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

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

XP capecitabine plus cisplatin

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

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

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

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

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References

1. Foundation for Promotion of Cancer Research. Cancer Statistics in Japan 2010. Available from http://ganjoho.ncc.go.jp/public/statistics/backnumber/2010_en.html. Accessed 30 June 2011.
2. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol. 2008;9:215–21.
3. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med. 2007;357:39–51.
4. Bang Y-J, Chung HC, Xu JM, Lordick F, Sawaki A, Lipatov O, et al. Pathological features of advanced gastric cancer: relationship to human epidermal growth factor receptor 2 positivity in the global screening programme of the ToGA trial. J Clin Oncol. 2009;27:abstract 4556.
5. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989;244:707–12.
6. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial. Lancet. 2010;376:687–97.
7. Tsuburaya A, Narahara H, Imamura H, Hatake K, Imamoto H, Esaki T, et al. GC0301/TOP002 Study Group. Updated result on the 2.5-year follow-up of GC0301/TOP-002: randomized phase III study of irinotecan plus S-1 (IRI-S) versus S-1 alone as first-line treatment for advanced gastric cancer (AGC). J Clin Oncol. 2009;27:abstract 4544.
8. Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, et al. Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomized phase 3 study. Lancet Oncol. 2009;10:1063–9.
9. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol. 2006;24:4991–7.
10. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med. 2008;358:36–46.
11. Ohtsu A. Chemotherapy for metastatic gastric cancer: past, present, and future. J Gastroenterol. 2008;43:256–64.
12. Ohtsu A, Yoshida S, Saijo N. Disparities in gastric cancer chemotherapy between the East and West. J Clin Oncol. 2006;24:2188–96.

13. Sasako M, Inoue M, Lin JT, Khor C, Yang HK, Ohtsu A. Gastric Cancer Working Group report. Jpn J Clin Oncol. 2010;40(Suppl 1):i28–37.
14. Warneke VS, Behrens H-M, Hartmann JT, Held H, Becker T, Schwarz NT, et al. Cohort study based on the seventh edition of the TNM classification for gastric cancer: proposal of a new staging system. J Clin Oncol. 2011;29:2364–71.
15. Chau I, Norman AR, Cunningham D, Waters JS, Oates J, Ross PJ. Multivariate prognostic factor analysis in locally advanced and metastatic esophago-gastric cancer—pooled analysis from three multicenter, randomized, controlled trials using individual patient data. J Clin Oncol. 2004;22:2395–403.
16. Yoshida M, Ohtsu A, Boku N, Miyata Y, Shirao K, Shimada Y, et al. Long-term survival and prognostic factors in patients with metastatic gastric cancers treated with chemotherapy in the Japan Clinical Oncology Group (JCOG) study. Jpn J Clin Oncol. 2004;34:654–9.
17. Adachi Y, Yoshida K, Inomata M, Sato K, Shiraishi N, Kitano S, et al. Pathology and prognosis of gastric carcinoma. Well versus poorly differentiated type. Cancer. 2000;89:1418–24.
18. Sasako M, Sano T, Yamamoto S, Kurokawa Y, Nashimoto A, Kurita A, et al. Japan Clinical Oncology Group. D2 lymphadenectomy alone or with para-aortic nodal dissection for gastric cancer. N Engl J Med. 2008;359:453–62.
19. Lee SS, Lee JL, Ryu MH, Chang HM, Kim TW, Kang HJ, et al. Combination chemotherapy with capecitabine (X) and cisplatin (P) as first line treatment in advanced gastric cancer: experience of 223 patients with prognostic factor analysis. Jpn J Clin Oncol. 2007;37:30–7.

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ORIGINAL ARTICLE

Prospective evaluation of incidence and severity of oral mucositis induced by conventional chemotherapy in solid tumors and malignant lymphomas

