second-line advanced gastric carcinoma trials,[20,25,28,30] everolimus monotherapy exhibits less bone marrow suppression in this setting. These results suggest that everolimus monotherapy is suitable for use on an outpatient basis.

PK analyses in this trial suggested that although the rate of oral absorption seems to be faster in patients who had undergone gastrectomy compared with patients who did not [as evidenced by a higher C_{max} and $AUC_{(0-4)}$ and shorter T_{max}], no differences in the extent of oral absorption were observed because the mean C_{min} and steady-state AUC over the dosing interval values were similar between patients with and without gastrectomy. Further investigation is needed because the sample size of this study is limited. In conclusion, everolimus monotherapy showed a promising rate of disease control and was well tolerated in previously treated patients with advanced gastric cancer, warranting further evaluation in a phase III trial of everolimus monotherapy in this population.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Nobutsugu Ohno, Novartis (C); Yoshie Fujita, Novartis (C); Matthew Robson, Novartis (C) **Consultant or Advisory Role:** None **Stock Ownership:** Matthew Robson, Novartis Pharma **Honoraria:** None **Research Funding:** None **Expert Testimony:** None **Other Remuneration:** None

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J Cancer Res Clin Oncol (2010) 136:1059–1064
DOI 10.1007/s00432-009-0752-8

ORIGINAL PAPER

Progression-free survival in first-line chemotherapy is a prognostic factor in second-line chemotherapy in patients with advanced gastric cancer

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Received: 21 October 2009 / Accepted: 14 December 2009 / Published online: 27 December 2009

Abstract
Purpose First-line chemotherapy (Cx-1) in advanced gastric cancer (AGC) provides survival benefit. However, it is unclear who should proceed to second-line chemotherapy (Cx-2).
Methods We reviewed patients who received Cx-2 for AGC following progressive disease after Cx-1 from 2000 to 2005 at the National Cancer Center Hospital, Tokyo. To evaluate the prognostic factors in Cx-2, Cox regression multivariate analysis was performed.
Results Of 995 patients who received Cx-1 in this study period, 466 met the eligibility criteria. The median progression-free survival in Cx-1 (PFS-1) was 133 days. The median survival time from the date of starting second-line chemotherapy (MST-2) was 207 days. Multivariate analysis revealed that the factors affecting short survival time in Cx-2 were poor performance status (≥2), low serum albumin level (<3.5 mg/dL), elevated C-reactive protein level (≥1.0 mg/dL), patients with bone, liver or peritoneal metastasis, and patients without previous gastrectomy ($p < 0.01$). PFS-1 was an independent prognostic factor for survival (PFS-1 <120, MST-2 133 days, PFS-1 ≥120, MST-2 258 days, hazard ratio 0.71, 95% confidence interval 0.58–0.86, $p < 0.01$). The Cx-2 regimen (irinotecan vs. taxane) did not correlate with survival.
Conclusion PFS-1 is one of the prognostic factors of Cx-2 in patients with AGC.

Keywords Advanced gastric cancer · Second-line chemotherapy · Prognostic factor · Time to progression · Survival

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Introduction

Gastric cancer is the fourth most common malignancy with approximately 940,000 new patients in the world and ranks second in all causes of death from cancer, with about 700,000 confirmed deaths annually (Kamangar et al. 2006). In 2002, there were about 100,000 gastric cancer patients in Japan and roughly half died from the malignancy in the same year.

For the first-line chemotherapy (Cx-1) of advanced gastric cancer (AGC), some randomized controlled trials have revealed survival benefit (Glimelius et al. 1997; Pyrhonen et al. 1995). Combination chemotherapy with fluorouracil (FU) (including S-1 and capecitabine) and platinum analogs (e.g., cisplatin and oxaliplatin) is presently the most widely accepted regimen for Cx-1 (Glimelius et al. 1997; Koizumi et al. 2008; Ohtsu et al. 2003; Pyrhonen et al. 1995; Ross et al. 2002; Vanhoefer et al. 2000). Although there is still no established second-line chemotherapy (Cx-2) for AGC, some promising agents such as irinotecan, taxane, and cisplatin have been used (Boku et al. 1999; Kodera et al. 2007; Lee et al. 2007; Shirao et al. 1997; Sulkes et al. 1994).

Several potential prognostic factors for short survival time in Cx-1 have been proposed. These include performance status (PS) ≥2, presence of liver or peritoneal metastasis, and

elevated alkaline phosphatase (ALP) level ≥100 U/L (Chau et al. 2004; Louvet et al. 2003; Yamamura et al. 2002). Ichikawa and Sasaki (2006) suggested that the response to Cx-1 affects progression-free survival and overall survival. However, the prognostic factors in Cx-2 remain unclarified.

In the present study, we determined the prognostic factors of survival at the start of Cx-2. Identification of these factors may assist oncologist in selecting patients for subsequent chemotherapy following progressive disease in Cx-1.

Patients and methods

Patient population

We extracted AGC cases meeting the eligibility criteria from the database of the National Cancer Center Hospital in Tokyo. The eligibility criteria were as follows (1) histologically proven gastric adenocarcinoma and received Cx-2 after Cx-1 failure due to progressive disease (PD) between January 2000 and December 2005, (2) <75 years of age, with an Eastern Cooperative Oncology Group (ECOG) PS range of 0–2 at the start of Cx-1, (3) maintained adequate main organ function for chemotherapy when Cx-1 was started. Adequate main organ function was indicated by the following: serum creatinine level <1.5 mg/dL; serum asparate aminotransferase (AST) level <100 U/L; alanine aminotransferase (ALT) level <100 U/L; white blood cell count (WBC) $>3.0 \times 10^9/\mu L$ but $<12.0 \times 10^9/\mu L$; platelet count $>100 \times 10^9/\mu L$; total bilirubin <1.5 mg/dL; able to take oral medication; no symptoms of brain metastasis or pleural effusion.

Data collection

Chart reviews for all patients were performed to obtain laboratory data on Cx-1 and Cx-2, chemotherapy regimens, treatment duration, and reason for chemotherapy discontinuation. Data included the following: WBC, hemoglobin, platelet count, ALP, lactate dehydrogenase (LDH), AST, ALT, serum creatinine, C-reactive protein (CRP), bilirubin, metastatic site (liver, peritoneum, and bone), number of metastatic sites, and tumor differentiation on histopathology. Baseline characteristics such as sex, age, PS and history of previous gastrectomy when starting Cx-2 were also evaluated. This study was conducted in accordance with Japanese ethics guidelines for clinical and epidemiological studies, which took effect in August 2007.

Chemotherapy regimens and treatment evaluation

Chemotherapy regimens were divided as follows: (1) Taxane-based: paclitaxel or docetaxel, (2) Irinotecan-based: irinotecan monotherapy, or combination with S-1, mitomycin C, or cisplatin, (3) 5FU-based: oral S-1 monotherapy or combination with cisplatin; continuous 5-FU infusion or combination with methotrexate.

In Cx-1, PD was defined as clinical progression (e.g., increased ascites or pleural effusion; deteriorated general condition due to bone metastasis; elevated tumor marker level) or radiographic progression according to response evaluation criteria in solid tumors.

Statistical analysis

Univariate and multivariate analyses were performed using the Cox regression model to determine the prognostic factors for survival. Hazard ratio (HR) and 95% confidence interval (CI) were also determined. Progression-free survival in first-line chemotherapy (PFS-1) was defined as the interval between the start of first-line chemotherapy to recognition of PD. Overall survival (OS) was defined as the interval between the start of chemotherapy to death or the date of last follow-up. Median survival time in patients who received first-line chemotherapy (MST-1) was calculated by the median interval from the start of first-line chemotherapy to death. Median survival time in patients who received second-line chemotherapy (MST-2) was the median interval from the start of second-line chemotherapy to death. PFS-1 was dichotomized according to the median PFS-1. Statistical significance was set at alpha = 0.05 for a two-sided test. OS and PFS were estimated by the Kaplan–Meier method.

Results

Baseline characteristics of patients

Of 995 patients who received Cx-1 in the study period, 466 met the eligibility criteria. Patient clinical characteristics at the start of Cx-2 are shown in Table 1. The median age of the patients was 60 years. S-1 monotherapy was the most commonly used regimen in Cx-1 of 178 patients (38.2%). Only eight patients underwent S-1 plus cisplatin combination chemotherapy (1.7%). The median PFS-1 was 129 days (95% CI 119–142 days) (Table 1). In Cx-2, taxane alone ($n = 201$) and irinotecan with or without cisplatin ($n = 141$) were the most frequently used chemotherapeutic agents.

Survival analysis

MST-1 was 371 days (95% CI 340–418 days) (Fig. 1) and MST-2 was 207 days (95% CI 182–227 days). MST-2 was significantly longer in the group with PFS-1 ≥120 than in the group with PFS <120 (Fig. 2) [i.e., 258 days (95% CI 229–287) and 133 days (95% CI 117–156), respectively ($p < 0.001$)].

