study, a total of 713 patients who underwent complete resection of primary GIST > 3 cm in size were assigned to receive either imatinib or placebo. The dosing regimen of imatinib was the same as ours: imatinib was given at 400 mg/day for 1 year postoperatively. Of the 359 patients in the imatinib group, 262 (73%) completed the assigned treatment. The fact that the treatment completion rate (76.6%) in our clinical study is very similar to that (73%) in the Z9001 study reveals that Japanese patients are able to receive postoperative adjuvant therapy with imatinib in the same manner as European and American patients.

The ACTS-GC study, which was a phase III trial of adjuvant therapy for gastric cancer patients, showed that the treatment completion rate was 65.8% for patients who were assigned to 1-year treatment with S-1, an oral fluoropyrimidine [7]. Furthermore, in a phase III trial for patients with stage I lung cancer, the compliance of adjuvant therapy was reported to be 74% at 1 year [5]. Based on the previous results of these pivotal clinical trials on the adjuvant therapy for other malignancies, the treatment completion rate of 76.6% shown by the present study seemed common and acceptable for the use of an oral anticancer agent in the adjuvant setting.

The present study also revealed safety profiles that were specific to Japanese patients with GISTs. The overall incidence of adverse drug reactions and the incidence of grade ≥3 adverse drug reactions in the present study were 100 and 34%, respectively, which are very similar to those in the Z9001 study (99 and 31%, respectively) [8]. However, the adverse drug reaction profile showed that neutropenia of grade ≥3 was observed in 14% of the patients in our study, considerably higher than the 3% observed in the Z9001 study. The B1202 study [9], which was a phase II trial involving Japanese patients with unresectable and metastatic GISTs, also showed a similar finding in that the incidence of grade ≥3 neutropenia was 21.6%, much higher than that in reported clinical studies conducted in Europe and the USA [3, 14]. It is suggested, therefore, that Japanese patients may be more sensitive to the hematotoxicity of imatinib than their European and US counterparts. Fortunately, the incidence of febrile neutropenia, which could potentially interfere with the continuation of the treatment, was only 1.6%. Many of the neutropenia events were afebrile and manageable by interruption of the treatment. Conversely, the frequent onset of asymptomatic neutropenia of grade 3 in the present study indicates that attention should be directed to the importance of hematological monitoring when adjuvant therapy with imatinib is administered in Japanese patients.

The present study provided clinically significant information on the safety of adjuvant therapy with imatinib in Japanese patients but could not provide clear information on the efficacy of this therapy. The 3-year recurrence rate was 42.7 and 95% CI was between 29.2 and 56.3%. As we had assumed that the expected recurrence rate for imatinib-treated patients was 20%, the clinical efficacy of the adjuvant therapy with imatinib could not be proven as the 3-year recurrence rate exceeded the expected recurrence rate in this single-arm phase II study. Takahashi et al. [15] have reported the prognostic data of 303 consecutive patients who underwent resection of primary GIST between 1987 and 2003. On the basis of their retrospective study, the 3-year disease-free survival rate of patients with high-risk GISTs was 60%. By comparing with this data, the 1-year treatment with imatinib seemed to contribute little to the improvement of the RFS rate of patients with high-risk GISTs. These findings should be primarily interpreted as showing that the present study failed to demonstrate the potential of adjuvant therapy in Japanese GIST patients. However, what should not be overlooked is that the RFS curve in the present study was very similar to that plotted with data from the Z9001 study. The Z9001 study, in which patients with primary GISTs > 3 cm were enrolled, also conducted subset analysis according to tumor size. The RFS rates of the treatment group having a tumor size ≥10 cm were 92% at 1 year, 77% at 2 years, and 41% at 3 years. These RFS rates are very similar to those obtained in our study. This high reproducibility found between the two RFS curves from the Z9001 study and our study suggests that we might have overestimated the expected recurrence rate of our single-arm phase II study. To enable a clear interpretation of the efficacy of the adjuvant chemotherapy, a randomized trial would be required in Japanese patients as well as western patients.

The present study highlighted a problem in postoperative adjuvant therapy with imatinib. Recurrence was observed in only 2 of the 64 patients during the course of treatment with imatinib but seen in 18 after the treatment. The RFS curve of this study depicted a biphasic pattern, reflecting the above change in recurrence rates. The RFS rate slowly declined during the first one and a half years and rapidly declined thereafter. A similar finding was noted in the Z9001 study. This problem should be taken into consideration when determining the optimal postoperative adjuvant therapy for GISTs.

The BFR14 trial [16], a study that aimed to determine treatment duration for patients with unresectable and metastatic GISTs, has shown that discontinuation of imatinib therapy caused rapid exacerbation of tumors even after 3 years of treatment. Rutkowski et al. [17] surgically excised residual tumors after a markedly good response was obtained. They reported the pathological observation that in 87% of their patients, active tumor cells were found in the degenerated tissues of the surgically excised tumors. On the basis of these findings, it is recommended that the treatment of metastatic GISTs with imatinib be continued

as long as possible [4, 18, 19]. Considering this unique feature of imatinib, namely that it is a competitive inhibitor of the KIT receptor, the therapy may have to be continued for a long time, exceeding 1 year, even if it is used as a postoperative adjuvant therapy for subclinical minor metastasis. One study that compares no treatment and 2-year treatment (European Organization for Research and Treatment of Cancer trial 62024), another study that compares 1-year treatment and 3-year treatment (Scandinavian Sarcoma Group trial XVIII), and yet another 5-year treatment study (PERSIST-5) are ongoing. It is expected that the optimal duration of postoperative therapy with imatinib will be determined by those clinical studies.

In conclusion, postoperative adjuvant therapy with imatinib was associated with the frequent onset of grade $\geq$3 neutropenia in Japanese patients with GISTs, although the feasibility of this therapy is satisfactory. The postoperative RFS rate of Japanese patients with high-risk GISTs shown in this study was similar to that of a previous western trial. The high reproducibility of the efficacy may be a rational basis for extrapolating new results that will be obtained from ongoing international phase III studies to the treatment of GIST patients in East Asia and Japan.

Acknowledgments This work was sponsored by Novartis Pharma K.K.

Conflict of interest T. Nishida received lecture fees and research funding from Novartis Pharma, K.K.; T. Sugiyama and S. Hirota received research funding from Novartis Pharma, K.K.; all other authors declare that they have no conflict of interest.

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Cancer Chemother Pharmacol
DOI 10.1007/s00280-011-1783-9

ORIGINAL ARTICLE

Pharmacokinetic analysis of capecitabine and cisplatin in combination with trastuzumab in Japanese patients with advanced HER2-positive gastric cancer

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Received: 22 April 2011 / Accepted: 8 November 2011

Abstract
Purpose To evaluate the pharmacokinetics (PK) of capecitabine and cisplatin, administered in combination with or without trastuzumab, in Japanese patients with HER2-positive advanced gastric cancer (AGC).
Methods Patients eligible for this PK study (study JP19959), which was carried out during treatment Cycle 1 of the ToGA study, received either capecitabine and cisplatin (XP arm) or trastuzumab plus capecitabine and cisplatin (HXP arm). All patients received capecitabine (1,000 mg/m^2 orally, twice daily for 14 days) and cisplatin (80 mg/m^2 intravenous infusion on Day 1). Patients in the HXP arm also received trastuzumab (8 mg/kg intravenous infusion on Day 1), concurrently with capecitabine. No further study medication was administered during study JP19959. Serial plasma samples for PK analysis were obtained at intervals before and after the administration of capecitabine and cisplatin on Day 1.
Results Twenty-two patients were enrolled in this PK study: eight in the HXP arm and 14 in the XP arm. All blood samples were available for PK analysis. Co-administration of trastuzumab resulted in no statistically or clinically significant changes in the PK profiles of capecitabine or its metabolites, or of cisplatin (total or unbound platinum).
Conclusions Variability in the AUC_{last} and C_{max} values for the capecitabine was consistent with the known PK profile of capecitabine and fell within established limits. Concurrent trastuzumab therapy is unlikely to alter the PK or safety profile of capecitabine or cisplatin in Japanese patients with HER2-positive AGC.

Keywords Capecitabine · Cisplatin · Trastuzumab · ToGA study · Pharmacokinetics · Advanced gastric cancer

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Introduction

Gastric cancer is one of the most frequent causes of cancer-related deaths, and chemotherapy is the standard treatment for advanced disease. In Europe, the combination of epirubicin, cisplatin, and 5-fluorouracil (5-FU) (ECF) is widely accepted as the standard chemotherapy regimen on the basis of the results from studies in patients with advanced esophagogastric cancer [1, 2]. In contrast, in the

Published online: 25 November 2011

United States, combinations of docetaxel, cisplatin, and 5-FU (DCF), including modified DCF regimens, are generally used as reference regimens [3, 4]. In Japan, Korea, and China, S-1 (a new oral antitumor agent that consists of tegafur, 5-chloro-2,4-dihydroxypyridine, and oxonic acid) is also available for the treatment of gastric cancer. Thus, there is no global standard regimen for the first-line treatment of advanced gastric cancer (AGC). Regimens combining 5-FU and cisplatin (FP) are, however, commonly used in routine clinical practice in many countries for the first-line treatment of AGC [5–7].

