

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  $\chi^2$  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 (IHC 0 and FISH-positive or IHC 1+ and FISH-negative). 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				Total
	IHC 0	IHC 1+	IHC 2+	IHC 3+	
FISH-positive, <i>n</i>	14	19	36	37	106
FISH-negative, <i>n</i>	155	57	14	1	227
NE, <i>n</i>	48	12	8	8	83
Total, <i>n</i>	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 ( <i>n</i> = 51)	XP/FP ( <i>n</i> = 50)	Trastuzumab plus XP ( <i>n</i> = 243)	XP/FP ( <i>n</i> = 240)
Sex				
Male, <i>n</i>	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, <i>n</i>	0 (0.0%)	1 (2.0%)	10 (4.1%)	9 (3.8%)
Metastatic, <i>n</i>	51 (100.0%)	49 (98.0%)	233 (95.9%)	231 (96.3%)
Primary tumor site				
Stomach, <i>n</i>	49 (96.1%)	44 (88.0%)	187 (77.0%)	198 (82.5%)
Gastroesophageal junction, <i>n</i>	2 (3.9%)	6 (12.0%)	56 (23.0%)	42 (17.5%)
Measurability of disease				
Measurable, <i>n</i>	45 (88.2%)	41 (82.0%)	224 (92.2%)	216 (90.0%)
Nonmeasurable, <i>n</i>	6 (11.8%)	9 (18.0%)	19 (7.8%)	24 (10%)
ECOG performance status				
0–1, <i>n</i>	51 (100.0%)	50 (100.0%)	213 (87.7%)	213 (88.7%)
2, <i>n</i>	0 (0.0%)	0 (0.0%)	30 (12.3%)	27 (11.3%)
Chemotherapy regimen				
XP, <i>n</i>	51 (100%)	50 (100%)	205 (84.4%)	205 (85.4%)
FP, <i>n</i>	0 (0.0%)	0 (0.0%)	38 (15.6%)	35 (14.6%)
Number of lesions			( <i>n</i> = 242)	
1–4, <i>n</i>	16 (31.4%)	18 (36.0%)	112 (46.3%)	98 (40.8%)
>4, <i>n</i>	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			( <i>n</i> = 242)	
1–2, <i>n</i>	28 (54.9%)	32 (64.0%)	124 (51.2%)	114 (47.5%)
>2, <i>n</i>	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) <sup>a</sup>			( <i>n</i> = 242)	( <i>n</i> = 237)
Intestinal type, <i>n</i>	37 (72.5%)	42 (84.0%)	188 (77.7%)	171 (72.2%)
Diffuse type, <i>n</i>	5 (9.8%)	4 (8.0%)	21 (8.7%)	21 (8.9%)
Mixed type, <i>n</i>	9 (17.6%)	4 (8.0%)	33 (13.6%)	45 (19.0%)
Visceral metastasis (liver or lung)				
Yes, <i>n</i>	35 (68.6%)	33 (66.0%)	134 (55.1%)	139 (57.9%)
No, <i>n</i>	16 (31.4%)	17 (34.0%)	109 (44.9%)	101 (42.1%)
History of treatment for gastric cancer				
Prior gastrectomy, <i>n</i>	8 (15.7%)	13 (26.0%)	62 (25.5%)	49 (20.4%)
Prior chemotherapy, <i>n</i>	1 (2.0%)	0 (0.0%)	26 (10.7%)	12 (5.0%)
HER2 status				
IHC 0/FISH-positive, <i>n</i>	3 (5.9%)	9 (18.0%)	20 (8.2%)	29 (12.2%)
IHC 1+/FISH-positive, <i>n</i>	10 (19.6%)	7 (14.0%)	28 (11.5%)	25 (10.4%)
IHC 2+/FISH-positive, <i>n</i>	18 (35.3%)	13 (26.0%)	62 (25.5%)	66 (27.5%)
IHC 3+/FISH-positive, <i>n</i>	16 (31.4%)	17 (34.0%)	115 (47.3%)	108 (45.0%)
IHC 3+/FISH-negative, <i>n</i>	1 (2.0%)	0 (0.0%)	8 (3.3%)	6 (2.5%)
IHC unknown/FISH-positive, <i>n</i>	0 (0.0%)	0 (0.0%)	5 (2.1%)	2 (0.8%)
IHC 3+/FISH unknown, <i>n</i>	3 (5.9%)	4 (8.0%)	5 (2.1%)	4 (1.7%)
Region of origin				
Japanese, <i>n</i>	51 (100%)	50 (100%)	0 (0.0%)	0 (0.0%)
Non-Japanese, <i>n</i>	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

<sup>a</sup> 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

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

	Trastuzumab plus XP ( <i>n</i> = 51)	XP ( <i>n</i> = 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

**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

	Hazard ratio (95% CI)		<i>P</i> 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

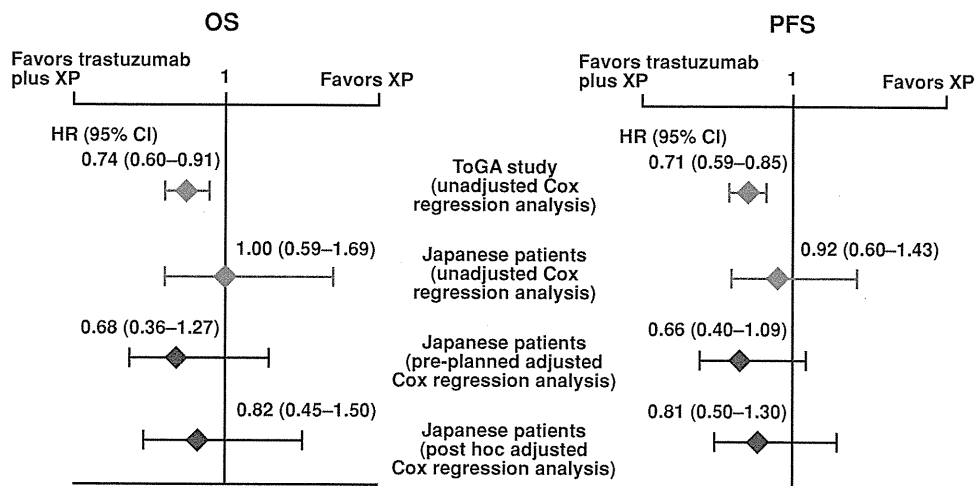
(95% CI 0.40–1.24) and 0.82 (95% CI 0.47–1.42), 0.68 (95% CI 0.39–1.22) and 0.76 (95% CI 0.43–1.34), and 0.67 (95% CI 0.37–1.22) and 0.78 (95% CI 0.44–1.36), respectively. Influential covariates chosen in the well-fitting models included sex, prior gastrectomy, and number of lesions. Table 5 shows the 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.

