60 mg/m^2) on day 1 plus oral S-1 (40 mg/m^2 twice per day) for 2 consecutive weeks repeated every 3 weeks for four cycles (CS group) within 8 weeks after surgery. The primary endpoints were relapse-free survival (RFS) at 2 years and identification of predictive biomarkers whose expressions have been reported to be associated with CDDP or fluoropyrimidine sensitivity.

Results: The RFS rate at 2 years was 65.6% (95% confidence intervals; CI, 55.3–74.0%) in the S group and 58.1% (95% CI, 47.7–67.2%) in the CS group. The only gene with interaction of $P < 0.05$ was uridine monophosphate synthase (UMPS; $P = 0.0348$). The benefit that members of the S group had over members of the CS group was higher expression of UMPS. *In vitro* and *in vivo* experiments confirmed that overexpression of UMPS enhanced the antitumor effect of fluoropyrimidine.

Conclusions: Adjuvant S-1 monotherapy might be preferable to CDDP+S-1 for patients with completely resected NSCLC. UMPS expression may define a patient subset that would benefit from long-term postoperative S-1 monotherapy. *Clin Cancer Res; 21(23); 5245–52. ©2015 AACR.*

[1]Department of Medical Oncology, Hiroshima City Hospital, Hiroshima, Japan. [2]Department of Thoracic Surgery, Kinki University Faculty of Medicine, Osakasayama, Japan. [3]Department of Genome Biology, Kinki University Faculty of Medicine, Osakasayama, Japan. [4]Department of Biostatistics, Yokohama City University, Yokohama, Japan. [5]Department of Respiratory Medicine, Kurashiki Central Hospital, Kurashiki, Japan. [6]Department of General Thoracic Surgery, Osaka City General Hospital, Osaka, Japan. [7]Department of Thoracic Surgery, Hyogo Cancer Center, Akashi, Japan. [8]Department of General Thoracic Surgery, Chiba University Graduate School of Medicine, Chiba, Japan. [9]Department of Medical Oncology, Kinki University Faculty of Medicine, Osakasayama, Japan. [10]Department of Pulmonary Medicine, Sendai Kousei Hospital, Sendai, Japan. [11]Department of Respiratory Medicine, Graduate School of Medicine, Osaka City University, Osaka, Japan. [12]Division of Thoracic Oncology, Shizuoka Cancer Center, Shuntougun, Japan. [13]Department of Thoracic Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Japan. [14]Clinical Research Institute, National Kyushu Cancer Center, Fukuoka, Japan. [15]Department of Thoracic Oncology, Kinki-chuo Chest Medical Center, Sakai, Japan. [16]Department of Surgical Oncology, Hiroshima University Graduate School of Medicine, Hiroshima, Japan. [17]Department of Medical Oncology, National Hospital Organization Nagoya Medical Center, Nagoya, Japan. [18]Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Corresponding Author: Tetsuya Mitsudomi, Department of Thoracic Surgery, Kinki University Faculty of Medicine, 377-2, Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Phone: 81-72-366-0221; Fax: 81-72-368-3382; E-mail: mitsudom@surg.med.kindai.ac.jp

doi: 10.1158/1078-0432.CCR-14-3160

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, and non–small cell lung cancer (NSCLC) accounts for 85% of all lung cancers. Complete surgical resection is the most effective strategy for early-stage NSCLC. However, the 5-year survival rate of patients with completely resected NSCLC of pathologic stages I to IIIA ranges from 32.8% to 83.9% (1). The main reason for this poor outcome is development of distant metastasis, so controlling the micrometastases that are assumed to be present at the time of surgery with adjuvant drug therapy may lead to improvements in overall survival (OS) rates.

In the past decade, several randomized phase III studies have demonstrated that adjuvant cisplatin (CDDP)-based chemotherapy improves OS in patients with pathologic stage II and IIIA NSCLC (2–4). Pignon and colleagues reported an 11% reduction in the risk of death with the use of CDDP-based chemotherapy. However, they also observed a small number of deaths related to chemotherapy toxicity and cardiopulmonary complications (5), mainly caused by CDDP. Thus, a challenge for researchers is to develop safer and more efficacious treatment regimens.

In Japan, it has been shown that adjuvant chemotherapy with tegafur-uracil (UFT, Taiho Pharmaceutical Co. Ltd) improves 5- and 7-year survival rates in patients with completely resected stage

Translational Relevance

S-1 is an oral anticancer agent that consists of tegafur, gimeracil, and oteracil potassium at a molar ratio of 1:0.4:1. In this randomized phase II trial, we compared long-term S-1 monotherapy with cisplatin+S-1 in patients with completely resected pathologic stage II and III non–small cell lung cancer and explored predictive biomarkers. The primary endpoint—relapse-free survival at 2 years—was similar in both groups, and the toxicities were mild in the S-1 group. We identified expression levels of uridine monophosphate synthase (UMPS) as a predictive biomarker for long-term S-1 monotherapy. We were also able to confirm the utility of UMPS in *in vitro* and *in vivo* experiments. Our study suggests that long-term S1 monotherapy is an attractive adjuvant therapy option and that UMPS is a useful biomarker for identifying patients most likely to benefit from it.

I adenocarcinoma (6). Fluoropyrimidines, such as tegafur, are known to have time-dependent antitumor effects (7–9), and it is thought that long-term postoperative administration will be beneficial. However, how useful UFT will be in postoperative adjuvant chemotherapy for patients at more advanced disease stages is not clear (10, 11).