Capecitabine (Xeloda, F. Hoffmann-La Roche) is an oral fluoropyrimidine and a prodrug for 5-FU, which is designed to mimic a continuous infusion of 5-FU and enhance activity in tumor tissues. It is currently approved globally for the treatment of metastatic breast cancer, adjuvant colon cancer, metastatic colorectal cancer, metastatic pancreatic cancer, and AGC. Capecitabine can replace infused 5-FU in triplet combinations for the treatment of AGC [8]. A regimen combining capecitabine and cisplatin (XP) was shown to be an effective and well-tolerated therapy for the first-line treatment of AGC in the ML17032 trial [9].

Trastuzumab (Herceptin, Genentech) is a humanized monoclonal antibody that binds to human epidermal growth factor receptor 2 (HER2), resulting in anticancer effects. In the Trastuzumab for GAstric cancer (ToGA) phase III international study, trastuzumab in combination with chemotherapy demonstrated a significant and clinically relevant survival benefit in patients with HER2-positive AGC, without new or unexpected side effects [10]. In October 2010, the United States Food and Drug Administration (FDA) approved Trastuzumab in combination with cisplatin and capecitabine, for the treatment of patients with HER2-positive metastatic gastric cancer. A regimen combining trastuzumab, capecitabine, and cisplatin is, therefore, a new therapeutic option for patients with HER2-positive AGC.

There is evidence to suggest that, although the pharmacokinetic (PK) interactions between capecitabine and cisplatin lead to the accumulation over time when the two agents are co-administered, this PK interaction does not lead to a negative pharmacodynamic effect (i.e., increased toxicity) [11]. Furthermore, a study in patients with inoperable esophagogastric carcinoma has demonstrated that the PK profile of capecitabine is not significantly influenced when this drug is co-administered with epirubicin and cisplatin [12]. However, it is not known whether the PK profiles of capecitabine and cisplatin are affected by concomitant administration of trastuzumab in patients with AGC.

The PK profile of capecitabine, administered as a component of combination chemotherapy, has not previously been studied in Japanese patients with gastric cancer. While previous studies suggest that race does not influence the PK profile of capecitabine administered as monotherapy [13–15], we conducted a study to evaluate the PK profiles of capecitabine and cisplatin administered in combination with or without trastuzumab in patients enrolled into the ToGA study at Japanese centers.

Patients and methods

Patient characteristics

This Japanese pharmacokinetic study (JP19959) was a substudy of the ToGA study (BO18255; NCT01041404). Patients enrolled in the ToGA study had inoperable locally advanced or recurrent and/or metastatic adenocarcinoma of the stomach or gastro-esophageal junction, had HER2-positive tumors, and had not received any previous treatment for their advanced/metastatic disease. Additional eligibility criteria of the ToGA study were as follows: age $\geq$18 years; Eastern Cooperative Oncology Group (ECOG) performance status $\leq$2; no adjuvant chemotherapy within 6 months; no radiotherapy or major surgery within 4 weeks; no investigational anti-cancer therapy within 4 weeks; adequate end-organ function and renal function; baseline left ventricular ejection fraction (LVEF) >50%; measurable or evaluable disease according to the Response Evaluation Criteria In Solid Tumors (RECIST); and a life expectancy $\geq$3 months. All patients gave written informed consent to participate in the ToGA study and in this substudy. The protocol and consent were reviewed and approved by the institutional review boards at each participating institution.

Treatment

All treatments (trastuzumab, capecitabine, and cisplatin) were included in the ToGA study. Patients eligible to participate in this substudy received capecitabine and cisplatin during treatment Cycle 1 of the ToGA study. While patients randomized to the XP arm received no additional therapy, patients randomized to the trastuzumab plus XP (HXP) arm received trastuzumab. No further study medication was administered during the ToGA study.

All patients in the JP19959 study received capecitabine (1,000 mg/m^2 orally) twice daily for 14 days, beginning on the morning of Day 1 of Cycle 1. Patients randomized to the HXP arm received a concurrent intravenous infusion of trastuzumab (8 mg/kg loading dose over 90 min on Day 1; subsequent doses: 6 mg/kg). Cisplatin was administered at the same dose in both treatment groups (80 mg/kg via intravenous infusion over 2 h on Day 1), beginning 2.0–2.5 h after administration of capecitabine. Thus, in the

HXP arm, 30–60 min elapsed between completion of the trastuzumab infusion and initiation of dosing with cisplatin.

Plasma sampling and drug assay

Blood samples (5 mL) for the measurement of capecitabine and its metabolites were collected at the following time points on Day 1 of Cycle 1: before administration of capecitabine; 1, 2, 3, and 8–12 h after administration of capecitabine; at the end of cisplatin infusion; and 2 h after the end of cisplatin infusion. These blood samples were collected into sampling tubes containing Na-EDTA, gently inverted several times to mix thoroughly, and immediately centrifuged at 1,500×*g* for 10 min at 4°C. The supernatant plasma was then placed in a designated tube and stored frozen at −20°C or colder until shipping to Covance Laboratories (WI, USA) for analysis. The concentrations of capecitabine and its metabolites (5′-deoxy-5-fluorocytidine [5′-DFCR], 5′-deoxy-5-fluorouridine [5′-DFUR], 5-FU, and α-fluoro-β-alanine [FBAL]) in plasma were determined using liquid chromatography tandem mass spectrometry (LC/MS–MS) [16].

Blood samples (5 mL) for the measurement of cisplatin PK were obtained at the following times on Day 1 of Cycle 1: before the cisplatin infusion; at the end of the cisplatin infusion; 1 and 2 h after the end of the infusion; and 8–12 h after administration of capecitabine. These blood samples were collected into sampling tubes containing heparin, gently inverted several times to mix thoroughly, and immediately centrifuged at 1,500×*g* for 10 min at 4°C. An aliquot (500 μL) of the obtained plasma was stored in a designated tube as a sample for total platinum concentration measurement, while the remaining plasma was dispensed into 4 designated centrifuge filter tubes (in portions of approximately 400 μL plasma) for ultrafiltration, and centrifuged at 1,500×*g* (not exceeding 2,000×*g*) for 30 min at 4°C. The obtained ultrafiltered plasma was placed in designated tubes as samples for measurement of unbound platinum concentration. All the samples for cisplatin measurements were stored frozen at −20°C or colder until shipping to Advion BioSciences (NY, USA) for the analysis. Total platinum and unbound platinum concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS).

Pharmacokinetic analyses

The PK parameter values for capecitabine (both prodrug and metabolites) and cisplatin (total platinum in plasma and unbound platinum in ultrafiltered plasma) were calculated with WinNonlin (Version 4.01, Pharsight, CA, USA) using non-compartmental models. For capecitabine and its metabolites, extravascular input (model 200) was used. For cisplatin constant infusion, model 202 was used. The PK parameter values calculated for capecitabine and its metabolites in plasma, and for total platinum in plasma and unbound platinum in ultrafiltered plasma for cisplatin, were the maximum plasma concentration (C_{max}), the time of maximum plasma concentration (T_{max}), the area under the plasma concentration–time curve (AUC) for time zero to infinity (AUC_{inf}), the AUC for time zero to last measured time (AUC_{last}), the elimination rate constant (K_{el}), the elimination half-life ($t_{½}$), clearance (CL), the apparent total clearance (CL/*F*), and the volume of distribution at steady state (V_{ss}). For each parameter, means and standard deviations (SD) of values for the 2 treatments were calculated. For between-group comparison, AUC_{last} values were natural-logarithmically transformed and the ratio and 90% confidence intervals (CI) for the XP arm were compared with those of the HXP arm.

The PK parameter values for capecitabine and its metabolites obtained in this study were compared with the PK parameter values for capecitabine obtained in a Japanese study of capecitabine monotherapy in gastric cancer [17] and with the published PK profile of capecitabine [13–15, 18]. It has previously been reported that the PK profile of capecitabine and its metabolites (5′-DFCR, 5′-DFUR, 5-FU, and FBAL) shows dose proportionality [13–15, 18]. The C_{max}, AUC_{last}, and AUC_{inf} values in the present study were, therefore, dependant on the dose of capecitabine. Statistical analysis were performed to test the difference of AUC_{last} values for capecitabine, 5-FU and FBAL among XP, HXP and X only group using Welch's test by TIBCO Spotfire S+ (Version 8.1 J,TIBCO Software Inc., CA, USA). Similarly, the PK parameter values for cisplatin were compared with those found in previous publications [19–22].

Results

Patient characteristics

A total of 22 patients from seven institutions in Japan were enrolled into this substudy between June 2006 and January 2008: eight patients were enrolled into the XP arm and 14 patients into the HXP arm, and all were evaluable for PK. The patient characteristics were similar in the two treatment groups (Table 1).