## 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

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

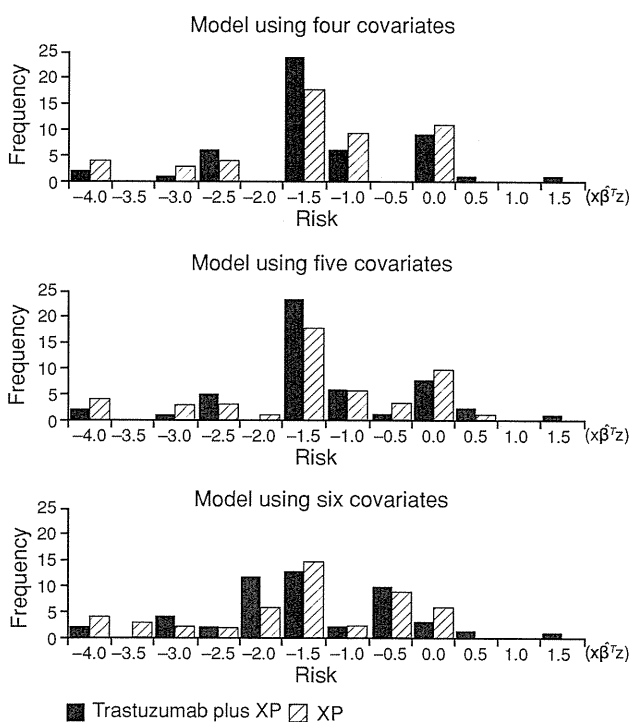
**Fig. 1** Unadjusted and adjusted hazard ratios for overall and progression-free survival. *CI* confidence interval, *HR* hazard ratio, *OS* overall survival, *PFS* progression-free survival, *XP* capecitabine plus cisplatin



**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  $\geq 10\%$  of Japanese patients in ToGA

	Trastuzumab plus XP ( <i>n</i> = 51)		XP ( <i>n</i> = 50)	
	All grade <i>n</i> (%)	Grade $\geq 3$ <i>n</i> (%)	All grade <i>n</i> (%)	Grade $\geq 3$ <i>n</i> (%)
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 erythrodysesthesia 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.

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# Risk Factors for Treatment-Related Death Associated with Chemotherapy and Thoracic Radiotherapy for Lung Cancer

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**Introduction:** The aim of the study is to evaluate the current status of treatment-related death (TRD) in lung cancer patients.

**Methods:** We retrospectively analyzed the incidence and risk factors of TRD in lung cancer patients who received chemotherapy and/or thoracic radiotherapy using logistic regression analyses.

**Results:** Between January 2001 and December 2005, 1225 (222 small cell and 1003 non-small cell lung cancers) patients received chemotherapy and/or thoracic radiotherapy as the initial treatment. Of these, 43 patients receiving chemotherapy followed by thoracic radiotherapy were included into both the chemotherapy-alone and radiotherapy-alone groups. There were a total of 23 (1.9%) TRDs. Chemotherapy-related deaths occurred in 7 of 927 (0.8%) patients, including 4 from drug-induced lung injury, 2 from pneumonia, and 1 from unknown cause. Concurrent chemoradiotherapy-related deaths occurred in 12 of 245 (4.9%) patients, including 11 from radiation pneumonitis and 1 from pneumonia. Thoracic radiotherapy-related deaths occurred in 4 of 96 (4.2%) patients. The incidence of chemotherapy-related death was correlated with poor performance status (odds ratio [OR]: 11.4, 95% confidence interval [CI]: 3.53–37.1), the presence of hypoxia (OR: 19.3, CI: 6.06–61.7), hyponatremia (OR: 45.5, CI: 13.4–154), and treatment with epidermal growth factor receptor-tyrosine kinase inhibitors (OR: 8.56, CI: 2.48–29.5), whereas the incidence of concurrent chemoradiotherapy-related death was correlated with pulmonary fibrosis (OR: 22.2, CI: 5.61–87.8). Radiotherapy results were not analyzed because there were too few patients.

**Conclusions:** TRD occurred in 1.9% of the patients as a result of treatment-related lung injury in the majority of the cases.

**Key Words:** Lung cancer, Treatment-related death, Risk factor, Chemotherapy, Thoracic radiotherapy.

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Before any medical interventions are undertaken in patients with lung cancer, they must be clearly informed about the risks and benefits of the intervention(s) and about alternative treatment options. Careful delivery of this is particularly important if the planned treatment may not only result in cure but may also be harmful. Provision of accurate information to help patients make the most appropriate decision is therefore crucial. However, the risks of death from drug toxicity and the incidences of such events tend to be uncertain<sup>1–4</sup> and also constantly change with the wide use of newer agents, such as third-generation chemotherapy agents, and molecular-targeted agents. In addition, the incidence of treatment-related deaths (TRDs) has not been thoroughly examined in clinical settings outside of clinical trials. Prospective clinical trials for poor-risk patients are often difficult to perform because of poor accrual, reflecting the reluctance of physicians to subject patients with underlying comorbid illness to the toxic effects of chemotherapy and radiation.

Our ultimate goal is to prospectively identify individuals who are at a high risk of TRD so as to provide the most precise estimation of the possible risks to each patient. In this study, we retrospectively examined the data of patients with locally advanced or metastatic lung cancer who were treated at the National Cancer Center Hospital, Tokyo, Japan, focusing on the risks and incidences of TRD associated with chemotherapy and radiotherapy.

## PATIENTS AND METHODS

### Patients

Between January 2001 and December 2005, a total of 1623 lung cancer patients were admitted to the thoracic oncology ward at the National Cancer Center Hospital. All patients were admitted in this period to be treated as part of standard practice in Japan. Patients who received chemotherapy alone usually stayed in the hospital for 7 to 10 days for one cycle of chemotherapy, and those who received concurrent chemoradiotherapy stayed for 6 weeks. Among these, a total of 1225 patients who had received first-line chemotherapy and/or radiotherapy on an inpatient basis were extracted from the institutional database. Additional details about the patients, including the diagnostic imaging findings, were then reviewed from the patients' medical records. The data of patients receiving chemotherapy and/or thoracic radiotherapy

as the initial treatment were evaluated. They included patients with stage III to IV disease and postoperative recurrent disease who received chemotherapy; those with stage III disease who received chemoradiotherapy or radiotherapy alone; and those with stage III disease who received preoperative induction therapy or postoperative adjuvant therapy. All the patients had been followed for at least 4 weeks after the completion of treatment.

### Treatment Selection

After a thorough evaluation of the operability and/or curability, the eligibility of each patient for enrollment in an open clinical trial was determined. Although patient recruitment for protocol treatments is a priority of ours, patients were free to refuse treatment. If no appropriate clinical trials were scheduled or under way, the known best standard treatments were administered.