S-1 (TS-1, Taiho Pharmaceutical Co. Ltd), which consists of tegafur, gimeracil, and oteracil potassium at a molar ratio of 1:0.4:1 (12, 13), is a modified version of UFT. Gimeracil is added to reduce the degradation of tegafur by inhibiting dehydropyrimidine dehydrogenase, and oteracil is added to lower fluoropyrimidine levels in the gut to reduce gastrointestinal toxicity. A phase II study of combination chemotherapy with S-1 and CDDP for previously untreated patients with advanced NSCLC showed an objective response of 47%, and a median survival time (MST) of 11.2 months (14). A recent phase III trial demonstrated that CDDP+S-1 was not inferior to CDDP+docetaxel in terms of OS in patients with advanced NSCLC (15). Although S-1 is more active and toxic than UFT (16), it can be administered for a year as a postoperative adjuvant therapy for gastric cancer, resulting in prolongation of both PFS and OS when compared with surgery alone (17). Thus, it would be useful to know how good long-term S-1 is as an adjuvant therapy for patients with stage II–III lung cancer who would otherwise be candidates for cisplatin doublet chemotherapy.

In this randomized phase II trial, we compared long-term S-1 monotherapy (S) with CDDP+S-1 (CS) in patients with completely resected pathologic stage II and III NSCLC, hypothesizing that long-term S-1 would be at least as effective as cisplatin doublet. Although the fact that fluoropyrimidine is a time-dependent drug makes CDDP+long-term S1 appear to be an attractive alternative, we decided to abandon this strategy over concerns about increased toxicity due to long-term administration of S-1.

Even with cisplatin doublet adjuvant therapy, the survival benefit 5 years after surgery is about 5%, meaning that the remaining 95% of patients experience no benefit. This has led to a search for biomarkers that would allow selection of patients who were suitable for adjuvant chemotherapy as a co-primary endpoint.

Materials and Methods

Patients with completely resected stage II or IIIA (metastasis to a single mediastinal lymph node only) NSCLC, as classified according to the TNM staging system version 6, aged 20 to 74 years, and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1 were eligible. Patients had to have adequate bone marrow and organ function. The main exclusion criteria were a history of drug allergies, interstitial pneumonia, and concomitant malignancies: heart failure, uncontrolled diabetes mellitus, and active infections. Patients who had undergone a pneumonectomy were also excluded. Written informed consent was obtained from all patients, and the study protocol was approved by the Institutional Review Board of each participating institution.

This study is registered on the UMIN Clinical Trial Database (Study ID: 000001658).

Procedures

Selected patients were randomly assigned to receive either oral S-1 (40 mg/m^2 twice per day) on day 1 to 14 every 3 weeks for a year (S group), or CDDP (60 mg/m^2) on day 1 plus oral S-1 (40 mg/m^2 twice per day) on 14 consecutive days repeated every 3 weeks for four cycles (CS group). Patients were required to start the protocol treatment within 8 weeks after surgery. Randomization (minimization method) was done at the central datacenter, and the stratification factors were pathologic disease stage (II vs. IIIA), type of histology (adenocarcinoma vs. others), and institutions. The primary endpoints were relapse-free survival (RFS) at 2 years, and identification of molecules whose expression was significantly associated with patient outcome. Secondary endpoints included OS, rate of adverse events/side effects, and medical treatment completion rate. Relapse was assessed by means of PET and a CT scan of the chest at 6, 12, 18, and 24 months after initiation of the protocol treatment.

Biomarker analysis

cDNA extracted from macro-dissected formalin-fixed paraffin-embedded specimens was obtained from 197 of 200 patients. Thirty genes, including 18 whose expressions have been reported to be potentially associated with CDDP (e.g. ERCC1, XRCC1, BRCA1, GSTpi, HMG1, and TBP), and 12 genes associated with fluoropyrimidine sensitivity (TS, DHFR, DPD, UMPS, and UPP1; Table 1), were measured by mass spectrometry (QGE analysis MassArray). Primer sequences for each gene are available on request.

Statistical analysis

In this study, we expected the 2-year RFS rates for II-IIIA to be 50% in both groups. In order to obtain a precision of approximately $\pm$ 10% width in 95% confidence intervals (95% CIs), a total of 100 patients per group were required. The following decision rules were adopted: if the Bayesian posterior probability that the 2-year RFS would exceed a threshold value of 40% was less than 0.85, neither regimen merited further evaluation; if only one regimen had a probability of more than 0.85, then that regimen merited further study; if both regimens had probabilities of more than 0.85, no formal comparison of the two 2-year RFSs was made, but the decision was made on the basis of information about the secondary endpoints.

Table 1. List of genes examined and percentage of patients with values above detection limit and $P_{interaction}$ values for each gene by uni- and multivariate analysis