Effect of trastuzumab and cisplatin on the pharmacokinetics of capecitabine

The PK parameter values obtained for capecitabine and its metabolites are summarized in Table 2. Following the administration of capecitabine, the mean T_{max} for

Table 1 Patient characteristics (mean ± SD)

Characteristic	XP arm	HXP arm
Number of patients (male/female)	8 (3/5)	14 (2/12)
Gastrectomy (yes/no)	2/6	0/14
Liver function (normal/mild-to-moderate dysfunction)	5/3	5/9
Weight (kg)	53.9 ± 13.7	54.1 ± 9.3
Height (cm)	160 ± 16.2	163 ± 7.5
Creatinine clearance (mL/min)	85.7 ± 22.2	86.4 ± 24.0
Body surface area (m^2)	1.55 ± 0.28	1.57 ± 0.16

capecitabine, 5′-DFCR (intermediate metabolite), 5′-DFUR, and 5-FU (metabolites with antitumor activity) was obtained between 1.36 and 1.62 h in the XP arm and between 1.98 and 2.20 h in the HXP arm. The mean C_{max} of FBAL (the main catabolite of 5-FU) was reached after 2.72 h in XP the arm and after 3.04 h in the HXP arm. The mean C_{max} for capecitabine was numerically greater in the HXP arm (4.60 ± 5.46 μg/mL) than in the XP arm (2.21 ± 0.85 μg/mL). Similarly, the AUC_{last} value for capecitabine was larger in the HXP arm (6.56 ± 5.63 μg h/mL) than in the XP arm (3.65 ± 1.42 μg h/mL). However, the expected variation in C_{max} and AUC_{last} values observed between treatment arms was not clinically significant and fell within established ranges. The C_{max} of FBAL was reached slightly later than the other metabolites and decreased slowly in both arms. The $t_{½}$ of FBAL was correspondingly longer than that of capecitabine or other metabolites: 2.07 h in the XP arm and 2.41 h in the HXP arm. Conversely, capecitabine, 5′-DFCR, 5′-DFUR, and 5-FU were all rapidly eliminated in both arms: the mean $t_{½}$ of capecitabine and its metabolites ranged from 0.44 to 0.74 h in the XP arm and from 0.87 to 0.93 h in the HXP arm.

Effect of trastuzumab and capecitabine on the pharmacokinetics of cisplatin

The PK parameter values obtained for cisplatin are summarized in Table 3. The mean C_{max} values for total platinum were 4.00 μg/mL in the XP arm and 3.70 μg/mL in the HXP arm, with mean $t_{½}$ values of 13.9 h and 17.9 h, respectively. For unbound platinum, the mean C_{max} values were 1.83 μg/mL in the XP arm and 1.97 μg/mL in the HXP arm, with mean $t_{½}$ values of 1.28 and 1.11 h, respectively. There was no difference between the 2 treatment arms in the PK for total platinum or for unbound platinum. The ratios (HXP arm/XP arm) of $\ln(AUC_{last})$ for total and unbound platinum are shown in Fig. 1. The 90% CI of these ratios included 100%, indicating that there was

Table 2 Pharmacokinetic parameters for capecitabine and its metabolites (mean ± SD)

Group	Compound	N	T_{max} (h)	C_{max} (μg/mL)	AUC_{last} (μg h/mL)	AUC^{a}_{inf} (μg h/mL)	$t^{a}_{½}$(h)	CL/F^{a} (L/h)
XP	Capecitabine	8	1.36 ± 0.75	2.21 ± 0.85	3.65 ± 1.42	3.65 ± 1.54[b]	0.44 ± 0.12[b]	252 ± 141[b]
	5′-DFCR	8	1.48 ± 0.76	5.03 ± 2.00	10.3 ± 1.86	10.3 ± 1.85	0.69 ± 0.12	52.5 ± 12.0
	5′-DFUR	8	1.62 ± 0.75	4.81 ± 2.74	8.34 ± 2.19	8.72 ± 2.17[c]	0.74 ± 0.42[c]	61.6 ± 20.1[c]
	5-FU	8	1.61 ± 0.92	0.19 ± 0.14	0.32 ± 0.14	0.36 ± 0.14[c]	0.73 ± 0.38[c]	924 ± 569[c]
	FBAL	8	2.72 ± 0.69	3.80 ± 0.85	15.1 ± 5.14	16.9 ± 6.61[b]	2.07 ± 0.52[b]	14.9 ± 5.14[b]
HXP	Capecitabine	14	1.98 ± 0.91	4.60 ± 5.46	6.56 ± 5.63	7.88 ± 6.48[d]	0.89 ± 0.47[d]	148 ± 83.0[d]
	5′-DFCR	14	2.05 ± 0.87	5.08 ± 2.70	12.0 ± 5.58	11.9 ± 5.72[e]	0.93 ± 0.32[e]	54.1 ± 28.1[e]
	5′-DFUR	14	2.12 ± 0.90	3.45 ± 1.73	7.60 ± 2.25	7.76 ± 2.17[f]	0.87 ± 0.36[f]	76.5 ± 39.8[f]
	5-FU	14	2.20 ± 0.84	0.15 ± 0.10	0.36 ± 0.12	0.33 ± 0.14[f]	0.88 ± 0.39[f]	972 ± 523[f]
	FBAL	14	3.04 ± 0.96	3.55 ± 1.02	13.9 ± 3.84	17.2 ± 5.02[f]	2.41 ± 0.59[f]	14.6 ± 5.06[f]

[a] Some samples were not able to calculate K_{el} by lack of data in the elimination phase, [b] $N = 7$, [c] $N = 6$, [d] $N = 9$, [e] $N = 13$, [f] $N = 10$

Table 3 Pharmacokinetic parameters for cisplatin (mean ± SD)

Group	Parameter	N	C_{max} (μg/mL)	AUC_{last} (μg h/mL)	AUC^{a}_{inf} (μg h/mL)	$t^{a}_{½}$ (h)	CL^{a} (L/h)	V^{a}_{ss} (L)
XP	Total platinum	8	4.00 ± 0.51	14.8 ± 2.20	60.1 ± 13.4[b]	13.9 ± 4.84[b]	2.26 ± 0.65[b]	41.5 ± 10.7[b]
	Unbound platinum	8	1.83 ± 0.30	3.46 ± 0.52	3.58 ± 0.52	1.28 ± 0.38	35.6 ± 9.52	55.1 ± 16.2
HXP	Total platinum	14	3.70 ± 0.89	13.4 ± 2.95	71.1 ± 91.2[c]	17.9 ± 23.9[c]	2.89 ± 1.39[c]	43.0 ± 4.53[c]
	Unbound platinum	14	1.97 ± 0.65	3.64 ± 1.11	3.79 ± 1.15[d]	1.11 ± 0.18[d]	35.3 ± 14.3[d]	52.2 ± 23.9[d]

[a] Some samples were not able to calculate K_{el} by lack of data in the elimination phase, [b] $N = 7$, [c] $N = 12$, [d] $N = 13$

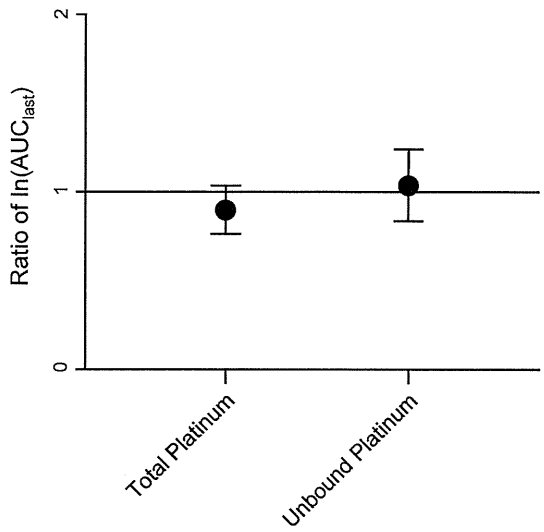

Fig. 1 The ratios (HXP arm/XP arm) of ln(AUC_{last}) for total and unbound platinum. *Error bars*, 90% CI

no clear difference in exposure to cisplatin between the XP and HXP arms.

Pharmacokinetics of capecitabine and cisplatin: comparison with the literature

Dose-normalized AUC_{last} values for capecitabine, 5-FU, and FBAL are displayed in Fig. 2. High interpatient variability in the PK profile of capecitabine is well documented. Consequently, limits of variability for AUC and C_{max} values for capecitabine treatment regimens have been defined and reported [16, 23, 24]. Briefly, for AUC values, acceptable between-treatment differences in 90% CIs fall within the range of 80 to 125%, whereas for C_{max} values, acceptable variability falls within the range of 70–143%. In this analysis, the 90% CIs for ratios of C_{max} and AUC_{last} values for capecitabine, with or without trastuzumab, fell within previously reported limits, indicating that there were no remarkable changes in the PK profile of capecitabine when co-administered with trastuzumab. Furthermore, the means and ranges of distribution of capecitabine metabolites were consistent, irrespective of whether capecitabine was administered alone (*X* only), with cisplatin (XP), or with trastuzumab and cisplatin (HXP). No significant differences of AUC_{last} values for capecitabine, 5-FU and FBAL among XP, HXP and *X* only group were observed.

After intravenous administration, cisplatin is rapidly and irreversibly bound to plasma proteins, and only the unbound fraction remains biologically active [19]. We therefore compared the PK parameter values obtained for unbound platinum in this study with those obtained in previously published studies (Table 4). In non-small-cell lung cancer patients who were treated with 80 mg/m^2 cisplatin, the mean unbound platinum C_{max} was 3.08 μg/mL and the mean AUC value was 2.0 μg h/mL [20]. Felici et al. [19] reported that, in patients with solid tumors treated with 75 mg/m^2 cisplatin, C_{max} was 1.22 and 1.18 μg/mL, and the value for AUC_{inf} was 3.72 and 3.67 μg h/mL for the docetaxel + cisplatin and docetaxel + cisplatin + 5-FU arms, respectively. Thus, the mean C_{max} values obtained in this study (1.83 μg/mL for the XP arm and 1.97 μg/mL for the HXP arm; Table 3) are similar to those previously reported, as are the AUC_{inf} values (3.58 μg·h/mL for the XP arm and 3.79 μg h/mL for the HXP arm; Table 3). In previous studies, Urien et al. [21] and Hanada et al. [22] reported that the mean CL values for unbound platinum were 35.5 and 18.5 L/h, respectively. The CL values from this study (35.6 and 35.3 L/h for the XP and HXP arm, respectively; Table 3) are, therefore, also consistent with previously reported data.