### Best Standard Treatments

For first-line treatment, patients with non-small cell lung cancer (NSCLC) who were deemed inoperable but curable with good local control with chemoradiotherapy received three to four cycles of cisplatin (CDDP) 80 mg/m<sup>2</sup> on day 1 + vinorelbine (VNR) 20 mg/m<sup>2</sup> on days 1 and 8, every 4 weeks, along with early concurrent thoracic radiotherapy, usually at a total dose of 60 Gy/30 fractions.<sup>5</sup> Sequential chemoradiotherapy, rather than concurrent chemoradiotherapy, was offered if the calculated percentage of the total lung volume receiving radiation in excess of 20 Gy (V<sub>20</sub>) was more than 40%.<sup>6</sup> Thoracic radiotherapy alone was selected if chemotherapy could not be given due to comorbidity. If the radiation field involved the contralateral hilum or if the patients had malignant effusion and/or distant metastasis, platinum doublet therapy was administered; the most common combination was four cycles of carboplatin (CBDCA) area under the curve = 6 on day 1 + paclitaxel (PTX) 200 mg/m<sup>2</sup> on day 1, every 3 weeks.<sup>7</sup> For limited-disease SCLC, four cycles of a combination of CDDP 80 mg/m<sup>2</sup> on day 1 + etoposide 100 mg/m<sup>2</sup> on days 1 to 3, every 4 weeks, were administered concurrently with hyperfractionated thoracic radiotherapy at a total radiation dose of 45 Gy in fractional doses of 1.5 Gy, administered twice a day.<sup>8</sup> In patients with extensive-disease SCLC, four cycles of a combination of CDDP 60 mg/m<sup>2</sup> on day 1 and irinotecan (CPT) 60 mg/m<sup>2</sup> on days 1, 8, and 15, every 4 weeks, were usually administered.<sup>9</sup> Radiotherapy was given using megavoltage photons (6–15 MV). The routine radiation schedule without chemotherapy for locally advanced NSCLC was a total radiation dose of 60 to 66 Gy, or as high as 70 Gy, administered in fractional doses of 2.0 Gy once a day.

### Definition of TRD

Chemotherapy-related death was defined as death occurring within 4 weeks of the completion of treatment, without clear evidence of any other cause of death, or death obviously caused by treatment toxicity. Radiotherapy-related death was defined as death secondary to hypoxia or to complications of corticosteroid administration after the diagnosis of radiation pneumonitis. Steroid therapy was adminis-

tered based on the attending physician's discretion, without a standardized treatment dose or duration, for the management of radiation-induced lung injury.<sup>10</sup>

### Definition of Treatment-Induced Lung Injury

The criteria of drug-induced lung injury in this study were as follows: (1) appearance of new symptoms and radiological abnormalities in the course of chemotherapy with the onset within a few months of the start of the therapy; (2) diffuse or multifocal ground-glass opacities and intralobular interstitial thickening without segmental distribution in computed tomography (CT) scans of the chest; and (3) no evidence of underlying heart disease, infection, or lymphangitic carcinomatosis. Lung biopsy was not routinely performed in our hospital because patients were frequently too frail to undergo biopsy. The criteria of radiation-induced lung injury were (1) appearance of new symptoms and radiological abnormalities with the onset within 6 months of the end of thoracic radiotherapy; (2) opacification, diffuse haziness, infiltrates, or consolidation conforming to the outline of the sharply demarcated irradiated area in CT scans; and (3) a reduction in lung volume within the irradiated area and linear, ground-glass opacities or reticular shadows beyond the irradiated area developing during clinical course. In contrast, the criteria of bacterial pneumonia were (1) clinical suspicion of pneumonia including rapidly developing fever and/or productive cough; and (2) consolidation spreading through anatomical structure of the lung in CT scans.

### Statistical Analysis

We investigated the associations between chemotherapy-related or concurrent chemoradiotherapy-related death and the potential risk factors at the time of diagnosis. The following potential risk factors were investigated: sex, age ( $\geq 70$  years versus  $< 70$  years), performance status (Eastern Cooperative Oncology Group criteria; 2–4 versus 0–1), smoking history (presence versus absence), partial pressure of oxygen (70 mmHg  $\leq$  PO<sub>2</sub> versus  $> 70$  mmHg), hemoglobin (Hgb  $< 13.7$  g/dl versus  $\geq 13.7$  g/dl), platelet (Plt  $> 367 \times 10^9/L$  versus  $\leq 367 \times 10^9/L$ ), albumin (Alb  $< 3.7$  g/dl versus  $\geq 3.7$  g/dl), sodium (Na  $< 138$  mEq/L versus  $\geq 138$  mEq/L), clinical trial (in versus out), and chemotherapy regimen (The cutoff values of hemoglobin, platelet, albumin, and sodium are the institutional normal limits [above or below]). For concurrent chemoradiotherapy-related factors, the presence of coincidental diseases such as emphysema (with versus without) or pulmonary fibrosis (with versus without) and the location of the primary tumor (lower lobe versus other lobes) were also included in the analyses. The diagnostic criteria of pulmonary fibrosis were a linear, ground-glass attenuation or reticular shadows on chest radiographs and CT scans before treatment that were predominant in the lower zone of the lung. Also, the influence of the chemotherapy regimens was evaluated.

In the univariate preliminary analysis, the relation between previously defined variables at the time of presentation and the occurrence of the outcome variable (toxic death) was assessed using the  $\chi^2$  test. To adjust for each factor, multivariate logistic regression analyses were planned. When the number of observed events was less than 10, multivariate

analysis was not performed. When the number of patients for each factor was small, the factor was excluded from the model, even when it appeared to be statistically significant. All the analyses were performed using the STATISTICA 4.1J program (StatSoft, Inc., Tulsa, OK).

## RESULTS

### Patient Characteristics

The patient characteristics before treatment are listed in Table 1. Of the 1225 patients (SCLC: 222; adenocarcinoma: 652; squamous cell carcinoma: 194; NSCLC not otherwise specified: 111; large cell carcinoma: 7; others: 39), chemotherapy alone was administered in 884 patients, concurrent chemoradiotherapy in 245, sequential chemoradiotherapy in 43, and thoracic radiotherapy alone in 53 patients. To evaluate the incidence of TRD among the patients who received chemotherapy, radiotherapy, or a combination of these modalities, we included the 43 patients who received sequential chemoradiotherapy into both the chemotherapy-alone group and the thoracic radiotherapy-alone group. Therefore, the patients who received sequential chemoradiotherapy were regarded as having been exposed to the risks of treatment

twice. The groups were therefore analyzed as chemotherapy alone in 927 patients, concurrent chemotherapy in 245 patients, and thoracic radiotherapy alone in 96 patients. In these groupings, the percentages of patients enrolled in clinical trials were 62, 53, and 23%, respectively.

### Cumulative Incidence and Causes of TRD

The cumulative incidence and causes of TRD are listed in Table 2. Of the 1225 patients, a total of 23 (1.9%) TRDs occurred. Chemotherapy-related deaths occurred in 7 of 927 (0.8%) patients, including 4 (0.4%) from drug-induced lung injury (gefitinib,  $n = 3$  and CBDCA + gemcitabine,  $n = 1$ ), 2 (0.2%) from pneumonia (CBDCA + PTX,  $n = 2$ ), and 1 (0.1%) from unknown cause. The patient who died of unknown cause experienced hemodynamic instability (shock) of unknown etiology within 24 hours of ingestion of the first dose of gefitinib (250 mg). No TRDs from sepsis occurred in this series.