			% of patients with above detection limit	Univariate *P*	Multivariate *P*
	Genes whose expression is potentially associated with fluoropyrimidine sensitivity				
1	DHFR	Dehydroforlate reductase	22%	—	—
2	DPD	Dihydropyrimidine dehydrogenase	52%	0.0491	0.0578*
3	ECGF1	Endothelial cell growth factor/thymidine phosphorylase	92%	0.1141	0.3647
4	FPGS	Folypolyglutamate synthase	99%	0.5339	0.4501
5	MTHFR	Methylenetetrahydrofolate reductase	3%	—	—
6	PRPS1	Phosphoribosyl pyrophosphate synthetase 1	99%	0.5807	0.8306
7	RRM1	Ribonucleotide reductase M1	61%	0.367	0.5305
8	TK1	Thymidine kinase 1	37%	—	—
9	TS	Thymidylate synthase	76%	0.1755	0.2383
10	UCK2	Uridine-cytidine kinase 2	78%	0.1087	0.2845
11	UMPS	Uridine monophosphate synthetase/orotate phosphoribosyl transferase and orotidine-5′-decarboxylase	82%	0.0243	0.0348**
12	UPP1	Uridine phosphorylase 1	69%	0.0645	0.0745*
	Genes whose expression is potentially associated with CDDP sensitivity				
13	ERCC1	Excision repair cross-complementing 1	97%	0.8732	0.7908
14	ERCC6	Excision repair cross-complementing 6/ Cockayne syndrome B	62%	0.0534	0.0697*
15	NP	Nucleoside phosphorylase	8%	—	—
16	XRCC3	X-ray repair complementing defective repair in Chinese hamster cells 3	25%	—	—
17	BRCA1	Breast cancer 1	27%	—	—
18	CSA	Cockayne syndrome A/ERCC8	28%	—	—
19	gammaGCS	Gamma-glutamylcysteine synthetase	20%	—	—
20	GSTalpha	Glutathione S-transferase alpha	24%	—	—
21	GSTpi	Glutathione S-transferase pi	60%	0.4552	0.6408
22	HMG1	High-mobility group 1	100%	0.0773	0.0621*
23	HMG2	High-mobility group 2	42%	—	—
24	MGMT	O-6-methylguanine-DNA methyltransferase	5%	—	—
25	MT1A	Metallothionein 1A	99%	0.2359	0.7555
26	MT2A	Metallothionein 2A	100%	0.6289	0.785
27	OGG1	8-Oxoguanine DMA glycosylase	21%	—	—
28	Rad51		28%	—	—
29	XPD	Xeroderma pigmentosum group D/ERCC2	66%	0.5428	0.5403
30	XRCC1	X-ray repair cross-complementing protein 1	39%	—	—

*, $P < 0.10$ by both uni- and multivariate analyses.
**, $P < 0.05$ by both uni- and multivariate analyses.

The Kaplan–Meier method was used to estimate the RFS and OS curves of the treatment groups. The 95% CIs of 2-year RFS were estimated according to the Greenwood formula. Stratified Cox regression with histology (adenocarcinoma vs. other) and pathologic stage (II vs. III) as strata was applied to estimate the HR between the two groups. All efficacy analyses were done for the full analysis set, which was defined as all randomly assigned patients with confirmed eligibility. Safety analyses were done for patients who were actually treated with at least one dose.

To evaluate potential biomarkers, the expression of each gene was dichotomized according to its median value of expression. *P* values for interaction between expression levels (high/low) and treatment groups were calculated using a Cox proportional hazards model. As we thought the power of statistical tests for interaction would be low due to the small number of relapses and deaths in this phase II study, we did not adopt a rigorous statistical threshold to take account of multiple comparisons. Instead, our strategy was to perform confirmatory *in vitro* and *in vivo* studies of promising but potentially false-positive genes identified in the current study. All *P* values are reported as two tailed, and statistical analyses were conducted with SAS (version 9.2, SAS Institute).

Creation of UMPS expressing cells *in vitro*

Plasmid constructs expressing Uridine monophosphate synthetase (UMPS) were generated in pIRES2-ZsGreen1 vectors (Clontech). Cells were transfected with empty pIRES2-ZsGreen1 or pIRES2-ZsGreen1-UMPS using FuGENE 6 (Roche Diagnostics). Forty-eight hours after transfection, the virus was harvested. NIH3T3 mouse fibroblasts and A549 lung adenocarcinoma cells were purchased from the ATCC. A549 cells were authenticated by short tandem repeat at Takara Bio Inc. These cells were maintained in RPMI-1640 (Sigma) supplemented with 10% heat-inactivated FBS, and were infected with viral supernatants; the GFP-positive cells were then sorted. The stable viral transfectant cells in each cell line were designated as NIH3T3-mock, NIH3T3-UMPS, A549-mock, and A549-UMPS.

In vitro growth inhibition assay

Cells were transferred to 96-well flat-bottomed plates and cultured for 24 hours before exposure for 72 hours to various concentrations of 5-FU, as indicated. Attached cells were fixed and then stained for 10 minutes with 0.5% crystal violet. The stain was eluted with 0.05 mol/L sodium dihydrogenphosphate dehydrate, and the absorbance at 540 nm was measured with a spectrophotometer.

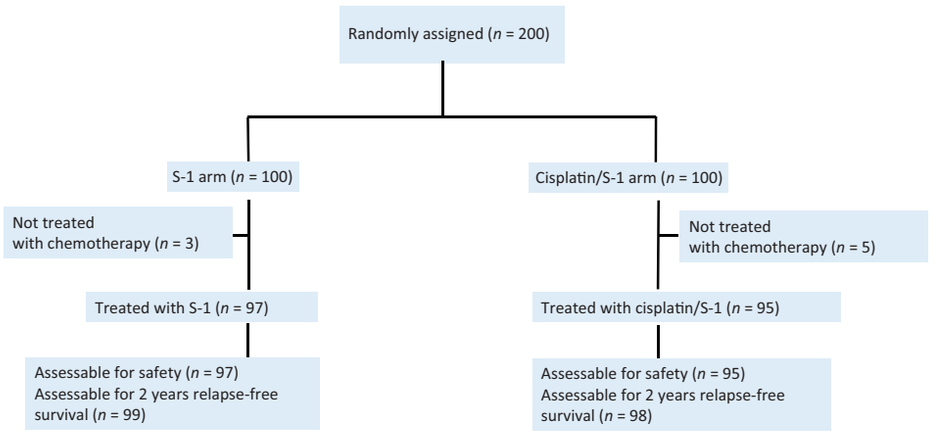

Figure 1.
CONSORT diagram for the study.