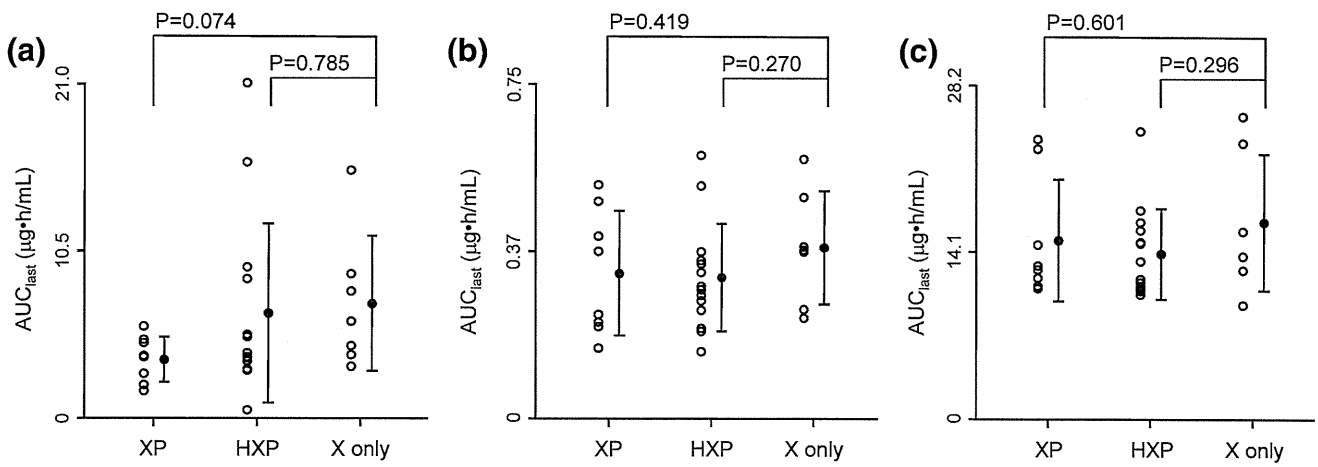

Fig. 2 Dose-normalized AUC_{last} for **a** capecitabine, **b** 5-FU, and **c** FBAL. Mean ± SD. *X*, capecitabine; *P*, cisplatin; *H*, trastuzumab. *P* values were calculated by Welch's test

Table 4 Comparison of pharmacokinetic parameters of unbound platinum (mean ± SD or mean)

	JP19959 (current study)		Previous data				
	XP	HXP	Felici et al. [19]		Kitajima et al. [20]	Urien et al. [21]	Hanada et al. [22]
			TC	TCF			
Cisplatin (mg/m^2)	80	80	75	75	80	15–80	60–100
AUC_{inf} (μg h/mL)	3.58 ± 0.52	3.79 ± 1.15	3.72	3.67	2.0	–	–
C_{max} (μg/mL)	1.83 ± 0.30	1.97 ± 0.65	1.22	1.18	3.08	–	–
CL (L/h)	35.6 ± 9.52	35.3 ± 14.3	39.2	39.9	–	35.5	18.5

T docetaxel, *C* cisplatin, *F* 5-fluorouracil

Discussion

Several chemotherapeutic agents are considered to be active in AGC. These include 5-FU, cisplatin, anthracyclines, oral fluoropyrimidines, intravenous fluoropyrimidines, irinotecan, oxaliplatin, and docetaxel. Although regimens containing 5-FU and cisplatin are widely accepted as potential standard therapies for advanced gastric and esophagogastric cancer, they are associated with response rates of 25–45% and a median overall survival time limited approximately 7–9 months [1–4]. Thus, there is a requirement for more efficacious treatments.

The efficacy and safety findings of the pivotal ToGA study have been reported [10]. Briefly, addition of trastuzumab to chemotherapy extended the median overall survival time of patients with HER2-positive AGC by 2.7 months compared with chemotherapy alone (hazard ratio 0.74, 95% CI: 0.60–0.91; $P < 0.005$). In patients limited to those with highly HER2-positive tumors (graded as immunohistochemistry [IHC] 2+/fluorescence *in situ* hybridization [FISH]-positive or IHC 3+), median overall survival was 16.0 months for patients receiving trastuzumab plus chemotherapy compared with 11.8 months for chemotherapy alone. Importantly, the overall treatment safety profiles of the 2 study arms were similar, indicating that the addition of trastuzumab did not adversely affect treatment safety [10].

The testing of any new combination of molecular-targeted agents must take into account drug–drug interactions that may negatively affect treatment-related adverse events. The primary analyses performed in the ToGA study focused on comparing treatment efficacy and safety, and included data obtained from 584 patients. The population of the ToGA study was broad and heterogeneous, enrolling patients from all over the world, with over 50% of the patients enrolled from Asian regions. The aim of the present study was to characterize the PK profiles of capecitabine and cisplatin when given in combination with trastuzumab in Japanese patients with HER2-positive AGC, and to identify any major drug–drug interactions. The data presented here are obtained from Japanese patients who were enrolled into the ToGA study and who agreed to participate in a PK-monitoring substudy.

The results of the present study show that the addition of trastuzumab to chemotherapy (XP) does not result in any consistent or clinically significant changes in the PK profile of either capecitabine (prodrug or metabolites) or cisplatin (total or unbound platinum) when administered concurrently in Japanese patients with HER2-positive AGC. This finding, coupled with safety profiles in ToGA study, suggests that drug–drug interactions are unlikely to occur.

Moderate variability in the PK profile of the capecitabine was observed between treatment arms, but this was not surprising because orally administered cytotoxic drugs (such as capecitabine) are slowly absorbed and extensively metabolized, resulting in high interpatient variability in exposure [24]. Furthermore, variations in AUC_{last} and C_{max} values for capecitabine were comparable with those observed in previous PK studies of capecitabine [23, 24].

The present study identified no consistent or clinically significant differences in the PK profiles of capecitabine metabolites, including 5-FU (the active antitumor metabolite), when capecitabine was administered concurrently with trastuzumab or cisplatin. In phase I studies evaluating the combination of capecitabine with paclitaxel [18] and docetaxel [25], the PK profiles of capecitabine and its metabolites were found to be unaffected by either drug. The data in the present study (Table 2) are in accordance with those reported previously [14, 18], showing that 5′-DFUR is the major circulating anabolite. Upon administration, capecitabine is hydrolyzed by carboxylesterase (primarily in the liver) to form 5′-DFCR. This is then converted to 5′-DFUR by cytidine deaminase, which is highly active in tumor cells and in the liver. Finally, thymidine phosphorylase, which is significantly more active in tumor tissue than in healthy tissue, converts 5′-DFUR to 5-FU [13, 14]. The dose-normalized AUC_{last} values for capecitabine, 5-FU, and FBAL, in both the XP and HXP arms of the present study, are similar to those previously observed in a Japanese phase II gastric cancer study [17]. These data indicate that trastuzumab and cisplatin do not affect the metabolism of capecitabine. This could be due to distinctive metabolic pathways for respective drugs.

Another study [11] highlighted that the presence of cisplatin with capecitabine could result in the accumulation of 5′-DFUR and 5-FU during multiple treatment cycles because 5′-DFCR (the precursor of 5′-DFUR) is excreted mainly via the kidney, an organ particularly sensitive to the presence of cisplatin [16]. In the event of renal toxicity resulting from cisplatin administration, the AUC values of 5′-DFUR and 5-FU might increase significantly throughout the course of treatment with co-administered capecitabine, as in the previous study [11]. Increased AUC values of 5′-DFCR and 5′-DFUR may result in a higher incidence of grade 3 or 4 peripheral neuropathy, hand–foot syndrome, and diarrhea.

In conclusion, there are no consistent or clinically significant changes in the PK profile of capecitabine or cisplatin when co-administered with trastuzumab. Variability in the AUC_{last} and C_{max} values for capecitabine was consistent with the known PK profile of capecitabine and fell within established limits. Concurrent trastuzumab therapy is unlikely to alter the PK or safety profile of capecitabine or cisplatin in Japanese patients with HER2-positive AGC.

Acknowledgments The authors would like to thank the patients who participated in this trial. Editorial support was provided by Health Interactions, with funding from Roche. This study was sponsored by Chugai Pharmaceutical Co., Ltd.