Table 1 Baseline characteristics

Characteristic	No.	%
Age (years)		
Median	60	
Range	22–73	
Sex		
Male	305	65.5
Female	161	34.5
Performance status		
<1	396	85.0
≥2	70	15.0
Tumor differentiation		
Intestinal	313	67.2
Diffuse	145	31.1
Undetermined	8	1.7
Recurrence or metastasis		
Recurrence	132	28.3
Primary metastasis	334	71.7
Previous gastrectomy		
Yes	201	43.1
No	265	56.9
Number of metastatic site		
<3	414	88.8
≥3	52	11.2
Peritoneal metastasis		
Yes	211	45.3
No	255	54.7
Bone metastasis		
Yes	42	9.0
No	424	91.0
Liver metastasis		
Yes	169	36.3
No	297	63.7
First-line treatment		
S1 only	178	38.2
5-Fluorouracil only	97	20.8
Cisplatin/Irrinotecan	80	17.2
Methotrexate/5-Fluorouracl	90	19.3
Others	21	4.5
Second-line treatment		
Taxane base	201	43.1
Paclitaxel	182	
Docetaxel	19	
Irinotecan base	141	30.3
Irinotecan/Cisplatin	108	
Irinotecan/Mitomycin	29	
Irinotecan/S1 or Irinotecan only	4	
5-Fluorouracil base	116	24.9
S1 only	66	
S1/Cisplatin	6	
5-Fluorouracil/Methotrexate	31	
5-Fluorouracil only	9	
5-Fluorouracil/Cisplatin	4	
Others	8	1.7
Monotherapy	285	61.2
Combination chemotherapy	181	38.8
Serum albumin (mg/dL)		
Median	3.5	
Range	1.7–4.7	
Alkaline phosphatase (mg/dL)		
Median	299	
Range	34–9,656	
Total birilubin (mg/dL)		
Median	0.6	
Range	0.1–8.8	
Hemoglobin (g/dL)		
Median	10.9	
Range	5.3–108	
White blood cell count ($\times 10^3$)/μL		
Median	6.3	
Range	2.4–25.3	
Platelet count ($\times 10^4$)/μL		
Median	27.85	
Range	1.9–116.1	
Lactate dehydrogenase (IU/L) (normal range 119–229)		
Median	252	
Range	10.3–9,300	
Serum creatinine (mg/dL)		
Median	0.8	
Range	0.3–2.8	
Asparate aminotransferase (IU/L)		
Median	25	
Range	1–646	
Alanine aminotransferase (IU/L)		
Median	18	
Range	4–246	
C-reactive protein (mg/dL)		
Median	0.6	
Range	0.1–25.9	
Progression-free survival in first-line chemotherapy (days)		
Median	129	
Range	6–1,199	

Univariate and multivariate analyses

Table 2 shows the results of the univariate analysis for survival from the start of Cx-2. The following data were significantly correlated with shorter survival time: PFS-1

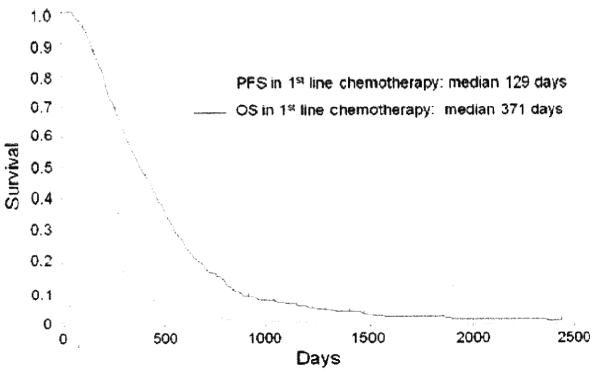

Fig. 1 Overall survival (OS) and progression-free survival (PFS) from the start of first-line chemotherapy

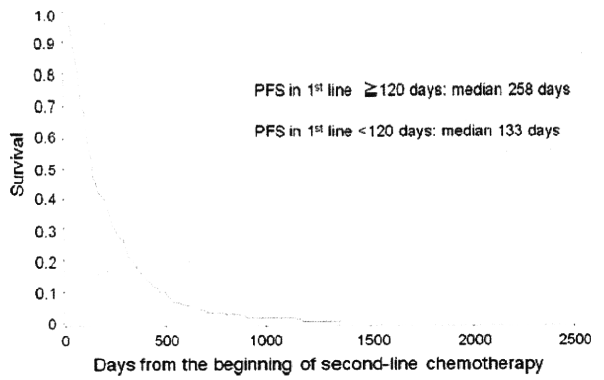

Fig. 2 Overall survival from the start of second-line chemotherapy. *Orange line* progression-free survival of first-line chemotherapy (PFS-1) ≥120 days; *green line* PFS-1 <120 days

<120 days, ECOG PS ≥2, site of metastasis ≥3, presence of peritoneal or bone metastasis, serum albumin <3.5 mg/dL, ALP ≥360 mg/dL, LDH ≥250 IU/L (above the normal range), bilirubin ≥1 mg/dL, AST >40 IU/L, ALT >40 IU/L, CRP ≥1.0 mg/dL, and WBC ≥6.5 × 10^3/μL. In the Cx-2 regimen, neither irinotecan nor taxane affected survival (irinotecan vs. taxane; HR 0.95; $p = 0.62$). We further analyzed monotherapy versus combination therapy in second-line treatment, which failed to detect the relation to survival in Cx-2.

Cox multivariate analysis included all variables that were found to have prognostic significance in univariate analysis. The results of the analysis identified the following eight independent prognostic factors that correlated with shorter survival time in Cx-2: PFS-1 <120 days (HR 0.71; $p < 0.001$); PS ≥2 (HR 0.51; $p < 0.001$); history of no previous gastrectomy (HR 0.78; $p = 0.02$); presence of peritoneal or bone metastasis (HR 0.60; $p < 0.001$ and HR 0.48; $p < 0.001$, respectively); presence of liver metastasis (HR 0.76; $p = 0.02$); serum albumin <3.5 mg/dL (HR 0.65; $p < 0.001$); CRP >1.0 mg/dL (HR 0.65; $p < 0.001$) (Table 3).

We also performed Cox regression analysis for the Cx-2 regimens to potentially identify predictive factors for survival in Cx-2; however, these factors showed no independent prognostic significance for survival (data not shown).

Discussion

We identified the following eight factors affecting the survival of AGC patients in Cx-2: PFS-1, PS, history of previous gastrectomy, peritoneal, liver, or bone metastasis, serum albumin, and CRP.

There are several studies that have described prognostic factors for the survival of AGC patients in Cx-1. These include PS, liver or peritoneal metastasis, elevated ALP level, and number of metastatic site (Chau et al. 2004; Lee et al. 2007; Louvet et al. 2003; Yamamura et al. 2002). Response to Cx-1 was previously reported to correlate with PFS-1 and survival from Cx-1 ($r = 0.56$, 0.47, respectively) (Ichikawa and Sasaki 2006). However, the prognostic factors for Cx-2 remain to be determined. In the present study, patients with longer PFS-1 had longer MST-2, resulting in longer survival from the start of Cx-1. Since gastric cancer consists of a heterogeneous population of neoplastic cells, tumor growth rate and the chemosensitivity of each tumor may vary. Longer PFS-1 in AGC may identify a chemosensitive cohort from this heterogeneous cell population.

Recently, Catalano et al. (2008) have reported five clinico-pathological factors affecting the survival of 175 AGC patients in Cx-2. These included PS, PFS-1 duration, hemoglobin, carcino embryonic antigen level, and number of metastatic sites. Their results also suggested that PFS-1 duration (HR 1.79, 95% CI 1.39–2.80, $p < 0.0001$) and PS (HR 1.79; 95% CI 1.16–2.77; $p = 0.008$) were independent prognostic factors for short survival time in Cx-2. PS has been identified as a prognostic factor in AGCs in several trials (Catalano et al. 2008; Koizumi et al. 2008) and also in other cancers (Bellmunt et al. 2002; Janisch et al. 1994; Sargent et al. 2009). PFS-1 was additionally identified as a prognostic factor in Catalano et al's analysis as well as in ours. Therefore, we conducted additional analysis focusing on two factors, namely, PS and PFS-1. Poor PS (≥2) with PFS-1 <120 days was considered to indicate dismal prognosis, with an MST-2 of only 60 days (95% CI 41–73 days) (data not shown). We consider that this cohort may not have survival benefit when using Cx-2.