Concurrent chemoradiotherapy-related deaths occurred in 12 of 245 (4.9%) patients, including 11 (4.5%) from radiation pneumonitis and 1 (0.4%) from pneumonia during the last planned cycle of CDDP + VNR. Radiotherapy-

TABLE 1. Patient Characteristics

Characteristics	Chemotherapy Alone <sup>a</sup> ( $n = 927$ )	Concurrent Chemoradiotherapy ( $n = 245$ )	Radiotherapy Alone <sup>a</sup> ( $n = 96$ )
Sex			
Male	639	201	43
Female	288	44	53
Age			
Median (range)	64 (27–86)	59 (18–77)	67 (35–81)
Performance status			
0–1	871	245	88
2	140	0	8
3–4	16	0	0
Stage			
III	297	235	71
IV	454	2	17
Postoperative recurrence	176	8	8
Histology			
Non-small cell carcinoma	760	191	88
Small cell carcinoma	167	54	8
Coincidental lung disease			
Pulmonary fibrosis	34	1	4
Pulmonary emphysema	69	30	1
Chemotherapy regimen			
Platinum + taxane	368	21	—
Platinum + irinotecan	133	1	—
EGFR-TKI	125	0	—
Platinum + etoposide	95	54	—
Platinum + antimetabolite	85	0	—
Platinum + vinca alkaloid	37	168	—
Others	84	1	—

<sup>a</sup> Forty-three patients who received sequential chemotherapy followed by radiotherapy are included in the analysis of both the chemotherapy-alone group and radiotherapy-alone group, as described in the text.

EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

**TABLE 2.** Treatment-Related Death and Its Cumulative Incidence

Characteristics	Chemotherapy Alone <sup>a</sup> (n = 927)	Concurrent Chemoradiotherapy (n = 245)	Radiotherapy Alone <sup>a</sup> (n = 96)
No. of treatment-related deaths	7	12	4
Cumulative incidence (%)	0.8	4.9	4.2
Sex			
Male	5	11	4
Female	2	1	0
Age of patients who died of treatment (yr)			
Median (range)	69 (46–77)	68 (50–77)	75 (65–77)
Causes			
Treatment-induced lung injury	4	11	4
Infectious pneumonia	2	1	0
Unknown	1	0	0
Chemotherapy regimen			
Platinum + taxane	2	2	—
EGFR-TKI	4	—	—
Platinum + antimetabolite	1	—	—
Platinum + etoposide	0	1	—
Platinum + vinca alkaloid	0	8	—
Others	0	1	—

<sup>a</sup> Forty-three patients who received sequential chemotherapy followed by radiotherapy are included in the analysis of both the chemotherapy-alone group and radiotherapy-alone group, as described in the text.

EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

related deaths occurred in 4 of 96 (4.2%) patients: all 4 (4.2%) patients died of radiation pneumonitis.

### Risk Factors for TRD from Chemotherapy

Statistically significant factors identified by the univariate analysis were a performance status of 2 to 4, hypoxia, hypoalbuminemia, hyponatremia, out of clinical trials, and treatment with epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) (Table 3). Although statistically significant, the degrees of hyponatremia in the events were neither clinically significant nor symptomatic for the range of 133 to 137 mEq/L. Pulmonary fibrosis and emphysema were noted in 34 and 69 patients, respectively, among the 927 patients. None of these patients with lung disease died of treatment in this study. Multivariate analysis was not performed because the number of observed events was too small ( $n = 7$ ).

### Risk Factors for TRD from Concurrent Chemoradiotherapy

None of the factors, except for pulmonary fibrosis, were found to be statistically significant in the univariate analysis, although a trend toward increase in the risk of TRD was observed in patients of advanced age (>70 years) and with lower lobe as the primary tumor site (Table 4). Pulmonary fibrosis appeared to be a statistically significant risk factor for TRD; however, it was excluded from the multivariate analysis because of its limited incidence. Thus, we did not perform multivariate analysis for chemoradiotherapy group, and an analysis of the risk of TRD associated with thoracic radiotherapy alone was not conducted because of the limited number of cases.

### DISCUSSION

We identified a total of 23 TRDs out of the 1225 patients (1.9%) enrolled in this study, which is lower than the rate (2.7%) indicated in a previous report, particularly in relation to the number of TRDs from infections, including pneumonia and sepsis.<sup>1</sup> The reason for the decrease in the incidence of infection-related deaths is likely explained by the infrequent use of triplet regimens when compared with previous studies. Especially, mitomycin-C-containing regimens are regarded as effective regimens in the treatment of lung cancer; however, prolonged neutropenia has been observed with these regimens. Ohe et al.<sup>1</sup> reported that combined mitomycin-C + vindesine + CDDP (MVP regimen) therapy is a risk factor for chemotherapy-related TRD (toxic deaths occurred in 9 of 301 patients; odds ratio [OR] = 9.36, 95% confidence interval [CI] = 1.29–68.0,  $p = 0.027$ ). In this study, only 35 patients, the majority (89%) of whom were enrolled in a clinical trial, received the MVP regimen. In the past, however, the MVP regimen was widely used as part of practice-based regimens (only 28% recorded under clinical trials). In most cases, patients who were not eligible for clinical trials ended up receiving the MVP regimen. Another reason is the relatively frequent use of EGFR-TKI (in 13.5% of the patients in this study) at present, which does not induce myelosuppression. The reduction in the frequency of TRD might also be explained by a progress in supportive care in the treatments given for cancer treatment toxicities.

This study revealed that drug-induced lung injury was the most frequent cause of TRD in the era of molecular-targeted therapy. Three (75%) of four TRDs from drug-induced lung injury were associated with gefitinib. The re-

**TABLE 3.** Risk Factors for Treatment-Related Death from Chemotherapy

Factors	No. of Patients	Cumulative Incidence (%)	Univariate Analysis	
			OR (95% CI)	p
Sex				
Female	288	0.8	1	
Male	639	0.7	1.13 (0.22–5.76)	0.89
Age				
<70	689	0.6	1	
≥70	238	1.3	2.17 (0.51–9.30)	0.30
PS				
0–1	870	0.5	1	
2–4	57	5.2	11.4 (3.53–37.1)	<0.001
Smoking history				
No	271	0.4	1	
Yes	656	0.9	2.49 (0.30–20.8)	0.40
PaO <sub>2</sub> (Torr)				
≥70	812	0.2	1	
<70	105	4.8	19.3 (6.06–61.7)	<0.001
Hemoglobin (g/dl)				
≥13.7	371	0.5	1	
<13.7	556	0.9	1.67 (0.33–8.39)	0.54
Albumin (g/dl)				
≥3.7	663	0.3	1	
<3.7	264	1.9	6.28 (1.51–26.1)	0.012
AST (IU/L)				
≤33	831	0.6	1	
>33	96	2.1	3.46 (0.75–16.0)	0.11
Na (mEq/L)				
≥138	819	0.1	1	
<138	108	5.6	45.5 (13.4–154)	<0.001
Clinical trial				
No	355	1.7	1	
Yes	572	0.2	0.10 (0.58–0.019)	0.001
Platinum + taxane				
No	559	0.9	1	
Yes	368	0.5	0.61 (0.12–3.14)	0.55
EGFR-TKIs				
No	802	0.4	1	
Yes	125	3.2	8.56 (2.48–29.5)	0.001
Platinum + antimetabolite				
No	842	0.7	1	
Yes	85	1.1	1.66 (0.20–13.9)	0.64

Multivariate analysis was not performed because the number of observed events was too small (n = 7).