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Gastric Cancer
DOI 10.1007/s10120-011-0118-1

ORIGINAL ARTICLE

Efficacy of trastuzumab in Japanese patients with HER2-positive advanced gastric or gastroesophageal junction cancer: a subgroup analysis of the Trastuzumab for Gastric Cancer (ToGA) study

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Received: 6 August 2011 / Accepted: 31 October 2011

Abstract
Background The Trastuzumab for Gastric Cancer (ToGA) study is the first international trial to include Japanese patients with human epidermal growth factor 2 (HER2) positive advanced/metastatic gastric or gastroesophageal junction cancer. ToGA showed that trastuzumab plus chemotherapy (capecitabine/cisplatin or 5-fluorouracil/cisplatin) improved overall survival in the overall population (hazard ratio 0.74). Regional differences in outcome in favor of Japanese populations were observed in other studies; therefore, subgroup analyses of ToGA may contribute to the evaluation of the potential benefits of this regimen in Japanese patients.
Methods We performed subgroup analyses on 101 Japanese patients enrolled into ToGA (trastuzumab plus chemotherapy, $n = 51$; chemotherapy, $n = 50$).
Results Median overall survival in the Japanese subgroup was 15.9 months (95% confidence interval 12–25) for trastuzumab plus chemotherapy and 17.7 months (95% confidence interval 12–24) for chemotherapy (hazard ratio 1.00; 95% confidence interval 0.59–1.69). After adjusting

Presented in part at the American Society of Clinical Oncology Gastrointestinal Cancer Symposium, San Francisco, 20–22 January 2011.

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Published online: 17 December 2011

for prespecified covariates, the estimated hazard ratio for overall survival was 0.68 (95% confidence interval 0.36–1.27). Further post hoc and exploratory examinations supported the robustness of the adjusted hazard ratios.
Conclusions After adjusting for imbalanced patient backgrounds between arms, overall survival of Japanese patients with human epidermal growth factor 2 positive advanced/metastatic gastric or gastroesophageal junction cancer who received trastuzumab plus chemotherapy was improved compared with patients who received chemotherapy alone.

Keywords Trastuzumab · Drug therapy · Stomach neoplasms · Randomized controlled trial

Background

Approximately 110,000 people in Japan develop gastric cancer each year [1], with 65,000 estimated deaths (which is second only to lung cancer among cancer-related deaths [1]). For advanced disease, the oral fluoropyrimidine S-1, in combination with cisplatin, has become the standard treatment for gastric cancer in Japan, based on the results of the SPIRITS trial [2]. However, the prognosis still remains poor, and therefore new therapies such as molecular-targeted drugs are needed. Trastuzumab is a recombinant monoclonal antibody that targets human epidermal growth factor receptor 2 (HER2). Trastuzumab derives its anticancer effects from inducing antibody-dependent cytotoxicity, inhibiting HER2-mediated signaling, and preventing cleavage of the extracellular domain of HER2 [3].

Trastuzumab has been approved for use in HER2-positive metastatic breast cancer and as a postoperative adjuvant therapy for HER2-positive breast cancer, and is now the standard of care worldwide for these indications, including in Japan. The Trastuzumab for Gastric Cancer (ToGA) study was the first international randomized controlled phase III trial to include Japanese patients with HER2-positive advanced/metastatic gastric or gastroesophageal junction (GEJ) cancer. The percentage of patients with HER2-positive gastric cancer, as assessed by immunohistochemistry (IHC; 3+ on a scale of 0 to 3+) or fluorescence in situ hybridization (FISH; *HER2*:CEP17 ratio $\geq$2.0) was 22.1% in the overall ToGA population. The proportion of patients with HER2-positive disease was similar for Europe (23.6%), Asia (23.5%), and Japan (27.6%) [4], and similar to that seen in patients with breast cancer in other trial populations (25–30%) [5]. ToGA showed that patients who received combination treatment with trastuzumab and chemotherapy [capecitabine plus cisplatin (XP) or fluorouracil plus cisplatin (FP)] had significantly improved survival compared with those who received chemotherapy alone: the median overall survival (OS) in the intent-to-treat (ITT) population was 13.8 months in the trastuzumab plus chemotherapy arm and 11.1 months in the chemotherapy-only arm [hazard ratio (HR) 0.74, 95% confidence interval (CI) 0.60–0.91; $P = 0.0046$] [6].

There were substantial differences in OS reported from recent phase III trials of chemotherapy for gastric cancer, and these are especially evident between Japan and other countries. Recent trials in Japan have demonstrated that combination therapy resulted in longer survival than was seen in studies outside of Japan, with a median survival exceeding 1 year [7, 8], as compared with around 10 months in Western trials [9, 10]. There are considered to be two reasons for the longer survival observed in Japanese trials. Firstly, up to 70% of Japanese patients receive subsequent chemotherapy following failure of first-line therapy [11–13]. Secondary, there may be differences in the eligibility criteria and baseline patient characteristics between the Japanese and non-Japanese trials; the studies in Japan included patients with and without measurable metastatic disease, whereas non-Japanese trials usually included patients with measurable metastatic disease only [11]. Since the primary endpoint of the ToGA study was OS, there is a possibility that the impact of trastuzumab on survival might be reduced in Japanese patients due to inherently longer survival in this population. To evaluate the efficacy of trastuzumab in combination with chemotherapy specifically in the Japanese population of ToGA, we conducted preplanned and post hoc subgroup analyses.

Patients and methods

The details of the ToGA trial design and methods have been reported elsewhere [6].

Japanese patient subgroup

To evaluate the efficacy and safety of the combination treatment (trastuzumab plus XP) in the Japanese population

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of the ToGA study, we performed subgroup analyses using data from patients who were enrolled from institutions in Japan.

Preplanned sample size for Japanese patients

In the ToGA study, the HR for OS was expected to be 0.77, the expected number of events was 460, and the target sample size was set at 584 patients [6]. Before starting the ToGA study, we set the sample size of Japanese patients to allow us to evaluate similarities between the overall ToGA results and our subgroup analysis in an exploratory manner. Assuming a 70% probability that the HR for OS in the Japanese subgroup would be less than 0.88 (the midpoint between 0.77 and 1.00), the expected number of events was 70. To reach this expected number of events within the study period, the minimum sample size was determined to be 89 patients to allow us to conduct four analyses: preplanned (unadjusted and adjusted), post hoc, and exploratory analyses of the HR.

Unadjusted analyses

We calculated the unadjusted OS and progression-free survival (PFS) of the Japanese sub-group using the same methods as those used for the overall ToGA study [6]. Objective response rate of the Japanese sub-group was analysed with a χ^2 test in patients with measurable disease ($n = 45$ in the trastuzumab plus XP arm and 41 in the XP arm).

Preplanned analyses

Prior to carrying out the Japanese subgroup analysis, we predicted an imbalance in the baseline patient characteristics. Therefore, we planned to calculate an adjusted HR and 95% CI in the Japanese subgroup using a multivariate Cox regression analysis with 15 factors: extent of disease, primary tumor site, measurability of disease, Eastern Cooperative Oncology Group Performance Status (ECOG PS), chemotherapy regimen (stratification factors), sex, age, number of lesions, number of metastatic sites, type of gastric cancer, visceral metastasis, prior gastrectomy, prior chemotherapy, HER2 status, and region of origin (other prespecified covariates). All factors were prespecified in the ToGA study protocol. Each covariate was also evaluated using a univariate Cox regression analysis.

Post hoc analyses

During the preplanned multivariate Cox regression analysis, we excluded patients for whom HER2 status was reported as IHC 3+/FISH unknown (no result). In addition, estimates of effects were extremely unstable for covariates that contained a category which included only one patient. Therefore, to target all of the enrolled patients and ensure the stability of the model, a post hoc analysis was conducted using a multivariate Cox analysis. Among covariates, HER2 status was divided into two categories: high expression (IHC 2+ and FISH-positive or IHC 3+) and low expression (IHC0 and FISH-positive or IHC 1+ and FISH-positive). Covariates that contained a category with only one patient (extent of disease and previous chemotherapy) were excluded from the model to ensure its stability.

Exploratory analyses to evaluate deviation of patient prognosis

To identify factors that affect prognosis specifically in the Japanese subgroup, and to confirm the robustness of our preplanned and post hoc analyses, an exploratory multivariate Cox regression analysis on the HR for OS with various combinations of covariates was carried out. We created a series of models that included the treatment group as a base covariate with 3–6 other covariates, and selected the top four models ranked by value following a chi-square test. The procedure was repeated for the models with three, four, five, and six covariates, and a total of 16 models were selected. From the well-fitting model that was obtained, we compared the HR for OS with the results of preplanned and post hoc analyses. To ensure that HER2 status was not a confounding variable, we carried out a multivariate Cox regression analysis with HER2 expression (high or low) as the stratification factor, and determined the HR for OS in which selected covariates were included in the model.

Furthermore, scoring of the prognosis of each patient in both study arms using the Cox regression model and estimation of the risk for each patient were carried out with the selected covariates. The risk was shown by the estimated value of logarithm HR for each patient. To eliminate the influence of treatment on the mortality risk, we set the treatment group as the stratification factor and produced a histogram plot according to the distribution of patient risk to evaluate potential bias between the treatment arms.

Safety

Adverse events and serious adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 and the International Conference on Harmonization guidelines, respectively.