In Japan, the standard Cx-1 for AGC includes S-1 and cisplatin (Koizumi et al. 2008), and most patients with good PS proceed to Cx-2. Although there is still no evidence that Cx-2 provides survival benefit in clinical practice, irinotecan

Table 2 Univariate analysis on survival

		HR	95% CI	*p* value
PFS-1	≥120 versus <120	0.64	0.53–0.77	<0.01
Age	≥60 versus <60	0.89	0.74–1.07	0.22
Sex	Male versus Female	0.95	0.78–1.16	0.61
PS	0–1 versus 2–4	0.36	0.28–0.47	<0.01
Histopathology	Intestinal versus Diffuse	1.08	0.89–1.32	0.43
	Unclassified versus Diffuse	1.32	0.64–2.69	0.45
Previous gastrectomy	Yes versus No	0.65	0.54–0.79	<0.01
Number of metastatic site	3< versus 3≥	0.54	0.40–0.72	<0.01
Peritoneal metastasis	No versus Yes	0.71	0.59–0.86	<0.01
Bone metastasis	No versus Yes	0.58	0.42–0.80	<0.01
Liver metastasis	No versus Yes	0.86	0.71–1.04	0.12
Serum albumin (mg/dL)	≥3.5 versus <3.5	0.44	0.37–0.54	<0.01
ALP (mg/dL)	<360 versus ≥360	0.60	0.49–0.72	<0.01
Total bilirubin (mg/dL)	<1 versus ≥1	0.77	0.60–0.98	0.03
Hemoglobin (g/dL)	≥11 versus <11	0.86	0.71–1.03	0.10
WBC ($\times 10^3$) (/μL)	<6.5 versus ≥6.5	0.77	0.64–0.92	<0.01
Platelet count ($\times 10^4$) (/μL)	≥15 versus <15	0.88	0.63–1.23	0.45
LDH (IU/L)	<250 versus ≥250	0.73	0.61–0.88	<0.01
Serum creatinine (mg/dL)	≥1 versus <1	0.95	0.75–1.20	0.65
AST (IU/L)	≤40 versus >40	0.63	0.51–0.78	<0.01
ALT (IU/L)	≤40 versus >40	0.71	0.55–0.90	<0.01
C-reactive protein (mg/dL)	<1 versus ≥1	0.44	0.36–0.53	<0.01
Second-line treatment	Irinotecan base versus Taxane base	0.95	0.76–1.18	0.62
	Others versus Taxane base	0.98	0.78–1.23	0.85
	Combination chemotherapy versus Monotherapy	1.11	0.92–1.34	0.29

PFS-1 progression-free survival in first-line chemotherapy, *PS* performance status, *ALP* alkaline phosphatase, *WBC* white blood cell, *LDH* lactate dehydrogenase, *AST* asparate aminotransferase, *ALT* alanine aminotransferase, *HR* hazard ratio, *CI* confidence interval

Table 3 Multivariate analysis on survival

		HR	95% CI	*p* value
PFS-1	≥120 versus <120	0.71	0.58–0.86	<0.001
Performance status	0–1 versus 2–4	0.51	0.38–0.69	<0.001
Previous gastrectomy	Yes versus No	0.78	0.64–0.96	0.02
Peritoneal metastasis	No versus Yes	0.60	0.49–0.74	<0.001
Bone metastasis	No versus Yes	0.48	0.33–0.67	<0.001
Liver metastasis	No versus Yes	0.76	0.60–0.95	0.02
Albumin (mg/dL)	≥3.5 versus <3.5	0.65	0.53–0.81	<0.001
ALP (mg/dL)	<360 versus ≥360	0.83	0.66–1.02	0.08
CRP (mg/dL)	<1 versus ≥1	0.65	0.52–0.80	<0.001

PFS-1 progression-free survival in first-line chemotherapy, *ALP* alkaline phosphatase, *CRP* C-reactive protein, *HR* hazard ratio, *CI* confidence interval

or taxane containing regimen is commonly used for Cx-2 (Boku et al. 1999; Kodera et al. 2007; Shirao et al. 1997; Sulkes et al. 1994). In our analysis, patients who received irinotecan tended to have liver metastasis but not peritoneal metastasis (data not shown); however, the prognosis of these patient groups was not significantly different. In the early 1990s when Cx-2 was not widely used, the median survival time of AGC patients was nearly 7 months (Ohtsu et al. 2003). A recent trial conducted in Japan suggested that median survival time was prolonged to almost 1 year with the same regimens of Cx-1 used previously (Koizumi et al. 2008). This prolongation may be attributed to changes in the Cx-2 regimen. In the JCOG9205 study, the median survival time of patients receiving the 5-FU arm was 7.1 months; however, this time increased to 9.0 months in the JCOG9912 study (Boku et al. 2009). These results imply that the Cx-2 regimen improved survival. Taken together, we believe that Cx-2 may show benefit for selected patients.

Because of the retrospective nature of the present analysis, some considerations must be taken into account in interpreting our findings. First, the regimens used may not be valid in other facilities or countries where S-1 is not used as standard chemotherapy for AGC. Second, PFS-1 may possibly become a substitute potential prognostic factor in Cx-1, that is, patients with poor PS or other risks when starting Cx-1 may have shorter PFS-1 than those without. To exclude this possibility, we did not include patients with poor organ function, advance age (over 75 years), and inadequate bone marrow function in Cx-1 from the cohort. Nevertheless, the results of our analysis are important from three

aspects. First, our findings can be used as a basis for excluding patients who will not likely benefit from subsequent chemotherapy following disease progression after Cx-1. Second, our results may help oncologists in providing advice to patients regarding their potential survival. Third, the present data suggest the need to stratify future studies of Cx-2 to adequately assess treatment response and survival data.

In conclusion, we demonstrated that PFS-1 is an independent prognostic factor for survival in Cx-2. Survival benefit from Cx-2 may be limited for patients with PFS-1 <120 days and other risk factors. Physicians should carefully consider PFS-1 and PS, as well as other potential variables, when considering Cx-2 and advising patients about the potential risks, harms and benefit of CX-2 for AGC.

Conflict of interest statement None declared.

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Cancer Science
The official journal of the Japanese Cancer Association JCA

Plasma concentrations of VCAM-1 and PAI-1: A predictive biomarker for post-operative recurrence in colorectal cancer

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(Received December 2, 2009/Revised March 29, 2010/Accepted April 9, 2010/Accepted manuscript online April 21, 2010/Article first published online May 17, 2010)

This prospective study used antibody suspension bead arrays to identify biomarkers capable of predicting post-operative recurrence with distal metastasis in patients with colorectal cancer. One hundred colorectal cancer patients who underwent surgery were enrolled in this study. The median follow-up period was 3.9 years. The pre-operative plasma concentrations of 24 angiogenesis-related molecules were analyzed with regard to the TNM stage and the development of post-operative recurrence. The concentrations of half of the examined molecules (13/24) increased significantly according to the TNM stage ($P < 0.05$). Meanwhile, a multivariate logistic regression analysis revealed that the concentrations of vascular cell adhesion molecule 1 (VCAM-1) and plasminogen activator inhibitor-1 (PAI-1) were significantly higher in the post-operative recurrence group. The VCAM-1 and PAI-1 model discriminated post-operative recurrence with an area under the curve of 0.82, a sensitivity of 0.75, and a specificity of 0.73. A leave-one-out cross-validation was applied to the model to assess the prediction performance, and the result indicated that the cross-validated error rate was 12.5% (12/96). In conclusion, our results demonstrate that antibody suspension bead arrays are a powerful tool to screen biomarkers in the clinical setting, and the plasma levels of VCAM-1 and PAI-1 together may be a promising biomarker for predicting post-operative recurrence in patients with colorectal cancer. (*Cancer Sci* 2010; 101: 1886–1890)

Colorectal cancer (CRC) is one of the leading causes of death in Japan (http://ganjoho.ncc.go.jp/public/statistics/index.html) and Western countries.[1] Despite recent advances in adjuvant chemotherapy and surgical techniques, 20–40% of patients die because of metastasis after curative surgery.[2] Tumor-node-metastasis (TNM) staging is well established and the most reliable system for predicting the outcome of CRC. In particular, the TNM staging system works very well for predicting the outcome of early stage I cancers and advanced stage IV cancers. However, the 5-year survival rate varies from 44% to 83% within TNM stage III, indicating that a wide variation in outcomes exists within each stage as a result of biological heterogeneity.[3] Thus, highly accurate predictors of post-operative recurrence are needed for patients with CRC who undergo curative surgery, as such predictors would likely contribute to the further improvement of the 5-year survival rate by justifying the addition of intensive adjuvant chemotherapy to the therapeutic regimens of subgroups with a high risk of post-operative recurrence. Therefore, the prediction of post-operative recurrence is regarded as one of the most important research themes in clinical settings and has been extensively studied, with particular attention given to the investigation of various molecular prognostic factors.

In addition to the TNM stage, the carcinoembryonic antigen (CEA) level is routinely used to monitor recurrence in patients with CRC.[4] A large clinical study demonstrated that pre-operative CEA levels provide prognostic information in addition to that provided by the TNM staging system and determined that the pre-operative CEA level was an independent predictor of survival and recurrence.[5] However, the study concluded that although an elevated pre-operative CEA level (>5 mg/mL) may be correlated with a poor prognosis, the available data was insufficient to support the use of the CEA level for determining whether a patient should undergo adjuvant therapy.[4] Other molecular markers, including the K-Ras mutation status,[6,7] microsatellite instability,[8] the loss of heterogeneity at 18q,[9] and p53,[10] have been examined with regard to predicting the outcome of subgroups; unfortunately, none of these molecular markers are suitable for routine clinical use. Thus, further investigations of novel molecular markers are eagerly awaited.

The Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA, USA) utilizes a series of color-coded beads, each of which is coupled to a unique antibody specific for a biochemical marker. This assay is capable of measuring the levels of multiple targets in a single well of a 96-well microplate using as little as 12.5 μL of serum, plasma, or other matrix. In the present study, 24 angiogenesis-related markers from this assay panel were used to evaluate plasma proteins and their potential associations with disease progression and the recurrence of CRC.

Materials and Methods

Patient selection. Patients with histologically confirmed colorectal cancer who were between the ages of 20 and 80 years and who were scheduled to undergo surgery were eligible for enrollment in this study. Additional inclusion criteria included an Eastern Cooperative Oncology Group performance status of 0–2. All the patients in this series underwent surgery. This prospective study was approved by the Institutional Review Board of the National Cancer Center Hospital and written informed consent was obtained from all the patients.