OR, odds ratio; CI, confidence interval; PS, performance status; AST, aspartate transaminase; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors.

ported risk factors for interstitial lung disease in NSCLC patients treated with gefitinib are male sex, history of smoking, and underlying interstitial pneumonitis.<sup>11</sup> In this study, however, none of these factors were associated with TRD from chemotherapy. Another TRD from drug-induced lung injury occurred in a patient who received gemcitabine, but this patient was also free from underlying pulmonary disease

**TABLE 4.** Risk Factors for Treatment-Related Death from Concurrent Chemoradiotherapy

Factors	No. of Patients	Cumulative Incidence (%)	Univariate Analysis	
			OR (95% CI)	p
Sex				
Female	44	2.3	1	
Male	201	5.2	2.41 (0.35–16.6)	0.37
Age (yr)				
<70	221	4.1	1	
≥70	24	12.5	3.07 (0.92–10.3)	0.069
PS				
0	114	5.3	1	
1	131	4.6	0.87 (0.29–2.62)	0.81
Smoking history				
No	32	3.2	1	
Yes	213	5.2	1.65 (0.23–11.9)	0.24
Fibrosis				
No	244	4.5	1	
Yes	1	100	22.2 (5.61–87.8)	<0.001
Emphysema				
No	215	4.7	1	
Yes	30	6.7	1.43 (0.33–6.25)	0.63
Location of the tumor				
Other lobes	189	3.7	1	
Lower lobe	56	8.9	2.41 (0.82–7.13)	0.11
Histology				
SCLC	54	1.9	1	
NSCLC	191	5.8	3.11 (0.47–20.6)	0.24
Hemoglobin (g/dl)				
≥13.7	146	4.1	1	
<13.7	99	6.1	1.48 (0.49–4.42)	0.48
Albumin (g/dl)				
≥3.7	198	4.5	1	
<3.7	47	6.4	1.40 (0.40–4.99)	0.6
Na (mEq/L)				
≥138	219	5.0	1	
<138	26	3.8	0.77 (0.11–5.60)	0.79
Clinical trial				
No	114	5.3	1	
Yes	131	4.6	0.87 (0.29–2.62)	0.81
Platinum + taxane				
No	224	4.5	1	
Yes	21	9.5	2.25 (0.46–11.0)	0.32
Platinum + vinca alkaloid				
No	77	5.2	1	
Yes	168	4.8	0.91 (0.27–3.13)	0.88

Multivariate analysis was not performed because only fibrosis was significant in univariate analysis.

OR, odds ratio; CI, confidence interval; PS, performance status; NSCLC, non-small cell lung cancer.

or concomitant use of taxanes, which are reported to be risk factors for gemcitabine-associated interstitial lung disease.<sup>12</sup>

For patients who receive concurrent chemoradiotherapy, we would like to emphasize the previous finding that the

presence of evidence of pulmonary fibrosis on a plain chest x-ray is an extremely strong risk factor for TRD (OR = 166, 95% CI = 8.79–3122,  $p < 0.001$ ).<sup>1</sup> In this study, only one patient with pulmonary fibrosis was identified, and pulmonary fibrosis was not included in the multivariate analysis because of the small number of patients with this factor, because we generally exclude patients with evidence of pulmonary fibrosis on the chest x-ray from consideration of concurrent chemoradiotherapy. This study also suggested that advanced age may be a risk factor for TRD. This is consistent with the results of previous studies.<sup>1,13–15</sup> The association between advanced age and fatal radiation-induced lung injury may be explained by the increased likelihood of these patients developing comorbid lung disease, particularly among patients with a history of heavy tobacco exposure. A meta-analysis of chemoradiotherapy using individual data from 1764 patients with locally advanced NSCLC showed that the benefit of chemoradiotherapy was obtained in elderly patients ( $\geq 71$  years) as well as in younger patients. However, it might be assumed that patients who are included in such trials are fit patients with minimal comorbidities. In addition, despite the increase in toxicity that accompanied chemoradiotherapy in elderly patients, it seemed that they had disease control and survival rates similar to those of younger patients.<sup>16</sup>

In conclusion, TRD occurred in a total of 1.9% of patients and was caused in the majority of the cases by treatment-related lung injury. This finding is in clear contrast with previous reports which suggested that the principal cause of TRD in lung cancer patients was septic shock.

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# Hepatocyte Growth Factor Expression in *EGFR* Mutant Lung Cancer with Intrinsic and Acquired Resistance to Tyrosine Kinase Inhibitors in a Japanese Cohort

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**Introduction:** This study was performed to determine the incidence rates of resistance factors, i.e., high-level hepatocyte growth factor (HGF) expression, epidermal growth factor receptor (EGFR) T790M secondary mutation, and *MET* amplification, in tumors with intrinsic and acquired EGFR tyrosine kinase inhibitor (TKI) resistance in *EGFR* mutant lung cancer.

**Methods:** Ninety-seven specimens from 93 *EGFR* mutant lung cancer patients (23 tumors with acquired resistance from 20 patients, 45 tumors with intrinsic resistance from 44 patients [nonresponders], 29 sensitive tumors from 29 patients) from 11 institutes in Japan were analyzed. HGF expression, *EGFR* T790M secondary mutation,

and *MET* amplification were determined by immunohistochemistry, cycleave real-time polymerase chain reaction, and fluorescence in situ hybridization, respectively.

**Results:** High-level HGF expression, *EGFR* T790M secondary mutation, and *MET* amplification were detected in 61, 52, and 9% of tumors with acquired resistance, respectively. High-level HGF expression was detected in 29% of tumors with intrinsic resistance (nonresponders), whereas *EGFR* T790M secondary mutation and *MET* amplification were detected in 0 and 4%, respectively. HGF expression was significantly higher in tumors with acquired resistance than in sensitive tumors ( $p < 0.001$ , Student's *t* test). Fifty percent of tumors with acquired resistance showed simultaneous HGF expression with *EGFR* T790M secondary mutation and *MET* amplification.

**Conclusions:** High-level HGF expression was detected more frequently than *EGFR* T790M secondary mutation or *MET* amplification in tumors with intrinsic and acquired EGFR-TKI resistance in *EGFR* mutant lung cancer in Japanese patients. These observations provide a rationale for targeting HGF in EGFR-TKI resistance in *EGFR* mutant lung cancer.