In vivo antitumor activity

All animal studies were performed in accordance with the Recommendations for Handling of Laboratory Animals for Biomedical Research compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, Kinki University (Osaka, Japan). The ethical procedures followed conformed to the guidelines of the United Kingdom Coordinating Committee on Cancer Prevention Research.

Tumors cells (5×10^6) were injected subcutaneously into the axilla of athymic nude mice (BALB/c nu/nu, CLEA Japan). Treatment was initiated when tumors in each group of 6 mice achieved an average volume of 100 to 200 mm^3. S-1 was administered orally 5 days per week for 4 weeks at doses of 5 and 8.3 mg/kg. 5-FU was administered intraperitoneally 5 days per week for 4 weeks at doses of 10 and 20 mg/kg. Tumor volume was determined from measurements of tumor length (L) and width (W) according to the following formula: $LW^2/2$. Both tumor size and body weight were measured twice per week.

Results

Patient characteristics

A total of 200 patients from 30 institutions in Japan were enrolled in the study between September 2007 and December 2009 (Fig. 1). More than half of the patients were enrolled from the top five contributing institutions. The baseline demographic characteristics of the subjects were well balanced between the two treatment groups, except that the patients in the CS group had higher rates of extensive lymph node dissection and stage IIA disease, and a lower rate of IIB disease (Table 2). Because 3 patients in the S-1 group and 5 patients in the CDDP + S-1 group did not receive any chemotherapy, 192 patients were eligible for the safety analysis (Fig. 1).

Delivered chemotherapy

In total, 51 patients (52.6%) completed 15 cycles of adjuvant chemotherapy in the S-1 group, and 71 patients (74.7%) completed four cycles of adjuvant chemotherapy in the CDDP+S-1 group. The median relative dose intensities were high in both the S-1 (85.2%) and CDDP+S-1 groups (86.4% and 94.1%, respectively). The main reasons for discontinuation of the protocol treatment were patient withdrawal and medical decision based on toxicity (data not shown) in both groups, and recurrence in the S-1 group. The dose of S-1 was reduced for 25 of the 96 patients (26%) in the S group, whereas the dose of CDDP was reduced for 10 patients because of nonhematologic toxicities.

Survival analyses

In the 197 patients evaluated, a total of 89 relapses were observed. The 2-year RFS rate (one of the co-primary endpoints) was 65.6% (95% CI, 55.3–74.0%) in the S-1 group and 58.1% (47.7–67.2%) in the CDDP+S-1 group (Fig. 2A). The Bayesian posterior probability that the 2-year RFS would exceed a threshold value of 40% was 99.9% in the S-1 group and 99.9% in the CDDP+S-1 group. The HR between the two groups by stratified Cox regression was 0.90 (0.59–1.37). The 5-year OS rates of the two groups were also comparable: 72.6% (95% CI, 64.3–82.0) in the S-1 group and 72.2% (95% CI, 63.8–81.7) in the CDDP+S-1 group (Fig. 2B).

Safety

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria version 3.0. The incidence of anemia or neutropenia of grade 3 or 4 was significantly lower among the patients in the S-1 group than among those in the CDDP + S-1 group (anemia: 1.0% vs. 8.4%; neutropenia: 13.4% vs. 27.4%, respectively; Table 3). As for nonhematologic toxicities, grade 3 or 4 anorexia and nausea occurred more commonly in the CDDP+S-1 group than in the S-1 group (anorexia: 9.5% vs. 2.1%; nausea: 6.3% vs. 0%; febrile neutropenia: 5.3% vs. 0%, respectively). Conversely, treatment with S-1 was associated with a higher rate of grade 1 or 2 skin rash than treatment with CDDP+S-1 (26.8% vs.9.5%, respectively). There were 17 deaths in the S-1 group and 18 deaths in the CDDP+S-1 group during the study period, but they were not treatment related.

Biomarkers

There were 14 genes whose expression levels were below the detection limit in more than half of the patients, and these genes were not subjected to further analyses (Table 1). Of the remaining 16 genes, the only one with interaction of $P < 0.05$ was UMPS (also known as orotate phosphorybosyl transferase; OPRT; $P = 0.0348$; Table 1). P values of <0.1 were obtained for DPD, UPP1, ERCC6, and HMG1. RFS at 2 years (95% CI) in

Table 2. Patient demographic and clinical characteristics

Characteristic	Category	S-1 ($n = 99$) N %	CDDP+S-1 ($n = 98$) N %	P
Age	Median	62	62	0.916
Sex	Male	72 (72.7)	77 (78.6)	
	Female	27 (27.3)	21 (21.4)	0.407
P stage	IIA	17 (17.2)	29 (29.6)	
	IIB	40 (40.4)	27 (27.6)	0.061
	III	42 (42.4)	42 (41.8)	
Histologic type	Adeno	66 (66.7)	66 (67.3)	
	Squamous	22 (22.2)	23 (23.5)	0.934
	Other	11 (11.2)	9 (9.2)	
Surgery	Lobectomy	99 (100)	98 (100)	
Lymph node dissection	ND0-1[a]	6 (6.1)	4 (4.1)	0.747
	ND2[a]	93 (93.6)	94 (96.9)	

[a]ND0-1 and ND2 designate no lymph node dissection, dissection to the N1 level or N2 level, respectively.

patients with high levels of UMPS/OPRT was 69% (95% CI, 54–80%) in the S group and 53% (37–66%) in the CS group (Fig. 2C), whereas 2-year RFS in patients with low levels was 64% (49–76%) in the S group and 61% (46–73%) in the CS group (Fig. 2D). However, molecules such as ERCC1 and GSTpi (18, 19), whose expressions have previously been associated with CDDP sensitivity, did not emerge as predictive markers ($P = 0.7908$, 0.6406, respectively).