Clinical and pathologic features. Clinical features including age, sex, primary site of tumor, histologic type of tumor, TNM stage, and post-operative recurrence were recorded. A pathologist reviewed the microscopic slides. Post-operative recurrence was defined when distant metastasis was observed. The median follow-up period of post-operative recurrence was 3.9 years.

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doi: 10.1111/j.1349-7006.2010.01595.x

Preparation of plasma samples. Two milliliters of whole blood were collected into EDTA-containing tubes before surgery (within 2 weeks) and were centrifuged at 1500 *g* for 10 min to obtain the plasma samples. The samples were stored at −80°C until further use.

Angiogenesis-related molecules. The 24 plasma markers used in this study were as follows: interleukin 6 receptor (IL-6R), matrix metallopeptidase 9 (MMP-9), TIMP metallopeptidase inhibitor 1 (TIMP-1), TIMP metallopeptidase inhibitor 2 (TIMP-2), endostatin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), Tie-2, plasminogen activator inhibitor-1 (PAI-1), macrophage migration inhibitory factor (MIF), plasminogen activator urokinase receptor (uPAR), angiopoietin 2 (Ang-2), follistatin, hepatocyte growth factor (HGF), interleukin 8 (IL-8), colony stimulating factor 3 (G-CSF), platelet-derived growth factor beta polypeptide (PDGF-BB), vascular endothelial growth factor (VEGF), leptin, platelet/endothelial cell adhesion molecule (PECAM-1), interleukin 12 (IL-12), fibroblast growth factor 2 (FGF-basic), and tumor necrosis factor (TNF-α). Ang-2, Follistatin, The nine markers of HGF, IL-8, PDGF-BB, VEGF, Leptin, PECAM-1, and G-CSF are commercially available as the Human Premixed Angiogenesis (9) panel (Bio-Rad Laboratories). The others are available for customization or in developing markers.

Antibody suspension bead arrays system. The plasma concentrations of each molecule were measured using a Bio-Plex suspension array system (Bio-Rad Laboratories), which permits the simultaneous measurement of multiple circulating proteins in a single well using only 12.5 μL of plasma. The assay was performed according to the manufacturer's instructions and a previously described method.[11] All plasma samples were diluted 1 in 4 with the appropriate diluents prior to assay. The samples were tested in duplicate.

Statistical analysis. The correlations between the plasma concentrations and the TNM stages were analyzed using a linear trend analysis and the proportional odds model. In the linear trend analysis, we used a one-way ANOVA model with a linear contrast, which consisted of the TMN stage scores. A *t*-test was used to compare the post-operative recurrence and the no recurrence groups. In the multivariate analysis, an analysis was performed for all the plasma markers but not for the clinical variables because too many explanatory variables for the sample size were included in this study. A logistic regression analysis was used to examine statistical differences according to post-operative recurrence and a stepwise method was used to select the most useful explanatory parameters. Analyses were performed using SAS software version 9.1.3 (SAS Institute, Cary, NC, USA). A *P*-value of <0.05 was considered statistically significant.

Results

Patient results. A total of 100 consecutive patients were enrolled in this study. The median age of the enrolled patients was 59 years (range, 31–79 years). Among them, 96 patients received curative operations and four patients had distal metastasis and received palliative operations. Eleven patients developed recurrences with distal metastasis during the follow-up period. Table 1 summarizes the characteristics of the patients and their tumors.

Plasma concentrations of 24 angiogenesis-related molecules. We measured the plasma concentrations of 24 angiogenesis-related molecules: IL-6R, MMP-9, TIMP-1, TIMP-2, endostatin, P-selectin, ICAM-1, VCAM-1, Tie-2, PAI-1, MIF, uPAR, Ang-2, follistatin, HGF, IL-8, G-CSF, PDGF-BB, VEGF, leptin, PECAM-1, IL-12, FGF-basic, and TNF-α (Table 2). Overall, 98.5% of the plasma samples were successfully quantified using a standard curve.

Table 1. Patient characteristics

Characteristics	Curative ope.		Palliative ope.	Total
	Rec+	Rec−		
Age (years)				
≥60	6	42	2	50
<60	5	43	2	50
Sex				
Male	3	54	3	65
Female	8	31	1	35
Primary site				
Colon	8	40	1	49
Rectum	3	45	3	51
Hist. type				
Well diff.	8	59	3	70
Others	3	26	1	30
TNM stage				
I	1	26	–	27
II	2	22	–	24
III	8	37	–	45
IV	–	–	4	4
Total	11	85	4	100

Hist. type, histology of primary tumor; Rec+, post-operative recurrence (+); Rec−, post-operative recurrence (−).

Tumor-node-metastasis (TNM) stage and plasma concentrations of angiogenesis-related molecules. The TNM stage can be accurately used to stratify patients at a high risk for cancer progression and is thought to reflect the malignant potential of each tumor. To estimate the contributions of the angiogenesis-related molecules to the malignant potentials of the tumors, we examined the correlation between the plasma concentrations of each molecule and the TNM stage. A linear trend analysis showed that the plasma concentrations of 13 molecules increased significantly with an increasing TNM stage ($P < 0.05$): IL-6R, TIMP-1, TIMP-2, P-selectin, Tie-2, PAI-1, uPAR, Ang-2, follistatin, HGF, IL-8, PDGF-BB, and VEGF (Table 3). Next, we performed an exploratory multivariate analysis using a proportional odds model with the TNM stage (I–IV) assigned as the objective variable and each of the angiogenesis-related molecules assigned as explanatory variables. The multivariate analysis identified TIMP-1, P-selectin, Ang-2, HGF, IL-8, PDGF-BB, and VEGF as being significantly correlated with the TNM stage. These results indicated that the plasma concentrations of several molecules increased significantly with an increasing TNM stage, strongly suggesting that these molecules might be candidate biomarkers for an unfavorable outcome in patients with CRC.

Post-operative recurrence and plasma concentrations of angiogenesis-related molecules. To predict post-operative recurrence using this system, we analyzed the 96 patients with CRC who underwent curative operations, excluding the four patients with distal metastasis. Among these 96 patients, 11 developed recurrences during the follow-up period; the remaining 85 patients did not show any signs of recurrence. When the plasma levels of the angiogenesis-related molecules were compared between the patients with recurrences and those without recurrences, *t*-tests demonstrated that the plasma concentrations of IL-6R (63.2 ± 13.8 and 51.3 ± 17.0 ng/mL, respectively), P-selectin (76.1 ± 24.4 and 60.0 ± 24.8 ng/mL, respectively), VCAM-1 (163.9 ± 61.0 and 134.6 ± 35.0 ng/mL, respectively), and PAI-1 (28.2 ± 15.4 and 19.8 ± 10.2 ng/mL, respectively) were significantly higher among the patients with recurrences (Fig. 1a–d, Table 4). A multivariate logistic regression analysis revealed that the plasma concentrations of VCAM-1 and PAI-1 were significantly higher among the patients with recurrences ($P = 0.039$ and $P = 0.028$, respectively). A stepwise

Table 2. Plasma concentrations of 24 angiogenesis-related molecules in 100 colorectal cancers

Molecules	Range	Average (SD)	25%	Percentile	
	pg/mL			Median	75%
IL-6R	23–149	54 (18)	42	52	62
MMP-9	6–189	35 (27)	20	27	41
TIMP-1	44–283	117 (35)	96	118	135
TIMP-2	9–47	24 (5)	21	24	28
Endostatin	90–456	187 (64)	140	177	223
P-selectin	0–164	64 (27)	48	62	78
ICAM-1	103–605	282 (81)	225	270	317
VCAM-1	76–333	138 (40)	110	135	163
Tie-2	9–1070	143 (164)	48	73	182
PAI-1	3–65	21 (12)	14	19	26
MIF	0–120	53 (26)	44	59	69
uPAR	1–130	24 (23)	8	15	37
Ang-2	0–6147	1381 (1337)	415	938	2197
Follistatin	235–2903	924 (614)	502	686	1197
HGF	201–12213	2700 (2516)	1076	1628	3930
IL-8	5–234	52 (41)	24	34	74
G-CSF	0–4775	832 (1133)	79	247	1161
PDGF-BB	6–6219	737 (831)	220	439	922
VEGF	0–724	186 (164)	67	120	262
Leptin	0–32847	3155 (4433)	1149	2134	3851
PECAM-1	1188–15837	5562 (3472)	2901	4487	7348
IL-12	0–32	5 (6)	2	3	6
FGF-basic	0–235	21 (30)	4	13	25
TNF-α	0–72	4 (9)	1	2	4

The concentrations are ng/mL for interleukin 6 receptor (IL-6R), matrix metallopeptidase 9 (MMP-9), TIMP metallopeptidase inhibitor 1 (TIMP-1), TIMP-2, endostatin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), Tie-2, plasminogen activator inhibitor-1 (PAI-1), macrophage migration inhibitory factor (MIF), and plasminogen activator urokinase receptor (uPAR), and the others are pg/mL. FGF-basic, fibroblast growth factor 2; G-CSF, colony stimulating factor 3; HGF, hepatocyte growth factor; PDGF-BB, platelet-derived growth factor beta polypeptide; PECAM-1, platelet/endothelial cell adhesion molecule; TNF-α, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Table 3. Tumor-node-metastasis (TNM) stage and plasma concentrations in 100 colorectal cancers

Molecules	TNM stage				Univariate	Multivariate
	I	II	III	IV	*P*-value	*P*-value
IL-6R	50	57	52	76	0.01	n.s.
MMP-9	34	50	28	33	n.s.	n.s.
TIMP-1	107	122	114	185	<0.0001	0.02
TIMP-2	24	24	24	33	0.003	n.s.
Endostatin	184	198	182	201	n.s.	n.s.
P-selectin	56	67	63	109	0.0003	0.04
ICAM-1	276	315	266	298	n.s.	n.s.
VCAM-1	135	136	141	144	n.s.	n.s.
Tie-2	116	162	129	375	0.005	n.s.
PAI-1	18	24	21	38	0.003	n.s.
MIF	53	55	51	78	n.s.	n.s.
uPAR	20	27	22	53	0.01	n.s.
Ang-2	978	1458	1447	2914	0.007	0.03
Follistatin	778	953	928	1704	0.006	n.s.
HGF	1933	2932	2803	5342	0.01	0.04
IL-8	38	56	53	108	0.002	0.02
G-CSF	595	1114	797	1132	n.s.	n.s.
PDGF-BB	442	822	802	1483	0.02	0.03
VEGF	129	209	192	375	0.006	0.03
Leptin	3236	2815	3235	3752	n.s.	n.s.
PECAM-1	5026	5914	5625	6356	n.s.	n.s.
IL-12	3	8	5	5	n.s.	n.s.
FGF-basic	19	32	16	28	n.s.	n.s.
TNF-α	4	6	3	2	n.s.	n.s.