Results

Patients

Between September 2005 and December 2008, 594 patients were enrolled in the primary ToGA study at 122

Table 1 HER2 testing results in the Japanese population of ToGA

FISH result	IHC score				
	IHC 0	IHC 1+	IHC 2+	IHC 3+	Total
FISH-positive, *n*	14	19	36	37	106
FISH-negative, *n*	155	57	14	1	227
NE, *n*	48	12	8	8	83
Total, *n*	217	88	58	46	409

FISH fluorescence in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *NE* not evaluable

centers in 24 countries, of whom 584 were included in the primary analysis. Four hundred twenty-one tumor samples were provided for HER2 testing from 16 centers in Japan. Twelve samples were not evaluated due to a lack of tumor tissue in the sample ($n = 7$), shipment failure ($n = 4$), or disease progression before shipment ($n = 1$). Of the 409 samples successfully screened, 115 (28.1%) were scored as HER2-positive (IHC 3+ or FISH-positive; Table 1) and 102 patients were registered into the study. After excluding one patient who did not receive the study drug, 101 Japanese patients (trastuzumab plus chemotherapy, $n = 51$; chemotherapy alone, $n = 50$) were included in this subgroup analysis. All patients received capecitabine as the chemotherapy partner of cisplatin.

Table 2 shows the baseline characteristics of the Japanese patients included in this subgroup analysis ($n = 101$) and the non-Japanese patients ($n = 483$). There is similarity in the baseline characteristics of patients from other countries between the study arms. On the other hand, number of metastatic sites, histologic type, and prior gastrectomy were imbalanced by approximately 10% between the study arms in the Japanese subgroup, and were considered to be prognostic factors. Median follow-up times were 18.6 months [interquartile range (IQR) 11–25] in the trastuzumab plus XP arm and 17.1 months (IQR 1–49) in the XP arm. The median number of cycles of trastuzumab therapy was eight (range 1–24). Forty-one patients in the trastuzumab plus XP arm (80.4%) and 41 patients in the XP arm (82.0%) received second-line treatment (at least one chemotherapy treatment after disease progression despite the study treatments).

Efficacy

Unadjusted analyses

Twenty-eight patients (54.9%) in the trastuzumab plus XP arm and 27 patients (54.0%) in the XP arm had died by the data cutoff point. As shown in Table 3, unadjusted median OS was 15.9 months (95% CI 12–25 months) in the trastuzumab plus XP arm and 17.7 months (95% CI 12–24 months) in the XP arm (HR 1.00, 95% CI 0.59–1.69). The number of PFS events (defined as disease progression or death) was 43 (84.3%) in the trastuzumab plus XP arm and 40 (80.0%) in the XP arm. Unadjusted median PFS was 6.2 months (95% CI 5–7 months) in the trastuzumab plus XP arm and 5.6 months (95% CI 5–7 months) in the XP arm (HR 0.92, 95% CI 0.60–1.43). The objective response rate was 64.4% (95% CI 48.8–78.1%) in the trastuzumab plus XP arm and 58.5% (95% CI 42.1–73.7%) in the XP arm.

Preplanned analyses

In the multivariate analysis, the HR for OS, adjusted by the 15 prespecified covariates above, was 0.68 (95% CI 0.36–1.27, $P = 0.2251$, Table 4). The adjusted HR for PFS was 0.66 (95% CI 0.40–1.09%), which was slightly improved compared with the results for the overall population. Among the covariates in the preplanned analysis, the univariate analysis showed that prior gastrectomy was the covariate most strongly associated with longer OS (HR 0.39, 95% CI 0.16–0.91). There were more patients with prior gastrectomy in the XP arm (26%) than in the trastuzumab arm (16%). After adjusting for gastrectomy only, the HR for OS between the treatment arms was 0.85 (95% CI 0.49–1.45).

Post hoc analyses

For the post hoc exploratory multivariate Cox regression analysis, the adjusted HRs for OS and PFS were 0.82 (95% CI 0.45–1.50) and 0.81 (95% CI 0.50–1.30), respectively (Fig. 1).

Exploratory analyses to evaluate deviation of patient prognosis

We evaluated the HR for OS with different combinations of covariates in the model. In the well-fitting models with high chi-square values, the HRs using three, four, five, and six covariates ranged between 0.79 (95% CI 0.49–1.38) and 0.89 (95% CI 0.52–1.54), 0.77 (95% CI 0.44–1.33) and 0.88 (95% CI 0.51–1.53), 0.68 (95% CI 0.39–1.20) and 0.80 (95% CI 0.45–1.42), and 0.68 (95% CI 0.38–1.20) and 0.76 (95% CI 0.44–1.33), respectively. In choosing the well-fitting models, the covariates sex, HER2 status, type of gastric cancer, prior gastrectomy, prior chemotherapy, and number of lesions tended to be chosen. The sets of covariates were similar to those used as prespecified covariates (15 factors). A similar analysis was carried out

Table 2 Baseline patient characteristics of the Japanese population and the non-Japanese population of ToGA

Characteristic	Japanese		Non-Japanese	
	Trastuzumab plus XP ($n = 51$)	XP/FP ($n = 50$)	Trastuzumab plus XP ($n = 243$)	XP/FP ($n = 240$)
Sex				
Male, *n*	40 (78.4%)	40 (80.0%)	186 (76.5%)	178 (74.2%)
Median age, years (range)	63.0 (29–76)	63.5 (45–81)	60.0 (23–83)	59.0 (21–82)
Extent of disease				
Locally advanced, *n*	0 (0.0%)	1 (2.0%)	10 (4.1%)	9 (3.8%)
Metastatic, *n*	51 (100.0%)	49 (98.0%)	233 (95.9%)	231 (96.3%)
Primary tumor site				
Stomach, *n*	49 (96.1%)	44 (88.0%)	187 (77.0%)	198 (82.5%)
Gastroesophageal junction, *n*	2 (3.9%)	6 (12.0%)	56 (23.0%)	42 (17.5%)
Measurability of disease				
Measurable, *n*	45 (88.2%)	41 (82.0%)	224 (92.2%)	216 (90.0%)
Nonmeasurable, *n*	6 (11.8%)	9 (18.0%)	19 (7.8%)	24 (10%)
ECOG performance status				
0–1, *n*	51 (100.0%)	50 (100.0%)	213 (87.7%)	213 (88.7%)
2, *n*	0 (0.0%)	0 (0.0%)	30 (12.3%)	27 (11.3%)
Chemotherapy regimen				
XP, *n*	51 (100%)	50 (100%)	205 (84.4%)	205 (85.4%)
FP, *n*	0 (0.0%)	0 (0.0%)	38 (15.6%)	35 (14.6%)
Number of lesions			($n = 242$)	
1–4, *n*	16 (31.4%)	18 (36.0%)	112 (46.3%)	98 (40.8%)
>4, *n*	35 (68.6%)	32 (64.0%)	130 (53.7%)	142 (59.2%)
Median value (range)	6 (1–15)	6 (1–15)	5 (1–20)	5 (1–16)
Number of metastatic sites			($n = 242$)	
1–2, *n*	28 (54.9%)	32 (64.0%)	124 (51.2%)	114 (47.5%)
>2, *n*	23 (45.1%)	18 (36.0%)	118 (48.8%)	126 (52.5%)
Median value (range)	2 (1–5)	2 (1–5)	2 (1–7)	3 (1–8)
Type of gastric cancer (central review)[a]			($n = 242$)	($n = 237$)
Intestinal type, *n*	37 (72.5%)	42 (84.0%)	188 (77.7%)	171 (72.2%)
Diffuse type, *n*	5 (9.8%)	4 (8.0%)	21 (8.7%)	21 (8.9%)
Mixed type, *n*	9 (17.6%)	4 (8.0%)	33 (13.6%)	45 (19.0%)
Visceral metastasis (liver or lung)				
Yes, *n*	35 (68.6%)	33 (66.0%)	134 (55.1%)	139 (57.9%)
No, *n*	16 (31.4%)	17 (34.0%)	109 (44.9%)	101 (42.1%)
History of treatment for gastric cancer				
Prior gastrectomy, *n*	8 (15.7%)	13 (26.0%)	62 (25.5%)	49 (20.4%)
Prior chemotherapy, *n*	1 (2.0%)	0 (0.0%)	26 (10.7%)	12 (5.0%)
HER2 status				
IHC 0/FISH-positive, *n*	3 (5.9%)	9 (18.0%)	20 (8.2%)	29 (12.2%)
IHC 1+/FISH-positive, *n*	10 (19.6%)	7 (14.0%)	28 (11.5%)	25 (10.4%)
IHC 2+/FISH-positive, *n*	18 (35.3%)	13 (26.0%)	62 (25.5%)	66 (27.5%)
IHC 3+/FISH-positive, *n*	16 (31.4%)	17 (34.0%)	115 (47.3%)	108 (45.0%)
IHC 3+/FISH-negative, *n*	1 (2.0%)	0 (0.0%)	8 (3.3%)	6 (2.5%)
IHC unknown/FISH-positive, *n*	0 (0.0%)	0 (0.0%)	5 (2.1%)	2 (0.8%)
IHC 3+/FISH unknown, *n*	3 (5.9%)	4 (8.0%)	5 (2.1%)	4 (1.7%)
Region of origin				
Japanese, *n*	51 (100%)	50 (100%)	0 (0.0%)	0 (0.0%)
Non-Japanese, *n*	0 (0.0%)	0 (0.0%)	243 (100%)	240 (100%)

ECOG Eastern Cooperative Oncology Group, *FISH* fluorescence in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *XP* capecitabine plus cisplatin

[a] Type of gastric cancer was described by the Lauren Classification

using HER2 expression (high or low) as the stratification factor. The HR was approximately 0.7, and the HRs using three, four, five, and six covariates were between 0.67 (95% CI 0.38–1.18) and 0.79 (95% CI 0.46–1.39), 0.70 (95% CI 0.40–1.24) and 0.82 (95% CI 0.47–1.42), 0.68 (95% CI 0.39–1.22) and 0.76 (95% CI 0.43–1.34), and 0.67 (95% CI 0.37–1.22) and 0.78 (95% CI 0.44–1.36), respectively. Influential covariates chosen in the well-fitting models included sex, prior gastrectomy, and number of lesions. Table 5 shows the covariate combinations that resulted in a good fit based on these analyses. Figure 2 shows the distribution of patient risk with these three models. The risk distribution is broad in each arm; however, the XP arm shows a somewhat greater distribution toward the left, indicating that this arm included a greater number of patients with better prognosis.