Confirmation of UMPS as a predictive factor for pyrimidine sensitivity *in vitro* and *in vivo*

UMPS/OPRT could have been falsely identified as a predictive biomarker on the basis of a P value of <0.05. Therefore, we decided to evaluate the effect of UMPS/OPRT overexpression on fluoropyrimidine sensitivity *in vitro* and *in vivo*. Overexpression of UMPS was confirmed by Western blotting (Fig. 3A).

Overexpression of UMPS made the A549 lung cancer cell line about 100 times more sensitive to 5-FU *in vitro* (Fig. 3B). *In vivo* experiments also confirmed that UMPS/OPRT is a determinant of sensitivity to 5FU as well as S-1 (Fig. 3C).

Discussion

The purpose of this randomized phase II study was to compare the efficacy of an oral fluoropyrimidine, S-1, with that of S-1 plus CDDP in adjuvant chemotherapy for NSCLC in two groups of patients. The primary endpoint, RFS at 2 years, was similar in both groups. The secondary endpoint, OS at 5 years, was also similar in both groups, and was reproducibly observed. The Bayesian posterior probability that 2-year RFS would exceed 40% was more than 99% in both groups.

The adverse events associated with S-1 and CDDP+S-1 were as expected: treatment with CDDP+S-1 resulted in higher incidences of neutropenia of grade 3 or 4 (27.4%) and anemia (8.4%) than treatment with S-1 alone, which gave incidences of only 13.4% and 1%, respectively. However, we found CDDP+S-1 to be less toxic than the results of previous adjuvant trials had indicated: grade 3–4 neutropenia was noted in 73% of subjects in the Adjuvant Navelbine International Trialist Association trial (ANITA) and the National Cancer Institute of Canada JBR.10 trial (3, 4), respectively, and grade 4 neutropenia was found in 17.5% of patients in the International Adjuvant Lung Trial (IALT; ref. 2). In terms of nonhematologic toxicity, CDDP+S-1 produced higher incidences of febrile neutropenia, anorexia, nausea, and vomiting. In contrast, S-1 resulted in a higher incidence of skin rash. However, the toxicities were mild and controllable. There were no treatment-related deaths in either of our groups, although the sample size was admittedly small. Treatment-related deaths were reported in the previous trials (IALT: 0.8%, JBR.10: 0.8%, ANITA: 2%).

Although medication compliance of UFT at one year in a previous adjuvant trial was 74% (6), more than half (53%) of our patients received S-1 for a year. That S-1 had a favorable toxicity profile and produced survival outcomes comparable with those of CDDP+S-1 suggests that long-term administration is feasible.

As a co-primary endpoint, we were able to identify UMPS/OPRT as a promising predictive biomarker for S-1 monotherapy. UMPS/OPRT catalyzes the conversion of 5FU to fluorouridine monophosphate (FUMP), which is a precursor of the active metabolites fluorodeoxyuridine monophosphate (FdUMP) and fluorouridine monophosphate (FUMP; ref. 20). Thus, high UMPS/OPRT expression appears to enhance the effect of 5FU. Indeed, SNPs and expression levels of UMPS/OPRT have been reported to predict the efficacy and toxicity of 5FU in other human cancers and cell lines (21–23). Several reports on NSCLC treated with 5FU have shown associations between expression levels of UMPS/OPRT and clinical outcomes (24, 25).

Conflicting opinions exist regarding the expression of OPRT and the response to oral 5-FU–derivative agents. Nakano and colleagues reported that the 5-year survival rate of patients with OPRT-positive stage II to III tumors was significantly higher than that of patients with OPRT-negative tumors (24). Conversely, Takeda and colleagues reported that there is no significant association between tumor response and expression levels of OPRT (25). Our findings indicate that high UMPS/OPRT expression might identify a subset of patients who would benefit from S-1 rather than CDDP+S-1, even though both the monotherapy and combination groups received S-1. Our explanation is that susceptibility to UMPS/OPRT activity depends on differences in the length of treatment with S-1 (i.e., 1 year vs. 12 weeks), and hence differences in the total dose of S-1. However, the clinical relevance of UMPS/OPRT to NSCLC treated with S-1–based chemotherapy has not been established. Further prospective studies of these biomarkers are necessary to confirm clinical outcomes and benefits.