Values indicate the average. Univariate: linear trend analysis, multivariate: proportional odds model. The concentrations are ng/mL for interleukin 6 receptor (IL-6R), matrix metallopeptidase 9 (MMP-9), TIMP metallopeptidase inhibitor 1 (TIMP-1), TIMP-2, endostatin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), Tie-2, plasminogen activator inhibitor-1 (PAI-1), macrophage migration inhibitory factor (MIF), and plasminogen activator urokinase receptor (uPAR), and the others are pg/mL. FGF-basic, fibroblast growth factor 2; G-CSF, colony stimulating factor 3; HGF, hepatocyte growth factor; n.s., not significant; PDGF-BB, platelet-derived growth factor beta polypeptide; PECAM-1, platelet/endothelial cell adhesion molecule; TNF-α, tumor necrosis factor; VEGF, vascular endothelial growth factor.

method selected VCAM-1 and PAI-1 as the most useful explanatory parameters, suggesting that the combination of these two molecules might synergistically improve the prediction of post-operative recurrence. Finally, a prediction model incorporating VCAM-1 and PAI-1 successfully discriminated post-operative recurrence, with an area under the curve (AUC) of 0.82, a sensitivity of 0.75, and a specificity of 0.73 (Fig. 2a). To assess the prediction performance, a leave-one-out cross-validation was applied to the model. The cross-validated error rate was 12.5% (12/96). In stage III patients, the prediction model had a sensitivity of 0.625 (5/8) and a specificity of 0.865 (32/37) for predicting post-operative recurrence (Fig. S2). On the other hand, when apparent distal metastases of CRCs were applied to the VCAM-1/PAI-1 prediction model, three out of four metastatic cases were determined as "recurrence (+) cases". These results suggest that apparent metastatic tumors could be discriminated using the model. Although a validation study is necessary, our results raise the possibility that the combined use of the pre-operative plasma concentrations of VCAM-1 and PAI-1 might be useful for predicting post-operative recurrence in patients with CRC. Finally, we retrospectively analyzed the plasma PAI-1 concentrations of metastatic and non-metastatic CRC in another study of an independent cohort using conventional ELISA. The plasma concentrations in the metastatic CRC patients were significantly higher than those in the non-metastatic patients ($P = 0.005$), even in the independent cohort (Fig. S1). The CEA level was not significantly different between the recurrence (+) *versus* the recurrence (−) groups ($P = 0.335$) in our study.

Discussion

Vascular cell adhesion molecule 1 (VCAM-1)/CD106 is a member of the Ig superfamily and encodes a cell surface sialoglycoprotein expressed by cytokine-activated endothelium. This type I membrane protein mediates leukocyte-endothelial cell adhesion and signal transduction, and may play a role in the development of atherosclerosis[12] and rheumatoid arthritis.[13] In the field of oncology, accumulating evidence suggests that VCAM-1 is associated with a poor outcome.[14–17] Recently, Shariat *et al.*[18] reported that standard clinical variables alone exhibited an accuracy of 71.6% for predicting the risk of biochemical recurrence following a radical prostatectomy in patients with prostate cancer, whereas the addition of preoperative blood levels of TGF-β1, sIL-6R, IL-6, VCAM-1, VEGF, endoglin, and uPA increased the predictive accuracy by 15–86.6%. Vascular endothelial growth factor (VEGF) and uPA were not significant predictors of post-operative recurrence in our data set for CRC, but VCAM-1 and sIL-6R were significant, consistent with Shariat's study. The mechanism by which VCAM-1 mediates an unfavorable phenotype remains unclear, but the most probable explanation is that the tumor cells escape T-cell immunity by

doi: 10.1111/j.1349-7006.2010.01595.x

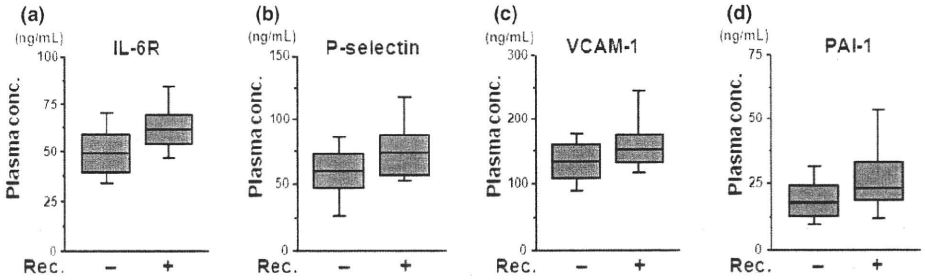

Fig. 1. The concentrations of (a) interleukin 6 receptor (IL-6R), (b) P-selectin, (c) vascular cell adhesion molecule 1 (VCAM-1), and (d) plasminogen activator inhibitor-1 (PAI-1) were significantly higher in the recurrence group in colorectal cancer. The upper bar, box, and lower bar represent the 90%, 75%, 50%, 25% and 10% percentiles. The plasma concentrations of each molecule were measured using a Bio-Plex suspension array system. Rec, recurrence.

Table 4. Results of multivariate analysis for recurrence after curative surgery in 96 colorectal cancers

Molecules	Recurrence*		Univariate		Multivariate
	+	–	*t*-test	Logistic	Stepwise
			P-value	*P*-value	*P*-value
IL-6R	63	51	0.03	n.s.	
MMP-9	37	35	n.s.	n.s.	
TIMP-1	119	113	n.s.	n.s.	
TIMP-2	25	24	n.s.	n.s.	
Endostatin	193	186	n.s.	n.s.	
P-selectin	76	60	0.04	n.s.	
ICAM-1	285	281	n.s.	n.s.	
VCAM-1	164	135	0.02	0.039	0.009
Tie-2	183	127	n.s.	n.s.	
PAI-1	28.3	19.8	0.02	0.005	0.005
MIF	64	51	n.s.	n.s.	
uPAR	30	21	n.s.	n.s.	
Ang-2	1514	1292	n.s.	n.s.	
Follistatin	962	883	n.s.	n.s.	
HGF	3155	2517	n.s.	n.s.	
IL-8	53	49	n.s.	n.s.	
G-CSF	892	810	n.s.	n.s.	
PDGF-BB	972	671	n.s.	n.s.	
VEGF	211	174	n.s.	n.s.	
Leptin	2623	3196	n.s.	n.s.	
PECAM-1	6159	5447	n.s.	n.s.	
IL-12	4	5	n.s.	n.s.	
FGF-basic	16	22	n.s.	n.s.	
TNF-α	4	4	n.s.	n.s.	

*Values indicate the average. Recurrence, post-operative recurrence; logistic, logistic regression model. The concentrations are ng/mL for interleukin 6 receptor (IL-6R), matrix metallopeptidase 9 (MMP-9), TIMP metallopeptidase inhibitor 1 (TIMP-1), TIMP-2, endostatin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), Tie-2, plasminogen activator inhibitor-1 (PAI-1), macrophage migration inhibitory factor (MIF), and plasminogen activator urokinase receptor (uPAR), and the others are pg/mL. FGF-basic, fibroblast growth factor 2; G-CSF, colony stimulating factor 3; HGF, hepatocyte growth factor; n.s., not significant; PDGF-BB, platelet-derived growth factor beta polypeptide; PECAM-1, platelet/endothelial cell adhesion molecule; TNF-α, tumor necrosis factor; VEGF, vascular endothelial growth factor.