**Key Words:** EGFR-TKI, EGFR mutation, HGF, Acquired resistance, Intrinsic resistance.

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Epidermal growth factor receptor (EGFR)-activating mutations, in-frame deletion in exon 19, and L858 point mutation in exon 21 are selectively expressed in a population with lung cancer.<sup>1,2</sup> *EGFR*-activating mutations are detected considerably more frequently in nonsmokers, females, adenocarcinomas, and patients from East Asia, including Japan.<sup>3–5</sup> The reversible EGFR tyrosine kinase inhibitors (EGFR-TKIs) gefitinib and erlotinib show dramatic therapeutic efficacy, response rates of 70 to 80%, and significant prolongation of progression-free survival (PFS) compared



with standard first-line cytotoxic chemotherapy in patients with *EGFR* mutant lung cancer.<sup>6–9</sup> However, patients almost always develop acquired resistance to EGFR-TKIs after varying periods.<sup>6,9,10</sup> In addition, 20 to 30% of patients with *EGFR*-activating mutations show intrinsic resistance to EGFR-TKIs.<sup>4</sup> Therefore, intrinsic and acquired resistance to EGFR-TKIs are major problems in management of *EGFR* mutant lung cancer.

Two genetically conferred mechanisms—*EGFR* T790M secondary mutation (T790M secondary mutation)<sup>11,12</sup> and *MET* gene amplification<sup>13</sup>—induce acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer. In addition, we recently demonstrated the occurrence of hepatocyte growth factor (HGF)-induced resistance.<sup>14</sup> HGF, a ligand of *MET*,<sup>15</sup> induces EGFR-TKI resistance by activating *MET*, which restores phosphorylation of downstream MAPK-ERK1/2 and PI3K-Akt pathways,<sup>14</sup> using Gab1 as an adaptor.<sup>16</sup> HGF may be involved in both intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer.<sup>14</sup>

T790M secondary mutation, *MET* amplification, and high-level HGF expression were detected in clinical specimens from *EGFR* mutant lung cancer patients who acquired resistance to EGFR-TKIs,<sup>11–14,16–18</sup> indicating the clinical relevance of all three resistance mechanisms in lung cancer. Although the number of cases in each study was limited (<30 cases/study), probably because of low availability of biopsy specimens from resistant tumors, *EGFR* T790M secondary mutation and *MET* amplification were estimated to have occurrence rates of 50%<sup>11,12,17,19</sup> and up to 20%,<sup>13,16,17</sup> respectively, in patients showing acquired resistance to EGFR-TKIs. Nevertheless, the incidence of HGF-induced resistance has not been determined. In addition, the incidence rates of these three resistance factors in intrinsic resistance (nonresponders) are unknown.

Here, we performed a large-scale study in 23 tumors with acquired resistance from 20 patients, 45 tumors with intrinsic resistance from 44 patients (nonresponders), and 29 sensitive tumors from 29 patients to determine the incidences of the three resistance factors not only in acquired resistance but also in intrinsic resistance (nonresponders) to EGFR-TKIs in Japanese patients with *EGFR* mutant lung cancer.

## MATERIALS AND METHODS

Patient details are described in the Supplementary information (<http://links.lww.com/JTO/A197>).

### Definition of Sensitivity to EGFR TKI

Here, tumors with *EGFR* mutation known to be associated with drug sensitivity (i.e., G719X, exon 19 deletion, and L858R) were obtained from patients before or after treatment with a single EGFR-TKI.<sup>9</sup>

Sensitive tumors were defined as those obtained from patients whose tumors showed a decrease in diameter of at least 30% (either documented partial response or complete response) associated with EGFR-TKI treatment in imaging studies (Response Evaluation Criteria in Solid Tumors [RECIST] version 1.0). Tumor specimens were obtained before EGFR-TKI treatment.

Tumors with acquired resistance were defined as described previously.<sup>9</sup> Briefly, cases showing objective clinical benefit from treatment with an EGFR TKI as defined by either documented partial or complete response (RECIST) or significant and durable (>6 months) clinical benefit (stable disease as defined by RECIST) and systemic progression of disease (RECIST), while on continuous treatment with gefitinib or erlotinib within the last 30 days were defined as showing acquired resistance. Tumor specimens were obtained after systemic progression of disease.

As intrinsic resistance (nonresponders) has not been clearly defined, tumors without response to treatment with an EGFR TKI, i.e., either documented stable disease or progressive disease (RECIST), were defined as showing intrinsic resistance (nonresponders). Tumor specimens were obtained either before or after EGFR-TKI treatment.

## Patients

Ninety-seven tumor specimens with *EGFR* mutations were obtained from 93 lung cancer patients, all of whom provided written informed consent, at 11 institutes in Japan. This study was approved by the Institutional Review Boards of each institute.

Patients' characteristics are shown in Table 1. Eighty-seven patients had adenocarcinomas, one had large cell carcinoma, two had squamous cell carcinoma, two had adenosquamous carcinoma, and one had undifferentiated non-small cell carcinoma. As the first EGFR-TKI, gefitinib and erlotinib were given to 82 and 10 patients, respectively, and the dual inhibitor of EGFR and VEGFR2, vandetanib,<sup>20</sup> was given to 1 patient.

Exon 19 deletion and L858R point mutation in exon 21 of *EGFR* were detected in 40 and 57 of the 97 tumors, respectively (Table 1). Two of these tumors had both exon 19 deletion and L858R point mutation. Two tumors without exon 19 deletion or L858R had G719X. Twenty-three tumors with acquired resistance were obtained from 20 patients after EGFR-TKI treatment. Forty-five tumors with intrinsic resistance (nonresponders) were obtained from 44 patients either before (41 tumors from 41 patients) or after (four tumors from three patients) EGFR-TKI treatment. Twenty-nine sensitive tumors were obtained from 29 patients before EGFR-TKI treatment.

## Immunohistochemistry for HGF

Immunohistochemical staining was conducted on formalin-fixed, paraffin-embedded tissue sections (4  $\mu$ m thick) of tumor specimens with microwave antigen retrieval in 0.01 M citrate buffer (pH 6.0). We used rabbit polyclonal antibody against HGF- $\alpha$  (IBL, Gunma, Japan) at 1:20 dilution as a primary antibody and EnVision/HRP Polymer Reagent (Dako, Glostrup, Denmark) and DAB (3,3'-diaminobenzidine tetrahydrochloride) Liquid (Dako) for detection.