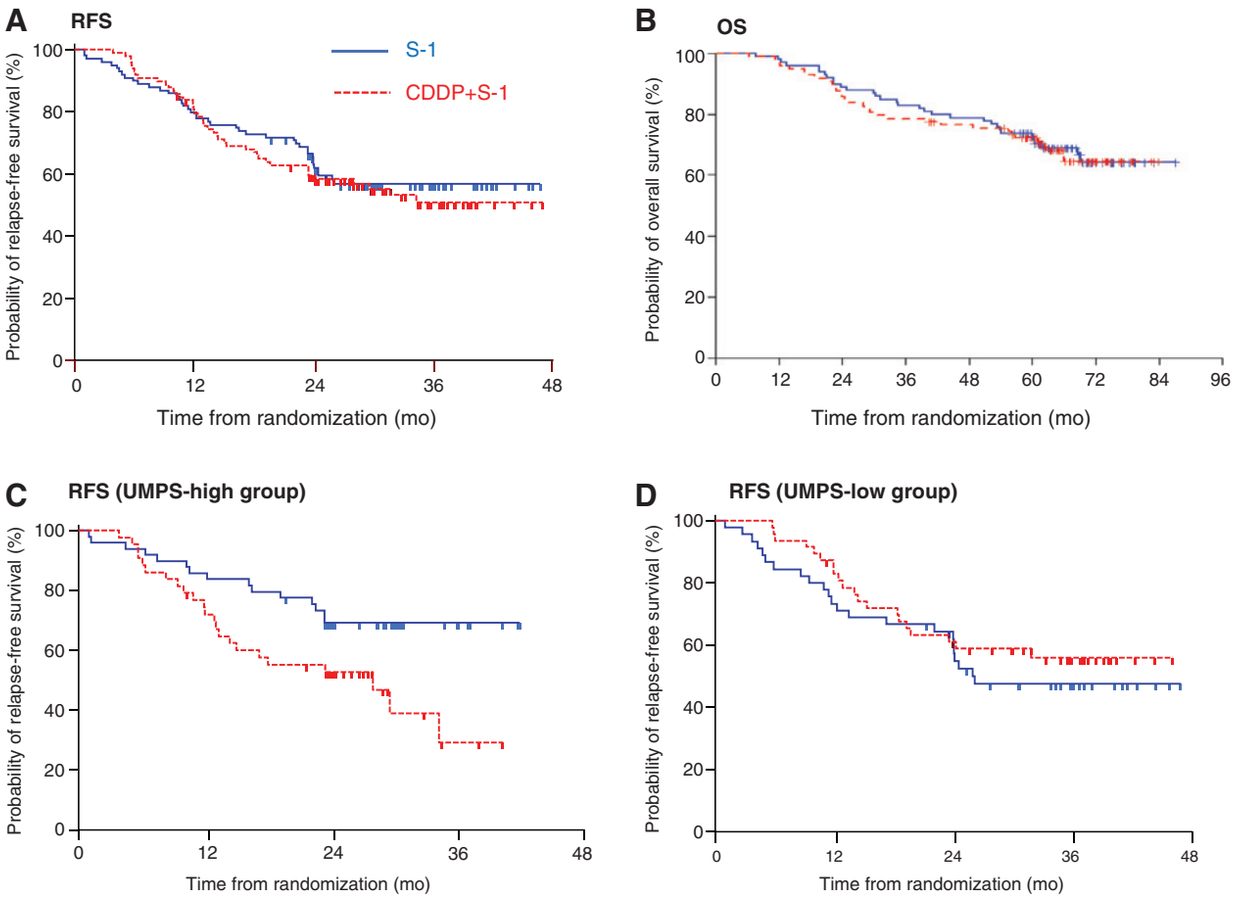

Figure 2.
RFS curve and OS curve for all patients (A and B, respectively), and for those with high and low expressions of UMPS/OPRT (C and D, respectively).

In conclusion, this study suggests that adjuvant long-term S-1 monotherapy or CDDP+S-1 for completely resected stage II–III NSCLC is a viable alternative to cisplatin doublet chemotherapy in terms of efficacy and toxicity. UMPS/OPRT expression may be a useful marker for identifying patients who would benefit most from adjuvant long-term S-1, although the fact that we did not adjust for multiple testing may limit the relevance of this finding. We plan to conduct a randomized phase III trial to compare adjuvant chemotherapy with S1 monotherapy versus CDDP+vinorelbine for completely resected stage II–III NSCLC.

Table 3. Incidence of drug-related toxicities in randomly assigned and treated patients

	Regimen by grade (%)									
	S-1 (n = 97)				CDDP+S-1 (n = 95)				P	
Toxicity	Grade I	Grade 2	Grade 3	Grade 4	Grade I	Grade 2	Grade 3	Grade 4	Any grade	Grade 3 or 4
General										
Fatigue	36.1	13.4	1	0	44.2	10.5	4.2	0	0.25	0.209
Anorexia	49.5	15.5	2.1	0	46.3	24.2	9.5	0	0.05	0.032
Nausea	39.2	13.4	0	0	46.3	22.1	6.3	0	0.002	0.014
Vomiting	13.4	3.1	0	0	21.1	16.8	2.1	0	<0.001	0.244
Skin rash	22.7	4.1	0	0	9.5	0	0	0	0.002	
Febrile neutropenia	0	0	0	0	0	0	5.3	0		0.028
Hematologic										
Anemia	15.5	10.3	1	0	22.1	25.3	6.3	2.1	<0.001	0.018
Neutropenia	29.9	19.6	13.4	0	26.3	25.3	22.1	5.3	0.017	0.02
Thrombocytopenia	9.3	3.1	0	0	16.8	9.5	1.1	1.1	0.007	0.244
Biochemical										
ALT elevation	30.9	5.2	1	0	27.4	1.1	0	0	0.221	1
Creatinine elevation	9.3	0	0	0	18.9	5.3	1.1	0	0.004	0.495