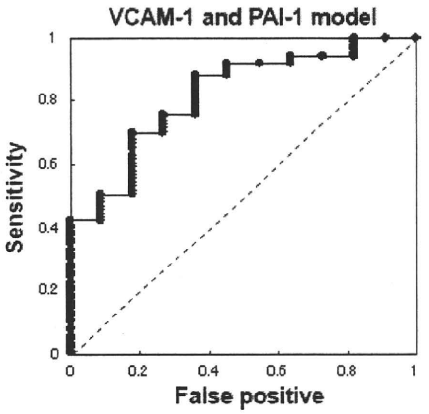

Fig. 2. The receiver–operator curve (ROC) for post-operative recurrence. A stepwise method selected vascular cell adhesion molecule 1 (VCAM-1) and plasminogen activator inhibitor-1 (PAI-1) as the most useful explanatory parameters; these two molecules were subsequently used to construct a prediction model. The ROC indicates the results of this model, which was capable of discriminating post-operative recurrence with an area under the curve (AUC) of 0.82, a sensitivity of 0.75, and a specificity of 0.73.

overexpressing the endothelial cell adhesion molecule VCAM-1, which normally mediates leukocyte extravasation to sites of tissue inflammation.[19]

Plasminogen activator inhibitor-1 (PAI-1)/SERPINE1 belongs to the plasmin/plasminogen system and is secreted into the blood, where it prevents the generation of plasmin, promoting the persistence and expansion of thrombi.[20] Plasminogen activator inhibitor-1 (PAI-1) is known as tumor biological prognostic factor and has been thoroughly validated with regard to its clinical utility in breast cancer.[21,22] The 2007 Breast Tumor Markers Guidelines recommend that uPA/PAI-1 be measured using an ELISA with a minimum of 300 mg of fresh or frozen breast cancer tissue for determining the prognosis of patients with newly diagnosed, node-negative breast cancer. Furthermore, CMF-based adjuvant chemotherapy provides a substantial benefit, compared with observation alone, in patients with a high risk of recurrence as determined by the presence of high levels of uPA and PAI-1.[23] Previous reports have demonstrated that higher levels of PAI-1, but not PAI-2, are associated with large tumors, metastatic stage, and a worse prognosis in patients with CRC.[24–26] Our study differed in that it evaluated the clinical parameter of post-operative recurrence in a prospective study. The biological mechanism by which PAI-1 promotes tumor progression is thought to involve a reduction in cell adhesion to the extracellular matrix as a consequence of excess PAI-1 interfering with uPAR binding to vitronectin, thereby facilitating cell invasion and migration.[27] Interestingly, accumulating data indicate that both VCAM-1 and PAI-1 promote tumor metastasis and cellular adhesion. These activities are likely involved in post-operative recurrence. We plan to perform

a validation study to predict post-operative recurrence using the plasma concentrations of VCAM-1 and PAI-1 in the near future.

In conclusion, we have demonstrated that a combination prediction model based on the plasma concentrations of VCAM-1 and PAI-1 was a useful biomarker for predicting post-operative recurrence in patients with colorectal cancer. Our strategy, which utilizes a multiplex immunoassay system, may be a powerful tool for identifying biomarkers in clinical settings.

Acknowledgments

This study was supported by the Third-Term Comprehensive 10-Year Strategy for Cancer Control, a Grant-in-Aid for Scientific Research; the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NiBio); a Grant-in-Aid for Cancer Research (H20-20-9) from the Ministry of Health, Labour and Welfare; and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (19209018). The following people have played very important roles in the conduct of this project: Hiromi Orita, Hideko Morita, and Mari Araake.

Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Metastasis (+) *vs* (−) in independent samples of colorectal cancer (CRC) (n = 28).

Fig. S2. Post-operative recurrence (+) *vs* (−) in stage III colorectal cancer (CRC) (n = 45).

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doi: 10.1111/j.1349-7006.2010.01595.x

Jpn J Clin Oncol 2010;40(8)732–738
doi:10.1093/jjco/hyq047
Advance Access Publication 29 April 2010

A Phase 1 Clinical Study of Temsirolimus (CCI-779) in Japanese Patients with Advanced Solid Tumors

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Received November 24, 2009; accepted March 18, 2010

Objective: Temsirolimus (CCI-779) is a novel inhibitor of the mammalian target of rapamycin. This Phase 1 study was aimed at investigating the maximum-tolerated dose, toxicity, pharmacokinetics and antitumor activity in Japanese patients with advanced solid tumors.
Methods: Temsirolimus was given as a 30 min intravenous infusion once a week. Patients with solid tumors not amenable to standard forms of treatment were eligible. Dose escalation of temsirolimus was planned from 15, 45, 80 to 165 mg/m^2. The pharmacokinetics of temsirolimus and sirolimus in whole blood were examined for cycles 1, 2, 4 and 5 of treatment.
Results: Ten patients (median age 60.5 years; range 41–69 years) with advanced solid tumors were enrolled. Their primary cancers were renal cell carcinoma (five patients), lung cancer (three patients) and colorectal cancer (two patients). The major toxicities were hypophosphatemia diarrhea, hyperglycemia, stomatitis, pyrexia, elevated aspartate aminotransferase, rash, reduced neutrophil count, elevated alanine aminotransferase, anorexia, hypertriglyceridemia and somnolence. Two of three patients who received temsirolimus 45 mg/m^2 developed dose-limiting toxicities of Grade 3 stomatitis (one patient) and Grade 3 diarrhea (two patients). The maximum-tolerated dose was 15 mg/m^2. The peak blood concentrations of temsirolimus and sirolimus, a major active metabolite, increased in a dose-dependent manner. The area under the concentration-versus-time curve of sirolimus, but not temsirolimus, increased in a dose-dependent manner.
Conclusions: The recommended dose for Phase 2 clinical studies of temsirolimus in Japanese patients with advanced solid tumors is 15 mg/m^2 intravenously once a week.

Key words: Phase 1 study – CCI-779 – temsirolimus – advanced solid tumor

INTRODUCTION

Temsirolimus (CCI-779), a novel inhibitor of mammalian target of rapamycin (mTOR), is an ester analogue of the immunosuppressive agent sirolimus (rapamycin, trade name: Rapamune®, sale: Pfizer Inc.), which was approved for the prophylaxis of organ rejection in patients receiving renal transplants in the USA. Temsirolimus inhibits several key signal transduction pathways regulating G1 phase of the cell cycle by inhibiting the activity of mTOR, a cell cycle regulatory kinase, and ultimately blocks progression from G1 to S phase of the cell cycle (1). Temsirolimus is also reported to show an inhibitory effect on the growth of various tumor cells, especially on the tumor cells with mutation or deletion of *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor gene (2–10).

On the basis of the inhibitory effect of temsirolimus on the growth of tumor cells in preclinical studies, clinical studies of temsirolimus have been conducted in patients with a variety of malignant tumors, including advanced renal cell carcinoma (13–20,22,23). In the USA, temsirolimus has been approved for the treatment of advanced renal cell carcinoma. In Europe, temsirolimus has been approved for the

first-line treatment of patients with advanced renal cell carcinoma who have at least three of six poor prognostic factors (Trade name: Torisel®, INN: temsirolimus, sale: Pfizer Inc. (21,24)). The recommended dose of temsirolimus is 25 mg/body infused intravenously over a 30–60 min period once a week.

On the basis of the above results, a Phase 1 study was also conducted in Japan to investigate the safety, tolerability, pharmacokinetics and antitumor activity of temsirolimus in patients with advanced solid tumors. Two different temsirolimus schedules were used in US, European and international studies (daily for 5 days every second week and once a week) and showed that temsirolimus was well tolerated and had antitumor activity (14,15,22,23). The weekly administration was chosen for the conduct of this clinical study in Japan because this is the schedule that has been approved in the USA and Europe and it is a more convenient schedule for patients than daily for 5 days every second week.

PATIENTS AND METHODS

Study Design

This was an open-label, single-center, Phase 1 study. The primary objective was to investigate the safety and tolerability of temsirolimus administered by a 30 min intravenous infusion once a week. The secondary objective was to collect preliminary data of the pharmacokinetics and antitumor activity of temsirolimus.

The study protocol was approved by the institutional review board of the National Cancer Center, and the study was conducted from October 2002 to March 2005 in accordance with Good Clinical Practice.

Patients

Patients were enrolled in the study only if they met the following entry criteria:

Inclusion criteria were: (i) histologically and cytologically confirmed diagnosis of advanced solid tumors; (ii) refractory to standard therapy or no appropriate therapy; (iii) measurable lesion; (iv) at least 4 weeks since any prior chemotherapy, hormonal therapy, radiation therapy and/or surgery, and any other investigational agent use (at least 6 weeks since nitrosourea and mitomycin C); (v) available to be hospitalized from the day before the first administration through the fourth week; (vi) age $\geq$20 and $<$75 years at the time of consent; (vii) neutrophil count $\geq$1500/mm^3, platelet count $\geq$100 000/mm^3, hemoglobin $\geq$8.5 g/dl; (viii) adequate renal function: serum creatinine $<$1.5 mg/dl, creatinine clearance (24 h method) $\geq$50 ml/min; (ix) adequate hepatic function: total bilirubin $<$2 mg/dl, glutamic oxaloacetic transaminase (aspartate aminotransferase, AST) and glutamic pyruvic transaminase (alanine aminotransferase, ALT) $<$3 times the institutional upper limit of the normal range; (x) serum cholesterol $\leq$350 mg/dl, triglyceride $\leq$500 mg/dl; (xi) performance status 0–2; (xii) estimated life expectancy of at least 3 months and (xiii) written informed consent for the study.