## Evaluation of HGF Expression

The percentages of cancer cells with positive cytoplasmic and/or membrane HGF immunoreactivity were evaluated (0 to 100%), and the modal intensity of the positively staining cells on a scale ranged from 0 to 3+ (0, complete

**TABLE 1.** Patient Characteristics

Number of Patients	Acquired Resistance ( <i>n</i> = 20)	Intrinsic Resistance ( <i>n</i> = 44)	Sensitive ( <i>n</i> = 29)	Total ( <i>n</i> = 93)
<b>Age</b>				
Median	59.5	65.5	65	64
Range	32–85	34–76	42–86	32–86
<b>Gender</b>				
Male	6	26	10	42
Female	14	18	19	51
<b>Smoking history</b>				
Former/current Smoker	3	21	11	35
Never smoker	17	23	18	58
<b>Histological type</b>				
Adeno	19	39	29	87
Large cell	0	1	0	1
Squamous cell	0	2	0	2
Undifferentiated non-small cell carcinoma, or adenosquamous	1	2	0	3
<b>EGFR-TKI treatment</b>				
Gefitinib	19	36	27	82
Erlotinib	1	7	2	10
Vandetanib	0	1	0	1
<b>Number of Tumors</b>	<b><i>n</i> = 23</b>	<b><i>n</i> = 45</b>	<b><i>n</i> = 29</b>	<b><i>n</i> = 97</b>
<b>EGFR mutation status</b>				
Exon 19 deletion	12	14 <sup>a</sup>	14 <sup>a</sup>	40
L858R	11	30	16	57
G719X	0	2	0	2

<sup>a</sup> One patient's tumor had both exon 19 deletion and L858R point mutation.

absence of staining; 1+, weaker staining than normal bronchial epithelium; 2+, similar staining to normal bronchial epithelium; and 3+, clearly more intense staining than normal bronchial epithelium) (Supplementary Figure 1, <http://links.lww.com/JTO/A197>). The percentage and intensity were multiplied to give a scoring index (*H* score) ranging from 0 to 300, according to a previously reported method with minor modifications.<sup>16</sup> Turke et al.<sup>16</sup> reported that HGF expression was significantly higher in specimens with acquired resistance (mean  $\pm$  SD: 205  $\pm$  106) compared with pretreatment (126  $\pm$  112). On additional evaluation with specimens showing acquired resistance from patients whose tumors were obtained only after acquiring EGFR-TKI resistance, HGF expression was similar (176  $\pm$  126) to that of specimens with acquired resistance in patients with paired tumor specimens; they concluded that these findings with clinical specimens supported the suggestion that HGF mediated resistance to EGFR-TKIs. Therefore, we defined high-level HGF expression as *H* score  $\geq$  200 in this study. Evaluation was performed independently by two investigators (KT and MN) blinded to individual clinical information.

#### Cycleave Real-Time Polymerase Chain Reaction Assay for T790M Mutation

Details of the cycleave real-time polymerase chain reaction (PCR) assay have been described previously.<sup>21</sup>

Briefly, tumor cell-rich areas in hematoxylin and eosin-stained sections were marked under a microscope, and tissues were scratched from the area of another deparaffinized unstained section. Pieces of the scratched tissue were incubated with 1  $\times$  PCR buffer containing 100  $\mu$ g/mL proteinase K for 1 hour at 54°C. After heat inactivation at 95°C for 3 minutes, the solution was used directly as the template DNA for the assay. Then, exon 20 of the *EGFR* gene was amplified by real-time quantitative PCR assay on a SmartCycler (Cepheid, Sunnyvale, CA) using Cycleave PCR Core kits (TaKaRa Co. Ltd., Ohtsu, Japan) with a T790M-specific cycling probe and a wild-type cycling probe. This assay detected as few as 5% cancer cells with T790M mutation in a background of cells with wild-type T790M in *EGFR*.

#### MET Amplification

Formalin-fixed, paraffin-embedded tissue sections (4  $\mu$ m thick) were subjected to dual-color fluorescence in situ hybridization using a MET/CEP7 probe cocktail (Kreatech Diagnostics, Amsterdam, The Netherlands) according to the manufacturer's instructions. Staining was evaluated as reported previously.<sup>22,23</sup>

#### Statistical Analysis

Statistical significance was determined by Student's *t* test. All statistical analyses were performed using GraphPad

**TABLE 2.** Expression of HGF, T790M Secondary Mutation, and *MET* Amplification in EGFR-TKI-Resistant Tumors Obtained from *EGFR* Mutant Lung Cancer Patients

	Acquired Resistance (n = 23)	Intrinsic Resistance (n = 45)	Sensitive (n = 29)
High-level HGF expression	14 (61%)	13 <sup>a</sup> (29%)	3 <sup>b</sup> (10%)
<i>EGFR</i> T790M secondary mutation	12 (52%)	0	0
<i>MET</i> amplification	2 (9%)	2 (4%)	0

<sup>a</sup> High-level HGF expression was detected in the stroma in two patients.  
<sup>b</sup> High-level HGF expression was detected in the stroma in one patient.

Prism Ver. 4.01 (GraphPad Software, Inc., San Diego, CA). All tests were two sided, and  $p < 0.05$  was taken to indicate statistical significance.

## RESULTS

### HGF Expression, T790M Secondary Mutation, and *MET* Amplification in Tumors with Acquired Resistance

Among 23 tumors with acquired resistance from 20 patients, *EGFR* T790M secondary mutation was detected in 12 tumors (52%) from 11 patients (60%) (Table 2). *MET* amplification was detected in two tumors (9%) from two patients (10%). As HGF is a soluble cytokine, evaluation of HGF is not as simple as that for genetically conferred T790M secondary mutation and *MET* amplification, which can be designated as plus or minus. As described in the Materials and Methods section, we defined high-level HGF expression as *H* score  $\geq 200$  in this study. High-level HGF expression was detected in 14 tumors (61%) from 13 patients (60%). In these 14 tumors, HGF was predominantly expressed in cancer cells.

The high HGF expression was simultaneously detected in 6 of 12 tumors positive for T790M secondary mutation (50%) (Table 3, Figure 1). High-level HGF expression was also detected simultaneously in one of two tumors positive for *MET* amplification (50%). These results suggested possible interactions among these three resistance factors, consistent with previous reports.<sup>16,17</sup>

### Expression of HGF, T790M Secondary Mutation, and *MET* Amplification in Tumors with Intrinsic Resistance (Nonresponders)

T790M secondary mutation was not detected in 45 tumors with intrinsic resistance from 44 patients (nonresponders), but *MET* amplification was detected in two tumors (4%) (Table 2). *EGFR* D761Y secondary mutation was detected in two tumors (4%) from one patient<sup>24</sup> (Supplementary Table 1, <http://links.lww.com/JTO/A197>). In contrast, high-level HGF expression in cancer cells was detected in 11 tumors (24%) from 11 patients. In addition, HGF was detected at high levels in stromal cells in two tumors (4%) from two patients (data not shown). In total, high-level HGF expression was detected in 13 tumors with intrinsic resistance

(29%). Notably, high-level HGF expression was simultaneously detected in one of two *MET* amplification-positive tumors (50%) (Table 2). These results suggested the involvement of HGF in intrinsic resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients.

### Expression of HGF, T790M Secondary Mutation, and *MET* Amplification in Sensitive Tumors

Neither *EGFR* T790M secondary mutation nor *MET* amplification was detected in 29 sensitive tumors from 29 patients. High-level HGF expression was detected in two tumors (7%) (Supplementary Table 2, <http://links.lww.com/JTO/A197>). High levels of HGF were detected in stromal cells in one tumor (3%). In total, a high level of HGF expression was detected in three sensitive tumors (10%). Thus, although high HGF expression level was detected even in sensitive tumors, the incidence of high HGF expression was much lower in sensitive tumors than in those with acquired or intrinsic resistance. In addition, mean *H* score of HGF in tumors with acquired resistance was significantly higher than that in sensitive tumors ( $p < 0.001$ , Student's *t* test) (Figure 2). There was no significant difference in mean *H* score of HGF between tumors with intrinsic resistance (nonresponders) and sensitive tumors.