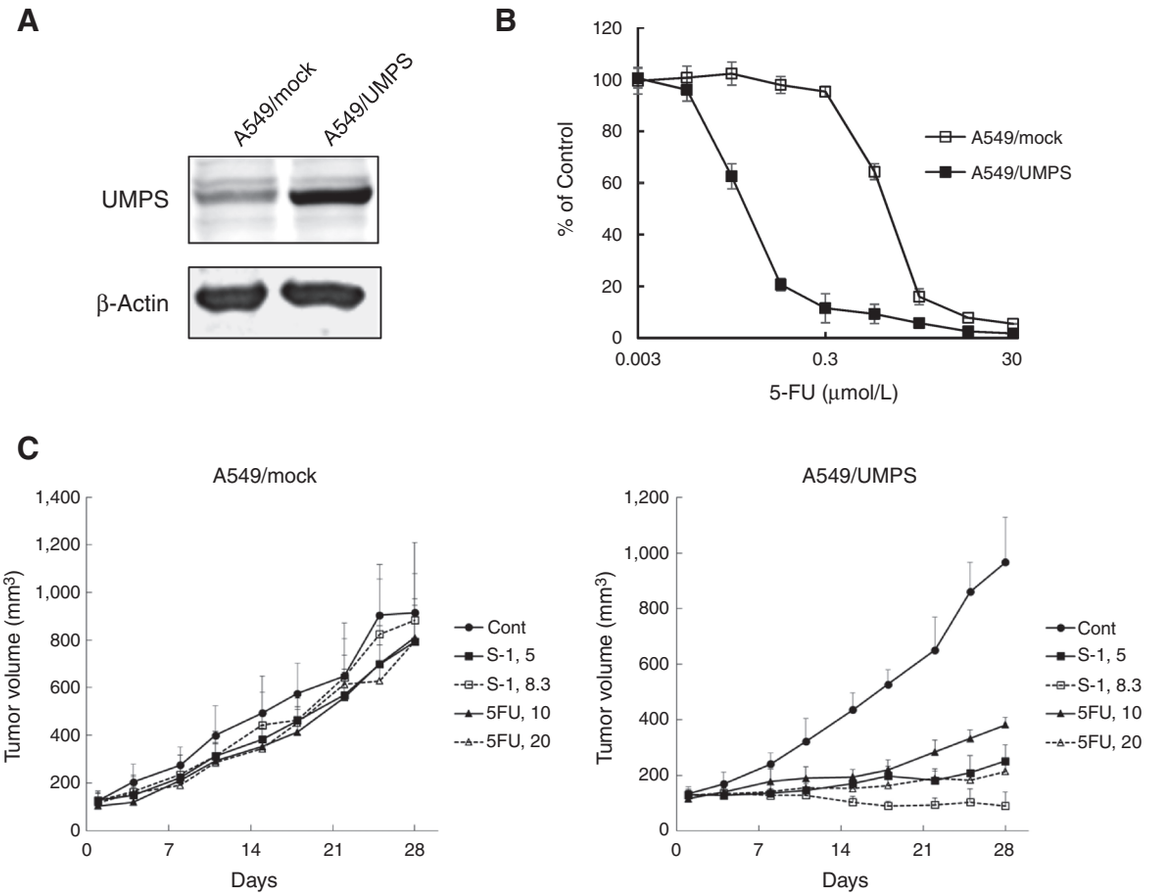

Figure 3.

A, expression of UMPS in mock- and UMPS-transfected A549 cells. Extracts of A549/mock and A549/UMPS cells were prepared, and 20 mg of cellular protein was loaded per lane. Proteins were separated by SDS-PAGE and immunoblotting using an anti-UMPS antibody. B, *in vitro* growth inhibition of 5-FU. Cells were treated with the indicated concentrations of 5-FU for 72 hours, and cell viability was tested by crystal violet staining. The 50% inhibition (IC_{50}) values of 5-FU against the A549/mock and A549/UMPS cells were 1.385 and 0.043 mmol/L, respectively. C, changes in tumor volume (left) and body weight (right) after administration of TS-1 and 5-FU in mice bearing A549/mock cells (top) and A549/UMPS tumors (bottom). A549/mock and A549/UMPS cells (5×10^6 cells/mouse) were inoculated s.c. into the flanks of BALB/cA nude mice. TS-1 was administered orally 5 days per week for 4 weeks at doses of 5 and 8.3 mg/kg. 5-FU was administered intraperitoneally 5 days per week for 4 weeks at doses of 10 and 20 mg/kg. Tumor volume (TV, expressed in mm^3) was calculated using the following equation: TV = (length × $width^2$)/2. Data, mean ± SD, $n = 5$.

Disclosure of Potential Conflicts of Interest

T. Mitsudomi reports receiving a commercial research grant and speakers bureau honoraria from Taiho Pharmaceutical. T. Yamanaka, H. Yoshioka, and T. Kawaguchi report receiving speakers bureau honoraria from Taiho Pharmaceutical. Y. Ichinose reports receiving a commercial research grant and speakers bureau honoraria from and is a consultant/advisory board member for Taiho Pharmaceutical. M. Okada reports receiving commercial research support from Taiho Pharmaceutical. K. Nakagawa reports receiving a commercial research grant and other research support from Taiho Pharmaceutical. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y. Iwamoto, T. Mitsudomi, T. Yamanaka, I. Yoshino, H. Saka, K. Nakagawa, K. Nishio

Development of methodology: Y. Iwamoto, K. Nishio

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Iwamoto, T. Mitsudomi, K. Sakai, H. Yoshioka, M. Takahama, M. Yoshimura, I. Yoshino, S. Sugawara, T. Kawaguchi, T. Takahashi, M. Ohta, Y. Ichinose, S. Atagi, M. Okada, H. Saka

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Iwamoto, T. Mitsudomi, T. Yamanaka, H. Yoshioka, M. Yoshimura, S. Sugawara, M. Okada, K. Nishio

Writing, review, and/or revision of the manuscript: Y. Iwamoto, T. Mitsudomi, T. Yamanaka, H. Yoshioka, I. Yoshino, M. Takeda, S. Sugawara, Y. Ichinose, S. Atagi, K. Nakagawa

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Iwamoto, S. Atagi, K. Nakagawa, Y. Nakanishi

Study supervision: Y. Iwamoto, T. Mitsudomi, H. Saka, K. Nakagawa, Y. Nakanishi, K. Nishio

Acknowledgments

The authors thank the patients who participated in this study and their families; Drs. Shinichiro Nakamura and Tokuzou Arao, Seiko Tanaka, and other staff members of the WJOG Data Center for their help with data management.