Exclusion criteria were: (i) concomitant use of CYP3A4 inhibitors or inducers, such as anticonvulsants and rifampicin, where the treatment could not be discontinued or switched to a different medication; (ii) symptomatic brain metastasis; (iii) positive HIV antibody, HBs antigen or HCV antibody; (iv) infection requiring systemic medication; (v) complications that were difficult to control by medication or other therapies; (vi) use of immunosuppressive agents within 3 weeks prior to enrollment; (vii) regular use of steroids; (viii) concurrent angina, myocardial infarction within 6 months prior to starting study or heart disease that was difficult to control with medication or other therapies; (ix) history of hypersensitivity to diphenhydramine or its structurally related antihistamine drugs; (x) history of hypersensitivity to polysorbate 80 or polyethylene glycol contained in the diluent of the investigational drug, (xi) women who were pregnant or lactating, or not willing to use acceptable contraception during the study and for at least 3 months following the last study drug administration and (xii) inappropriate patients for the study for any other reason such as screening test results by the investigator.

Dosage and Administration

Temsirolimus was supplied by Wyeth K.K. (Tokyo, Japan) in 5 ml vials for intravenous injection; each contained 25 mg/ml of temsirolimus, co-packaged with a specific diluent.

Diphenhydramine, an antihistamine drug, was intravenously infused at a dose of 30 mg over 30 min $\sim$1 h before the temsirolimus administration to prevent hypersensitivity reactions. Approximately 30 min after the diphenhydramine infusion, temsirolimus was administered by a 30 min intravenous infusion.

The doses of temsirolimus were 15, 45, 80 or 165 mg/m^2. The 15, 45 and 165 mg/m^2 doses correspond to flat doses of 25, 75 and 250 mg. The latter were used once a week in a Phase 2 clinical study in kidney cancer patients and tolerability and efficacy were observed at all doses (14). Thus, these doses were used in this study of Japanese patients. In addition, 80 mg/m^2 was used as an intermediate dose between 45 and 165 mg/m^2 so that there would not be a 3.7-fold increase in dose in the escalation. Temsirolimus was administered once a week as used previously (14,15,22) and at least three administrations were planned. The fourth or subsequent administrations of temsirolimus were allowed to continue until tumor progression or unacceptable toxicity occurred.

Dose Escalation Method

On the basis of the Guidelines for Clinical Evaluation of Anticancer Drugs (11), three patients were administered temsirolimus in each dose group. Additional patients were

treated at that dose level or dose escalation was performed according to the number of patients with unacceptable toxicity.

Definition of Dose-limiting Toxicity

Tolerability of temsirolimus was assessed on the basis of the safety evaluation of the initial three-weekly administrations. When the following events related to temsirolimus occurred before the fourth administration, the event was defined as unacceptable toxicity: (i) Grade 3 or 4 non-hematologic toxicity (exclusion: nausea or vomiting without the use of appropriate antiemetic drugs, and serum triglycerides <1500 mg/dl recovering to Grade 2 by the next week), (ii) Grade 4 thrombocytopenia, (iii) Grade 4 neutropenia lasting 5 days or more, (iv) Grade 4 febrile neutropenia or (v) delay of administration for 2 weeks or more due to prolonged toxicity.

The severity of toxicities was assessed according to National Cancer Institute Common Toxicity Criteria (NCI-CTC Version 2.0, 30 April 1999).

Efficacy Evaluation

On the basis of the guidelines of Response Evaluation Criteria in Solid Tumors (RECIST) (12), best overall response was assessed.

Pharmacokinetics

Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 24, 72, 96 and 168 h (just before the time of second administration) after the first administration of temsirolimus and at the time of fifth administration (168 h after the fourth administration). Blood concentrations of temsirolimus and sirolimus, a major active metabolite, were measured by Taylor Technology Inc. (NJ, USA) using a validated liquid chromatography/tandem mass spectrometry method. The limit of detection was 0.25 ng/ml for each compound. The pharmacokinetic parameters of temsirolimus and sirolimus in whole blood, including maximum concentration (C_{max}), time to maximum concentration (t_{max}), terminal half-life ($t_{1/2}$), area under the concentration-versus-time curve (AUC), apparent clearance (corrected for unknown fraction of drug metabolized; CL/f_m) and apparent volume of distribution at steady state (Vd_{ss}/f_m), were calculated using a non-compartmental analysis technique.

RESULTS

Patients' Characteristics

Ten patients with solid tumors were enrolled in this study (Table 1). The median age was 60.5 years with a range of 41–69 years. All patients had at least one prior chemotherapy and seven patients also had surgery for primary tumors.

Table 1. Patients' characteristics

	Number of patients ($n = 10$)
Sex	
Male	6
Female	4
Age (years)	
≥ 40 to <50	2
≥ 50 to <60	2
≥ 60 to <70	6
Median (range)	
60.5 (41–69)	10
Performance status	
0	2
1	8
Number of prior	
1	3
Chemotherapy	
2–3	2
Regimens	
≥ 4	5
Prior treatment	
Chemotherapy	1
Chemotherapy + radiation	2
Chemotherapy + surgery	7
Primary cancer	
Renal cell carcinoma	5
Lung cancer	1
Small cell lung cancer	1
Non-small cell lung cancer	1
Sigmoid colon cancer	1
Colorectal cancer	1

The primary cancer of five patients was renal cell carcinoma. Three patients had lung cancer and two had colon/colorectal cancer. With regard to prior chemotherapy, five patients had received four or more regimens.

Dose Escalation and Unacceptable Toxicity

The starting dose of 15 mg/m^2 was administered to three patients. One subject discontinued temsirolimus treatment after the first administration because of Grade 4 gastrointestinal perforation. The Efficacy and Safety Evaluation Committee determined that this adverse event was not an unacceptable toxicity, and the patient was excluded from tolerability evaluation and another patient was enrolled. The other three patients in the 15 mg/m^2 dose group did not develop unacceptable toxicity and the escalated dose of

45 mg/m^2 was administered to three patients. Two of them developed unacceptable toxicity. Grade 3 stomatitis and diarrhea occurred in one patient and Grade 3 diarrhea occurred in the other patient. According to the prescribed procedure, further dose escalation was discontinued, and three additional patients were enrolled in the starting dose of 15 mg/m^2 to confirm tolerability. None of these three additional patients developed unacceptable toxicity. From these results, temsirolimus administered as a monotherapy once a week at an intravenous dose of 15 mg/m^2 was judged to be acceptable.

In the 15 mg/m^2 dose group, six of seven patients received three or more weekly administrations of temsirolimus. Three received 15-, 26- and 47-weekly administrations. The patient in the 45 mg/m^2 dose group who did not have unacceptable toxicity received four-weekly administrations of temsirolimus.

Toxicities

All patients (10/10) experienced at least one adverse event related to temsirolimus administration (Table 2). A greater percentage of patients who received temsirolimus 45 mg/m^2 experienced Grade 3 or 4 adverse events (100%) than those who received 15 mg/m^2 (29%).

Temsirolimus-related adverse events of any grade that occurred in any cycle in at least six patients were hypophosphatemia (90.0%), diarrhea (80.0%), hyperglycemia (80.0%), stomatitis (70.0%), pyrexia (70.0%), elevated AST (70.0%), rash (70.0%), reduced neutrophil count (60.0%), elevated ALT (60.0%), anorexia (60.0%), hypertriglyceridemia (60.0%) and somnolence (60.0%) (Table 2).

The adverse events of Grade 3 or higher that were related to temsirolimus treatment are as follows. In the period from the first to the third dose of temsirolimus, two patients had diarrhea and one patient each had a perforated digestive tract, stomatitis, malaise, hyperglycemia, hypokalemia or hypophosphatemia (Table 2). In the fourth and subsequent administrations, two patients had supraventricular arrhythmia and one each had pulmonary infection and hypercholesterolemia. The perforated digestive tract, malaise, hypercholesterolemia and supraventricular arrhythmia (one patient) occurred in the 15 mg/m^2 group and the diarrhea, stomatitis, hyperglycemia, hypophosphatemia, hypokalemia, pulmonary infection and supraventricular arrhythmia (one patient) occurred in the 45 mg/m^2 group. These adverse events resolved when temsirolimus treatment was stopped and the patients were treated appropriately.

For Grade 2 or lower adverse events of hemoglobin decreased, hematocrit decreased, alkaline phosphatase increased, epistaxis and onychia, the frequency of occurrence increased with the number of administrations of temsirolimus. However, for the adverse events of Grade 3 or higher, the frequency of occurrence did not increase with the number of administrations.

The reasons for discontinuing temsirolimus treatment in this trial were adverse events (four patients), progressive disease (PD; five patients) and other (one patient). Diarrhea, perforated digestive tract, stomatitis and supraventricular arrhythmia were the adverse events that led to discontinuation. No patients died in this trial.

Antitumor Activity

All 10 patients who received temsirolimus were eligible for this study and were included in efficacy evaluation. Eight patients had stable disease (SD) and two patients had PD as their best overall responses. Of those with SD, 71% (5/7) received temsirolimus 15 mg/m^2 and 100% (3/3) received temsirolimus 45 mg/m^2. The median length of SD was 35 days (range 20+ to 371 days, Table 3). Three patients with renal cell carcinoma who received temsirolimus 15 mg/m^2 had a long period of SD (113–371 days).