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Abstract
Purpose Oral mucositis (OM), a complication frequently associated with cancer chemotherapy, may decrease treatment efficacy due to dose reduction or impair the patient's quality of life. The purpose was to determine the incidence and severity of OM and its sequelae in patients receiving conventional chemotherapy for various malignancies.
Methods Two hundred twenty-seven patients (male, 33%; female, 66%) who received chemotherapy for head and neck cancer, esophageal cancer, colorectal cancer, breast cancer, and malignant lymphomas at the Cancer Institute Hospital between January 2007 and December 2008 were examined with questionnaires, prospectively.
Results The incidence of OM was highest in patients with breast cancer (76.5%), then head and neck cancer (67.7%), colorectal cancer (63%), esophageal cancer (57.8%), and malignant lymphoma (42.9%). However, patients who experienced severe OM (≥grade 3) were rare: at most 4.8%. The high-risk regimens for OM were TPF (85.7%), FOLFIRI (80%), CAF (78.8%), AC (70.6%), and FOLFOX (60%). OM was associated with gastrointestinal adverse events, anorexia, diarrhea, and dysphagia, which aggravated quality of life. There was no correlation between incidence of OM and prior therapy, PS, oral care, or laboratory data. There was no statistically significant correlation between OM and overall survival. The predictive factor was history of OM in previous chemotherapy.
Conclusion OM frequently occurs in patients with various tumors receiving conventional chemotherapy. Despite low-grade OM, they might cause gastrointestinal adverse events. Adequate preventive treatment for OM is required depending on each chemotherapy regimen and each patient's OM history.

Keywords Oral mucositis · Incidence · Severity · Chemotherapy

Introduction

Aggressive combined modality therapies are used in patients with various malignancies and can help them achieve longer survival but may be accompanied by increasing frequency of adverse events. Of these, oral mucositis (OM) is one of the most frequent complications induced by chemotherapy, with or without radiotherapy [3]. Serious OM can cause considerable pain requiring opioid analgesia, sepsis in neutropenic patients [8], and dysphagia requiring placement of feeding tubes. Thus OM can limit the tolerated dose of chemotherapy with or without radiotherapy, which can in turn affect both progression-free survival and overall survival [7]. In 2004, the first evidence-based clinical guidelines for OM prevention and treatment were published by the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology [10]. Until then, OM had been underestimated. Therefore, major progress in recognizing OM as one of the important adverse events

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of chemotherapy and elucidating epidemiology, pathology, and clinical outcomes of OM has occurred recently. To prevent OM, the use of palifermin, keratinocyte growth factor-1, is recommended as level I evidence/grade A in patients with hematological malignancies receiving high-dose chemotherapy and total body irradiation with autologous stem cell transplantation. Other agents, such as Saforis (L-glutamine in a proprietary oral drug delivery system) [4] and RK-0202 (*N*-acetylcysteine in a proprietary mouth rinse formulation) are being developed [2]. These agents are expensive and may themselves cause adverse events including rash, pruritus, erythema, mouth and tongue disorders, as well as taste alteration. Therefore, we need to be careful in choosing these medications [11]. OM may occur in 70% to 100% of patients who have undergone high-dose chemotherapy plus total body irradiation followed by hematopoietic stem cell transplantation [9, 11] and in up to 100% of patients with head and neck cancer receiving chemoradiotherapy [6, 12]. However, there is no prospective study of the incidence of OM induced by standard chemotherapy for common solid tumors and malignant lymphomas. The aim of this study was to clarify the OM incidence and severity in patients with common solid tumors and malignant lymphomas and predict which patients would be indicated for emerging preventing medicines of OM.

Patients and methods

Patients

This study enrolled patients who were at least 20 years of age and had head and neck squamous cell cancer (HNSCC), esophageal cancer, breast cancer, colorectal cancer, gastric cancer, malignant lymphoma, or cancer of unknown primary site. They were all scheduled to undergo conventional chemotherapy for each cancer with or without radiotherapy from January 2007 to December 2008. Patients included were examined by self-report questionnaires about oral care, onset of OM, and gastrointestinal complications of anorexia, diarrhea, or dysphagia. OM was graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0) by clinical physicians. Patients were surveyed for detailed medical history, complete physical examination, blood counts, and chemistry profiles. The institutional review board at Cancer Institute Hospital approved the study protocol; all patients gave written informed consent before all study-related procedures. We excluded patients who gave inadequate consent or withdrew consent, died before chemotherapy, or were lost for follow-up.