Table 3 Overall survival in the Japanese population of ToGA (unadjusted Cox regression analysis)

	Trastuzumab plus XP ($n = 51$)	XP ($n = 50$)
Number of events (%)	28 (54.9)	27 (54)
Median OS, months (95% CI)	15.9 (12–25)	17.7 (12–24)
Survival rate (%)		
6 months	92	92
12 months	68	64
18 months	48	49
24 months	41	35
Hazard ratio (95% CI)	1.00 (0.59–1.69)	

CI confidence interval, *OS* overall survival, *XP* capecitabine plus cisplatin

Safety

Table 6 shows the adverse events in the Japanese population of ToGA, and indicates that all patients experienced at least one adverse event in each arm. Grade 3/4 adverse events occurred in 43 patients (84%) in the trastuzumab

Table 4 Preplanned multivariate Cox regression analysis of overall survival by extent of disease, primary tumor site, measurability of disease, ECOG status, chemotherapy regimen, and other prespecified covariates: sex, age, number of lesions, number of metastatic sites, type of gastric cancer, visceral metastasis, prior gastrectomy, prior chemotherapy, HER2 status, and region of origin

	Hazard ratio (95% CI)		*P* value
Trastuzumab plus XP versus XP	0.68	(0.36–1.27)	0.2251
Sex (male vs. female)	0.16	(0.07–0.41)	<0.0001
Age (<60 vs. ≥60)	1.07	(0.54–2.13)	0.8382
Extent of disease (locally advanced vs. metastatic)	0.00	(0.00–.)	0.9902
Primary tumor site (stomach vs. gastroesophageal junction)	0.68	(0.25–1.87)	0.4559
Measurability of disease (measurable vs. nonmeasurable)	0.95	(0.29–3.05)	0.9268
ECOG performance status	–	–	–
Chemotherapy regimen	–	–	–
Number of lesions (1–4 vs. >4)	0.49	(0.22–1.09)	0.0818
Number of metastatic sites (1–2 vs. >2)	0.79	(0.41–1.50)	0.4695
Type of gastric cancer			
Diffuse type versus intestinal type	3.24	(1.08–9.70)	0.0356
Mixed type versus intestinal type	0.91	(0.30–2.71)	0.8644
Visceral metastasis (yes vs. no)	1.15	(0.48–2.74)	0.7510
Prior gastrectomy (yes vs. no)	0.22	(0.06–0.75)	0.0159
Prior chemotherapy (yes vs. no)	27.72	(1.11–694.38)	0.0432
HER2 status			
IHC 0/FISH-positive versus IHC 3+/FISH-positive	5.31	(1.29–21.86)	0.0208
IHC 1+/FISH-positive versus IHC 3+/FISH-positive	4.87	(1.73–13.70)	0.0027
IHC 2+/FISH-positive versus IHC 3+/FISH-positive	1.53	(0.73–3.18)	0.2578
IHC 3+/FISH-negative versus IHC 3+/FISH-positive	25.66	(1.72–382.49)	0.0186
Region of origin	–	–	–

Among 15 prespecified factors, chemotherapy regimen, performance status, and region of origin were not calculated in this table because all Japanese patients received capecitabine as the chemotherapy partner of cisplatin, had Karnofsky performance status of 0–1, and were from Asia (Japan)

CI confidence interval, *ECOG* Eastern Cooperative Oncology Group, *FISH* fluorescence in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *XP* capecitabine plus cisplatin

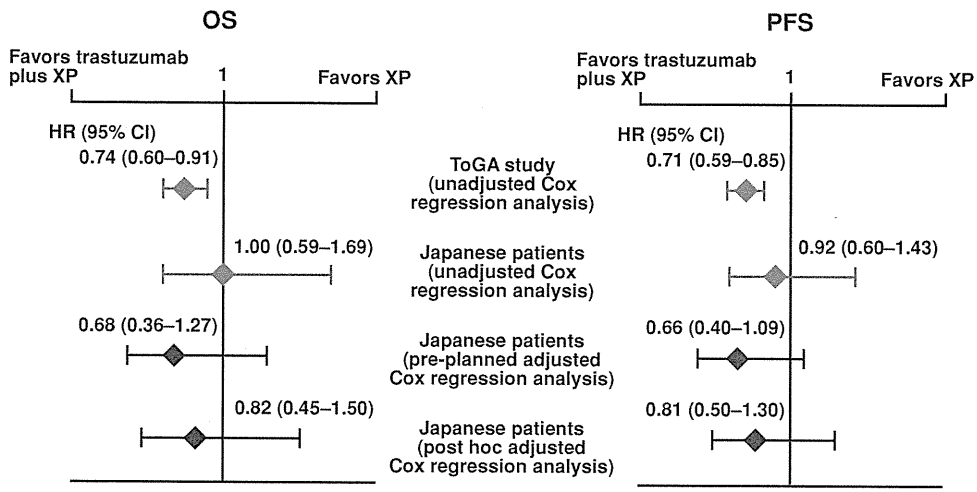

Fig. 1 Unadjusted and adjusted hazard ratios for overall and progression-free survival. *CI* confidence interval, *HR* hazard ratio, *OS* overall survival, *PFS* progression-free survival, *XP* capecitabine plus cisplatin

Table 5 Covariates included in the model

Number of covariates	Covariates included in the model
4	HER2 expression (low/high), sex (male/female), prior gastrectomy (yes/no), number of lesions (1–4/>4)
5	HER2 expression (low/high), sex (male/female), prior gastrectomy (yes/no), number of lesions (1–4/>4), type of gastric cancer (diffuse/intestinal)
6	HER2 expression (low/high), sex (male/female), prior gastrectomy (yes/no), number of lesions (1–4/>4), type of gastric cancer (diffuse/intestinal), number of metastatic sites (1–2/>2)

HER2 human epidermal growth factor receptor 2

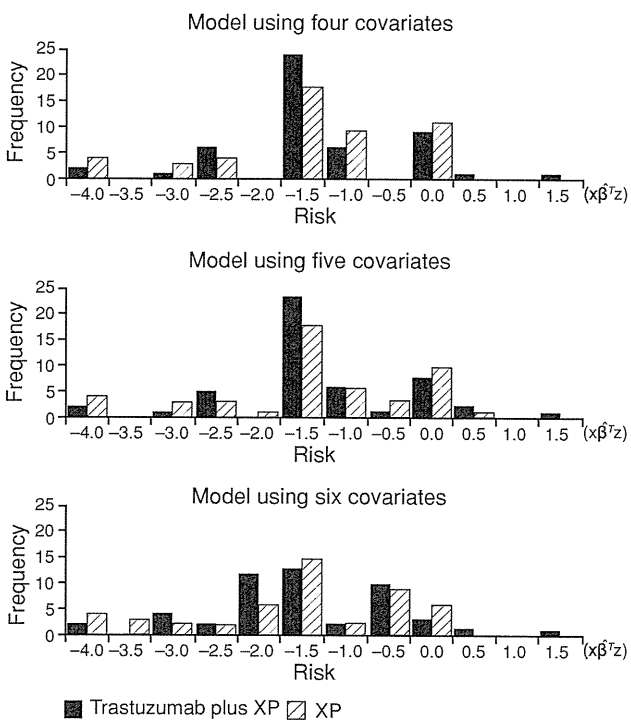

Fig. 2 Distribution of estimated values by linear predictor. *XP* capecitabine plus cisplatin. The ordinate is the number of patients and the abscissa is the risk score (estimated hazard number for each patient). The risk of mortality increases as the plot moves to the right

plus XP arm and 36 patients (72%) in the XP arm. Treatment was discontinued due to adverse events for one patient (2%) in the trastuzumab plus XP arm and four patients (8%) in the XP arm. Deaths due to adverse events occurred in two patients in the trastuzumab plus XP arm: one due to cardiac failure and unstable angina and the other due to gastrointestinal perforation. The case of cardiac failure and unstable angina was attributed to an adverse event likely related to trastuzumab.