Pharmacokinetics

Whole blood samples were collected from seven patients in the 15 mg/m^2 dose group and three patients in the 45 mg/m^2 dose group for measurement of concentrations of temsirolimus and sirolimus. After the intravenous infusion of temsirolimus was completed, concentrations of temsirolimus in whole blood decreased more rapidly than those of sirolimus. After a single dose of temsirolimus, mean C_{max} for temsirolimus increased in a dose-dependent manner but mean AUC did not (Table 4). Mean Vd_{ss} for temsirolimus increased with increasing dose from 83.85 l after the 15 mg/m^2 dose to 162.9 l after the 45 mg/m^2 dose. Mean CL also increased with increasing dose (8.48 l/h after 15 mg/m^2 and 27.19 l/h after 45 mg/m^2). Mean terminal half-life for temsirolimus was 14.77 and 13.47 h for 15 and 45 mg/m^2, respectively.

After a single dose of temsirolimus, sirolimus mean C_{max} and AUC were increased with increasing dose; however, they did not increase in a dose-proportional manner (Table 4). A similar dose-proportional increase in sirolimus was observed in the previous study (22). Mean Vd_{ss}/f_m and CL/f_m also appeared to increase with increasing dose. Mean terminal half-life for sirolimus was 67.04 and 59.15 h for 15 and 45 mg/m^2, respectively, and, therefore, was longer than $t_{1/2}$ for temsirolimus. After a single dose of temsirolimus, mean AUC_{sum} also exhibited a dose-dependent increase. Mean AUC_{ratio} was 2.94 after the 15 mg/m^2 dose and 4.79 after the 45 mg/m^2 dose. Inter-subject variability was generally modest after single and multiple doses of temsirolimus.

DISCUSSION

This Phase 1 trial in Japanese patients with advanced solid tumors showed that 15 mg/m^2 temsirolimus infused intravenously once a week was well tolerated. The result of evaluation by dose groups demonstrated that none of the patients

Table 2. Temsirolimus-related adverse events, including laboratory abnormalities, reported in any cycle in at least 30% of patients who received temsirolimus

Adverse event	Temsirolimus 15 mg/m^2 ($n = 7$)		Temsirolimus 45 mg/m^2 ($n = 3$)		Total ($n = 10$)	
	All grades[a] (n)	Grades 3 and 4[a] (n)	All grades[a] (n)	Grades 3 and 4[a] (n)	All grades[a] (n)	Grades 3 and 4[a] (n)
Any	7	2	3	3	10	5
Diarrhea	6		2	2	8	2
Nausea	2		1		3	
Stomatitis	5		2	1	7	1
Vomiting	3				3	
Malaise	2	1	3		5	1
Pyrexia	4		3		7	
Pharyngitis	3				3	
Weight loss	3		2		5	
Anorexia	3		3		6	
Dysgeusia	2		1		3	
Somnolence	4		2		6	
Headache	3		2		5	
Nasal bleeding	4		1		5	
Rash	5		2		7	
Flushing	3		2		5	
Hypertension	3		1		4	
Phlebitis	3				3	
Leukocytes decreased	2		3		5	
Lymphocytes decreased	2		1		3	
Monocytes increased	2		1		3	
Reduced neutrophil count	3		3		6	
Platelets decreased	2		2		4	
Hemoglobin decreased	3		2		5	
Hematocrit decreased	3		1		4	
Elevated ALT	4		2		6	
Elevated AST	5		2		7	
Creatinine increased	2		2		4	
LDH increased	3		2		5	
Alkaline phosphatase increased	3		1		4	
Hypercholesterolemia	4	1	1		5	1
Hyperglycemia	5		3	1	8	1
Hypertriglyceridemia	5		1		6	
Hypophosphatemia	6		3	1	9	1

In the 15 mg/m^2 group, one patient each had perforated digestive tract or supraventricular arrhythmia. In the 45 mg/m^2 group, one patient each had hypokalemia, pulmonary infection or supraventricular arrhythmia. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.
[a]NCI-CTC Ver 2.0.

in 15 mg/m^2 dose group experienced unacceptable toxicity, whereas two of three patients experienced Grade 3 stomatitis and diarrhea in 45 mg/m^2 dose group. For single doses of 15 or 45 mg/m^2, the exposure of temsirolimus and its major active metabolite sirolimus in whole blood increased in a dose-dependent but subproportional manner. Inter-subject variability between single (the first) and multiple (the fourth) dosing periods was low. The pharmacokinetic parameters for

temsirolimus and sirolimus that were measured in this study were very similar to those measured in the Phase 1 study of patients with solid tumors treated with intravenous temsirolimus on a weekly schedule (22) and in the Phase 2 study of patients with renal cell carcinoma treated with temsirolimus on a weekly schedule (14).

Table 3. Individual efficacy results of patients treated with temsirolimus

Temsirolimus dose	Primary cancer	Best overall response	Time to progression (days)
15 mg/m^2	Lung cancer	PD	20
	Colorectal cancer	PD	20
	Small cell lung cancer	SD	20+
	Sigmoid colon cancer	SD	23+
	Renal cell carcinoma	SD	113
	Renal cell carcinoma	SD	244
	Renal cell carcinoma	SD	371
45 mg/m^2	Renal cell carcinoma	SD	20+
	Renal cell carcinoma	SD	27+
	Non-small cell carcinoma	SD	42+

SD, stable disease; PD, progressive disease.

The common temsirolimus-related adverse events were hypophosphatemia, diarrhea, hyperglycemia, stomatitis, pyrexia, elevated AST, rash, reduced neutrophil count, elevated ALT, anorexia, hypertriglyceridemia and somnolence. These adverse events also have been reported in other studies of patients treated with temsirolimus (14,15,22,23). Because only 10 Japanese patients were treated in this study, we cannot accurately determine whether the incidence of these adverse events in this population is similar to the incidence in populations in other studies.

In the Phase 3, international, interferon-α-controlled study of temsirolimus conducted in patients with advanced renal cell carcinoma, the patients administered temsirolimus alone at the dose of 25 mg/body (15 mg/m^2) demonstrated a significant improvement in overall survival and progression-free survival when compared with the patients administered interferon-α alone (15). There was no significant difference in the objective response rate (percentage of patients with complete and partial responses) for patients treated with temsirolimus or interferon-α but patients treated with

Table 4. Pharmacokinetic parameters following intravenous administration of temsirolimus

	Single dose		Multiple doses[a]	
	15 mg/m^2	45 mg/m^2	15 mg/m^2	45 mg/m^2[b]
Temsirolimus (mean ± SD)				
C_{max} (ng/ml)	1013.9 ± 316.3	1793.3 ± 421.6	912.0 ± 50.0	1580.0
t_{max} (h)	0.51 ± 0	0.34 ± 0.2	0.43 ± 0.2	0.25
$t_{1/2}$ (h)	14.77 ± 0.68	13.47 ± 1.09	13.76 ± 0.15	11.65
AUC (ng h/ml)[c]	2873 ± 358	2750 ± 250	2203 ± 203	2403
CL (l/h)	8.48 ± 1.73	27.19 ± 6.37	11.54 ± 1.30	24.66
Vd_{ss} (l)	83.85 ± 10.91	162.9 ± 26.67	88.29 ± 9.61	153.4
Sirolimus (mean ± SD)				
C_{max} (ng/ml)	89.1 ± 40.5	157.3 ± 37.1	118.7 ± 28.5	190.0
t_{max} (h)	7.53 ± 11.3	1.87 ± 1.9	2.03 ± 1.7	0.52
$t_{1/2}$ (h)	67.04 ± 17.37	59.15 ± 28.94	71.36 ± 12.24	35.33
AUC (ng h/ml)[c]	8168 ± 2089	13 524 ± 9763	9061 ± 1788	7772
CL/f_m (l/h)	3.05 ± 0.61	7.11 ± 3.41	2.41 ± 0.90	7.37
Vd_{ss}/f_m (l)	189.6 ± 23.46	325.2 ± 103.4	139.0 ± 15.55	273.6
Composite				
AUC_{ratio} (sirolimus:temsirolimus)	2.94 ± 1.08	4.79 ± 3.03	5.20 ± 1.69	3.35
AUC_{sum} (ng h/ml)	11 041 ± 1935	16 274 ± 9970	13 583 ± 2715	10 469

SD, standard deviation; AUC, area under the curve.
[a]Following the fourth administration.
[b]A blood sample from only one patient was available.
[c]AUC for single dose means $AUC_{0-\infty}$ and for multiple doses means $AUC_{0-\tau}$.

temsirolimus had a significantly higher clinical benefit rate (percentage of patients with complete and partial responses and SD for at least 24 weeks) than did patients treated with interferon-α. Thus, long-term SD was an important component of the temsirolimus response in patients with advanced renal cell carcinoma. For patients treated with temsirolimus, the objective response rate was 9% and the clinical benefit rate was 32%. In this Phase 1 study, three (3) patients with renal cell carcinoma had long-term SD lasting 113–371 days. The fact that 3 of 10 Japanese patients had long-term SD and none had partial or complete responses agrees with the results obtained in the Phase 3 study and suggests that the antitumor activity of temsirolimus in Japanese cancer patients is similar to that in renal cell carcinoma patients of the international population.

From the above results, tolerability at 15 mg/m^2 was confirmed, and the recommended dose for Japanese patients with advanced solid tumors was determined to be temsirolimus 15 mg/m^2 as an intravenous infusion once a week. Further clinical studies will be required to investigate the safety and efficacy of temsirolimus in detail.

Acknowledgements

We thank all the patients and clinical personnel who participated in this study.

Funding

This clinical trial was supported by Wyeth K.K. (Tokyo, Japan), which was acquired by Pfizer Inc. in October 2009.

Conflict of interest statement

None declared.

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