Grant Support

This work was supported by the West Japan Oncology Group.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 11, 2014; revised June 12, 2015; accepted July 12, 2015; published OnlineFirst August 7, 2015.

References

1. Asamura H, Goya T, Koshiishi Y, Sohara Y, Eguchi K, Mori M, et al. A Japanese Lung Cancer Registrystudy: prognosis of 13,010 resected lung cancers. J Thorac Oncol 2008;3:46–52.
2. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351–60.
3. Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C , et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589–97.
4. Douillard JY, Rosell R, De Lena M, Riggi M, Hurteloup P, Mahe MA. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719–27.
5. Pignon JP, Tribodet H, Scagliotti GV, Douillard JY, Shepherd FA, Stephens RJ , et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552–9.
6. Kato H, Ichinose Y, Ohta M, Hata E, Tsubota N, Tada H, et al. A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. N Engl J Med 2004;350:1713–21.
7. Wilkoff LJ, Wilcox WS, Burdeshaw JA, Dixon GJ, Dulmadge EA. Effect of antimetabolites on kinetic behavior of proliferating cultured L1210 leukemia cells. J Natl Cancer Inst 1967;39:965–75.
8. Skipper HE, Schabel FM Jr, Mellett LB, Montgomery JA, Wilkoff LJ, Lloyd HH, et al. Implications of biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules. Cancer Chemother Rep 1970;54:431–50.
9. Shimoyama M, Kimura K. Quantitative study on cytocidal action of anticancer agents. Saishin Igaku 1973;28:1024–40.
10. Wada H, Hitomi S, Teramatsu T. Adjuvant chemotherapy after complete resection in non-small-cell lung cancer. West Japan study group for lung cancer surgery. J Clin Oncol 1996;14:1048–54.
11. Hamada C, Tanaka F, Ohta M, Fujimura S, Kodama K, Imaizumi M, et al. Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer. J Clin Oncol 2005;23:4999–5006.
12. Shirasaka T, Nakano K, Takechi T, Satake H, Uchida J, Fujioka A, et al. Antitumor activity of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. Cancer Res 1996;56:2602–6.
13. Fukushima M, Satake H, Uchida J, Shimamoto Y, Kato T, Takechi T , et al. Preclinical antitumor efficacy of S-1: a new oral formulation of 5-fluorouracil on human tumor xenografts. Int J Oncol 1998;13:693–8.
14. Ichinose Y, Yoshimori K, Sakai H, Nakai Y, Sugiura T, Kawahara M, et al. S-1 plus cisplatin combination chemotherapy in patients with advanced non-small cell lung cancer: a multi-institutional phase II trial. Clin Cancer Res 2004;10:7860–4.
15. Katakami N, Gemma A, Sakai H, Kubota K, Nishio M, Inoue A, et al. Randomized phase III trial of S-1 plus cisplatin versus docetaxel plus cisplatin for advanced non-small-cell lung cancer (TCOG0701). J Clin Oncol 30, 2002 (Suppl; abstr 7515).
16. Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of pyrimidine, barbituric acid and pyrimidine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer Res 1987;8:748–55.
17. Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, et al. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 2007;357:1810–20.
18. Friboulet L, Olaussen KA, Pignon JP, Shepherd FA, Tsao MS, Graziano S, et al. ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. N Engl J Med 2013;368:1101–10.
19. Goto S, Iida T, Cho S, Oka M, Kohno S, Kondo T. Overexpression of glutathione S-transferase pienhances the adduct formation of cisplatin with glutathione in human cancer cells. Free Radic Res 1999;31:549–58.
20. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003;3:330–8.
21. Ichikawa W, Takahashi T, Suto K, Sasaki Y, Hirayama R. Orotate phosphoribosyltransferase gene polymorphism predicts toxicity in patients treated with bolus 5-fluorouracil regimen. Clin Cancer Res 2006;12:3928–34.
22. Nio Y, Toga T, Maruyama R, Fukushima M. Expression of orotate phosphoribosyl transferase in human pancreatic cancer: implication for the efficacy of uracil and tegafur-based adjuvant chemotherapy. Oncol Rep 2007;18:59–64.
23. Sakamoto E, Nagase H, Kobunai T, Oie S, Oka T, Fukushima M, et al. Orotate phosphoribosyltransferase expression level in tumors is a potential determinant of the efficacy of 5-fluorouracil. Biochem Biophys Res Commun 2007;363:216–22.
24. Nakano J, Huang C, Liu D, Masuya D, Nakashima T, Yokomise H, et al. Evaluations of biomarkers associated with 5-FU sensitivity for non-small-cell lung cancer patients postoperatively treated with UFT. Br J Cancer 2006;95:607–15.
25. Takeda M, Okamoto I, Hirabayashi N, Kitano M, Nakagawa K. Thymidylate synthase and dihydropyrimidine dehydrogenase expression levels are associated with response to S-1 plus carboplatin in advanced non-small cell lung cancer. Lung Cancer 2011;73:103–9.