Discussion

In the original ToGA study, patients with HER2-positive advanced gastric or GEJ cancer who received the combination treatment of trastuzumab plus XP/FP had significantly longer OS and PFS than patients who received XP/FP alone [6]. No differences in OS or PFS were detected between the two treatment arms in this subgroup analysis of Japanese patients when unadjusted data were analyzed. However, in preplanned and post hoc analyses, the HRs were 0.68 and 0.82 for OS and 0.66 and 0.82 for PFS, respectively, after adjusting for baseline characteristics. These values were similar to the overall ToGA study results. Taken together, these results strongly suggest that

Table 6 Adverse events in ≥10% of Japanese patients in ToGA

	Trastuzumab plus XP ($n = 51$)		XP ($n = 50$)	
	All grade n (%)	Grade ≥3 n (%)	All grade n (%)	Grade ≥3 n (%)
Total	51 (100)	43 (84)	50 (100)	36 (72)
Gastrointestinal disorders				
Nausea	44 (86)	7 (14)	44 (88)	7 (14)
Vomiting	33 (65)	1 (2)	28 (56)	2 (4)
Constipation	24 (47)	1 (2)	24 (48)	–
Diarrhoea	23 (45)	4 (8)	24 (48)	2 (4)
Stomatitis	29 (57)	–	16 (32)	1 (2)
Blood and lymphatic system disorders				
Neutropenia	30 (59)	18 (35)	34 (68)	20 (40)
Thrombocytopenia	11 (22)	1 (2)	8 (16)	3 (6)
Anemia	15 (29)	13 (25)	11 (22)	8 (16)
Febrile neutropenia	5 (10)	5 (10)	3 (6)	3 (6)
Skin and subcutaneous tissue disorders				
Palmar–plantar erythrodysaesthesia syndrome	21 (41)	–	23 (46)	1 (2)
Alopecia	12 (24)	–	9 (18)	–
Skin hyperpigmentation	6 (12)	–	5 (10)	–
Rash	10 (20)	–	5 (10)	–
Pigmentation disorder	10 (20)	–	7 (14)	–
Nail disorder	5 (10)	–	5 (10)	–
Metabolism and nutrition disorders				
Anorexia	43 (84)	12 (24)	46 (92)	10 (20)
Dehydration	3 (6)	1 (2)	6 (12)	1 (2)
General disorders and administration site conditions				
Fatigue	31 (61)	4 (8)	26 (52)	4 (8)
Pyrexia	19 (37)	1 (2)	12 (24)	–
Chill	7 (14)	–	0 (0)	–
Edema	19 (37)	–	23 (46)	–
Nervous system disorders				
Peripheral neuropathy	16 (31)	1 (2)	10 (20)	–
Dysgeusia	13 (25)	–	8 (16)	–
Peripheral sensory neuropathy	2 (4)	–	11 (22)	–
Dizziness	5 (10)	1 (2)	5 (10)	–
Respiratory, thoracic, and mediastinal disorders				
Hiccups	21 (41)	–	16 (32)	–
Epistaxis	5 (10)	–	3 (6)	–
Renal and urinary disorders				
Renal impairment	32 (63)	2 (4)	27 (54)	–
Vascular disorders				
Hypertension	4 (8)	1 (2)	3 (6)	–
Investigations				
Weight decreased	27 (53)	2 (4)	13 (26)	1 (2)
Weight increased	10 (20)	1 (2)	9 (18)	–
Psychiatric disorders				
Insomnia	11 (22)	–	8 (16)	–
Infections and infestations				
Nasopharyngitis	18 (35)	–	6 (12)	–
Musculoskeletal and connective tissue disorders				
Back pain	5 (10)	–	1 (2)	–

XP capecitabine plus cisplatin

the same benefit of adding trastuzumab to chemotherapy was obtained in the Japanese patient subgroup as in the overall population.

In our subgroup analysis, the change in HR pre- and post-adjustment may have been due to an uneven distribution of prognostic factors between the two treatment arms. The XP arm included more patients with factors generally considered to be associated with a good prognosis (history of gastrectomy [14, 15], intestinal type cancer [16–19], and metastasis in fewer than two organs [19]). In the overall ToGA study and in the Japanese subgroup, gastric resection was shown to be the most influential factor affecting prognosis, as assessed by univariate Cox regression analyses (HRs of gastrectomy were 0.54 and 0.39, respectively). In the Japanese subgroup, the number of patients who had undergone gastric resection in the XP arm ($n = 13$, 26.0%) was approximately 10% higher than that of the trastuzumab plus XP arm ($n = 8$, 15.7%).

When multiple factors influence prognosis, different combinations of factors could affect the HR between two treatment groups. Therefore, to confirm that the HR is robust, it is necessary to analyze different combinations of factors. In this regard, we found that the HRs for OS were approximately 0.7 for all combinations of factors, thus supporting the robustness of our results.

Median OS in the XP/FP alone arm was 11.1 months (95% CI 10–13) in the overall ToGA population [6], but was approximately 6.5 months longer in the Japanese subgroup (XP arm: 17.7 months). These findings are consistent with results of recent trials reporting longer survival for patients with gastric cancer in Japan than for patients in Europe and the USA. One possible reason for this difference is that more Japanese patients receive second-line or later treatment after the failure of first-line treatment [11–13]. In the ToGA study, more than 80% of Japanese patients in both treatment arms underwent second-line or further treatment, which was considerably higher than the overall rates of second-line treatment in the overall ToGA population (42% of patients in the trastuzumab plus XP/FP arm and 45% in the XP/FP arm) [6]. In the present study of Japanese patients, the OS of patients who received XP only was similar to that reported in other recent Japanese trials [2, 7, 8]. Furthermore, after adjusting for imbalances between the baseline characteristics of treatment arms, we detected an additive effect of trastuzumab among Japanese patients, similar to that of the overall population. By further exploratory analyses, we confirmed that the HRs in favor of trastuzumab were consistently observed after adjusting for prognostic factors. These findings strongly suggest that the benefits of trastuzumab were of the same magnitude in Japanese patients as in the whole study population, although the absolute length of survival was longer in the Japanese subgroup. In conclusion, trastuzumab in combination with XP can be considered a new standard therapy for Japanese patients with HER2-positive advanced gastric or GEJ cancer.

Acknowledgments This study was sponsored by Chugai Pharmaceutical Co., Ltd. and F. Hoffmann-La Roche Ltd. We thank all of the patients and investigators who participated in the ToGA study in Japan.

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LUNG-3969; No. of Pages 8
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Lung Cancer xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Characterization of the immunophenotype of the tumor budding and its prognostic implications in squamous cell carcinoma of the lung

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ARTICLE INFO

Article history:
Received 24 June 2011
Received in revised form
20 September 2011
Accepted 8 November 2011

Keywords:
Tumor budding
Lung cancer
Squamous cell carcinoma
Epithelial Mesenchymal Transition (EMT)
Prognostic factor

ABSTRACT

Tumor budding is morphologically defined as infiltration by small clusters of cancer cells. While the biological properties of budding cells in adenocarcinoma (decreased expression of adhesion molecules and of differentiation markers) have been elucidated, those of the cells in squamous cell carcinoma (SqCC) of the lung still remain to be clarified. We examined the clinicopathological data of 217 patients with SqCC of the lung. Furthermore we evaluated the immunohistochemical properties of the budding cells. Tumor budding was observed in 83 (38.2%) patients. A statistically significant difference was observed in overall 5-year survival rates between the cases showing tumor budding and the cases not showing budding (45.6% vs. 64.0%, $p < 0.001$). As compared with cancer cells forming solid nests, budding cells (BCs) exhibited reduced expression levels of the cellular adhesion molecules (E-cadherin; $p = 0.004$, β-catenin; $p = 0.002$) and increased expression levels of laminin-5γ2 ($p = 0.001$). On the other hand, no significant differences in the staining scores for differentiation markers (p63 and podoplanin) were found between BCs and cancer cells forming nests. Multivariate analysis revealed that tumor budding was a significant independent prognostic factor in patients with SqCC of the lung ($p = 0.022$). Tumor budding is an independent adverse prognostic factor in patients with SqCC of the lung. Although budding cells in SqCC exhibited reduced expression levels of the cellular adhesion molecules, the expression levels of specific differentiation markers were retained, suggesting that the budding mechanism in SqCC may differ, at least in part, from that in adenocarcinoma.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality around the world. Adenocarcinoma and squamous cell carcinoma (SqCC) are the two major histopathological subtypes of NSCLC, and have different pathogenetic mechanisms and distinct biological characteristics. Recently, while custom-tailored therapy has been attempted for NSCLC depending on the biochemical characteristics, and newer agents have been developed for the treatment of adenocarcinoma, progress in SqCC treatment is insufficient [1–4]. In addition, the biological characteristics of SqCC are not yet completely understood. Therefore, elucidation of the molecular mechanisms and development of effective treatments for SqCC of the lung (SqCC-lung) are urgently needed.

The term "tumor budding" in colorectal cancer has been applied to single cells or small clusters of cells observed within the stromal tissue at the invasive margin [5]. Numerous studies on adenocarcinoma, including of the colon and lung, have reported tumor budding as an independent prognostic factor [6–9]. We recently reported that tumor budding in lung adenocarcinoma is a distinct morphologic feature with the following biologic characteristics and prognostic significance: (1) tumor budding is an independent prognostic factor; (2) budding cells display the Epithelial Mesenchymal Transition (EMT) phenotype and reduced expression levels of markers of differentiation [7]. On the other hand, there are no reports on the clinicopathological and biological characteristics associated with tumor budding in patients with SqCC-lung. Moreover, the immunohistochemical phenotypes of budding cells in SqCC-lung remain unclear. In this study, we reviewed the data of patients with SqCC-lung to examine the clinicopathological

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doi:10.1016/j.lungcan.2011.11.010

Please cite this article in press as: Taira T, et al. Characterization of the immunophenotype of the tumor budding and its prognostic implications in squamous cell carcinoma of the lung. Lung Cancer (2011), doi:10.1016/j.lungcan.2011.11.010