## DISCUSSION

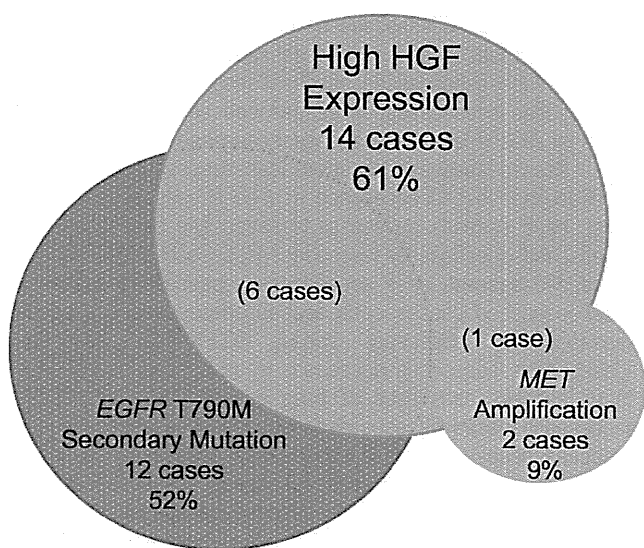
Our previous studies<sup>14,25,26</sup> documented HGF-mediated resistance to EGFR-TKIs in *EGFR* mutant lung cancer, which was also confirmed by other groups.<sup>16,27</sup> Here, we demonstrated that a high level of HGF expression was detected most frequently in tumors with intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients. Our data indicated that although T790M secondary mutation and *MET* amplification are predominantly responsible for acquired resistance, HGF may be responsible not only for acquired resistance but also for intrinsic resistance to EGFR-TKIs.

The mechanism of intrinsic resistance to EGFR-TKIs is not well understood. To our knowledge, this is the first study with more than 40 clinical specimens indicating the incidence of resistance factors in intrinsic resistance to EGFR-TKIs in *EGFR* mutant lung cancer. Here, we found that a high level of HGF expression was most frequently (29%) detected in tumors with intrinsic resistance, compared with T790M secondary mutation (0%) and *MET* amplification (4%). It is noteworthy that although the high HGF expression level was detected in cancer cells in tumors with acquired resistance, HGF expression was detected in both cancer cells (10/12 tumors) and host stroma cells (2/12 tumors) in tumors with intrinsic resistance (nonresponders). HGF was reported to be produced by not only cancer cells but also stromal cells.<sup>15</sup> Our data clearly indicated that both cancer cells and stromal cells are sources of HGF, which induces intrinsic EGFR-TKI resistance in *EGFR* mutant lung cancer. As HGF-induced resistance could be reversed by anti-HGF antibody and the natural HGF inhibitor NK4,<sup>25,27</sup> highly produced HGF in

**TABLE 3.** Summary of Tumors with Acquired Resistance

ID	Gender	Histological Type	EGFR Mutation Status	Treatment	BOR	PFS	HGF	T790M	MET Amplification
KZ-1	M	Ad	Exon 19 del	Erlotinib	PR	254	60	—	+
KZ-2	F	Ad	L858R	Gefitinib	CR	1041	40	—	—
KZ-3	F	Ad	L858R	Gefitinib	PR	366	200	—	—
OK1—1	M	Ad	Exon 19 del	Gefitinib	PR	351	290	—	—
OK1—2							300	—	—
OK4—2	F	Ad	Exon 19 del	Gefitinib	PR	57	210	+	—
TS-1—3	F	Ad	L858R	Gefitinib	PR	180	90	—	—
TS-1—4							280	+	—
SG2	M	Ad	Exon 19 del	Gefitinib	PR	174	150	+	—
SG3	F	Ad	L858R	Gefitinib	SD	368	110	+	—
SG4	F	Ad	L858R	Gefitinib	PR	60	220	—	+
SG6	M	Ad	Exon 19 del	Gefitinib	PR	352	140	+	—
SG8	F	Ad	L858R	Gefitinib	SD	210	90	+	—
SG9	F	Ad	Exon 19 del	Gefitinib	SD	221	200	+	—
SG10	F	Ad	L858R	Gefitinib	CR	210	210	—	—
TB1—2	M	Ad	Exon 19 del	Gefitinib	PR	1770	230	+	—
TB2—2	F	AdSq	Exon 19 del	Gefitinib	PR	300	300	—	—
AC29—1	M	Ad	L858R	Gefitinib	PR	533	250	—	—
AC29—2							270	+	—
AC24	F	Ad	Exon 19 del	Gefitinib	PR	98	170	+	—
AC26	F	Ad	Exon 19 del	Gefitinib	SD	448	180	+	—
AC28	F	Ad	Exon 19 del	Gefitinib	PR	357	200	+	—
AC31	F	Ad	L858R	Gefitinib	PR	894	200	—	—

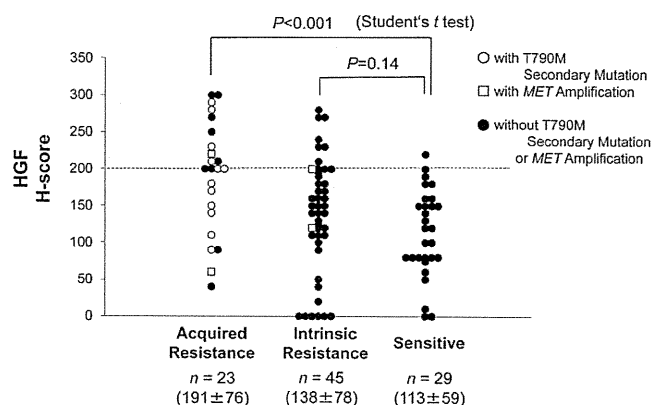
Ad, adeno; AdSq, adenosquamous; BOR, best overall response.



**FIGURE 1.** Incidences of high-level HGF expression, T790M secondary mutation, and MET amplification in 23 tumors with acquired resistance. Values in parentheses are the numbers of cases in which the tumors expressed two resistance factors simultaneously.

resistant tumors would be an ideal therapeutic target regardless of its origin.

It was of interest that a high level of HGF expression was detected in a small population of sensitive tumors. This



**FIGURE 2.** HGF expression score (H score) in EGFR-TKI-resistant tumors obtained from EGFR mutant lung cancer patients. Values in parentheses are mean ± SD of H score.

was consistent with a previous report<sup>16</sup> indicating high-level HGF expression (H score ≥200) in several specimens from responders. Although the reason for the high level of HGF expression in tumors from responders is unclear at present, there are several possible explanations as follows. First, although HGF was expressed at high levels, natural inhibitors such as cleaved HGF and truncated MET, both of which inhibit binding of HGF to MET, may be generated in the tumors.<sup>28,29</sup> Second, negative regulators of MET tyrosine kinase activity such as protein kinase C may be activated and negate the effect of HGF on induction of EGFR-TKI resis-