Treatment regimens and OM prophylaxis

Patients were treated by conventional chemotherapy for each pathologically confirmed primary malignancy. TPF regimen (docetaxel at a dose of 75 mg/m^2 i.v. on day 1, cisplatin at a dose of 75 mg/m^2 i.v. on day 1, and fluorouracil (5-FU) at a dose of 750 mg/m^2/day i.v. as a continuous infusion on days 1 to 5 were administered every 3 weeks) was used for patients with head and neck cancer. FOLFIRI regimen (irinotecan at a dose of 150 mg/m^2 i.v. on day 1, leucovorin at a dose of 200 mg/m^2 i.v. on day 1 followed by bolus 5-FU 400 mg/m^2, and a 46-h infusion of 5-FU at a dose of 2400 mg/m^2 on days 1 to 2 were administered every 2 weeks) or FOLFOX 4 regimen (oxiliplatin at a dose of 85 mg/m^2 i.v. on day 1, leucovorin at a dose of 100 mg/m^2 i.v. on days 1 to 2, followed by bolus 5-FU 400mg/m^2, and a 46-h infusion of 5-FU at a dose of 1200 mg/m^2/day as a continuous infusion on days 1 to 2 were administered every 2 weeks) were used for patients with metastatic colorectal cancer. CAF regimen (cyclophosphamide at a dose of 500 mg/m^2 i.v. on day 1, doxorubicin hydrochloride at a dose of 50 mg/m^2 i.v. on day 1, and 5-FU at a dose of 500 mg/m^2 intravenously on days 1 and 8 were administered every 3 weeks) and AC (doxorubicin hydrochloride at a dose of 60 mg/m^2 i.v on day 1 and cyclophosphamide at a dose of 600 mg/m^2, i.v. on day 1 were administered every 3 weeks) were used for patients with breast cancer. R-CHOP regimen (rituximab at a dose of 375 mg/m^2 i.v. on days 1, 8, and 15, cyclophosphamide at a dose of 750 mg/m^2 i.v. on day 1, doxorubicin hydrochloride at a dose of 50 mg/m^2 i.v. on day 1, vincristine sulfate at a dose of 1.4 mg/m^2 i.v., up to a maximal dose of 2 mg on day 1, and prednisone at a dose of 50 mg/m^2 i.v on day 1 followed by oral intake on days 2 to 5 every 3 weeks) was administered to patients with diffuse large B cell lymphoma; weekly rituximab administration was a clinical trial setting. TC regimen (carboplatin at an area under the curve dose of 6 mg/ml per minute and paclitaxel 200 mg/m^2 i.v. on day 1 every 3 weeks) was used for cancer of unknown primary site. Other regimens used for a few patients (at most three) were excluded from the summary table showing the OM incidence rate. Patients received some OM prophylaxis, including mouthwashes, gargling with sodium azulene sulfonate.

Endpoints

Primary endpoints were the incidence of OM of CTCAE at any grades and severity among each cancer patients. Secondary endpoints included the 2-year overall survival.

Statistical analysis

A chi square test for contingency tables was used to compare OM incidence in Table 2. Kruskal–Wallis test was

Table 1 Baseline patient characteristics

Total number of patients	227
Sex, no. (%)	
Male	76 (33%)
Female	151 (67%)
Age, years	
Median	57
Range	23–86
Diagnosis, no. (%)	
Head and neck squamous cell cancer	31 (14%)
Esophageal cancer	13 (6%)
Breast cancer	102 (50%)
Colorectal cancer	27 (12%)
Malignant lymphoma	49 (22%)
Cancer of unknown primary site (CUP)	5 (2%)

used to compare the incidence rate by primary focus of cancer and regimens. The mean data of C-reactive protein (CRP), white blood cell counts, and albumin concentration were calculated and compared between patients with and without OM using unpaired Student's *t* test. Differences were assessed with two-sided tests, with an alpha level of 0.05. The survival of patients with and without OM was estimated by Kaplan–Meier curves and was compared using the logrank test.

Results

Patients

A total of 237 cancer patients who were scheduled to receive chemotherapy or chemoradiotherapy were recruited into the study between January 2007 and December 2008. Of these, ten (4%) were ineligible (inadequate enrollment, $n=3$; lost for follow-up, $n=2$; withdrawal of consent, $n=1$; inadequate consent, $n=1$; discontinued treatment, $n=1$; death before treatment, $n=1$; unknown, $n=1$) Baseline patient characteristics at enrollment are listed in Table 1.

OM incidence and patient background

First we investigated which patient's background or OM prophylaxis affected the incidence rate of OM (Table 2). There was no significant difference in OM incidence rate between primary and recurrent cases, the presence or absence of past history of cancer, RT, chemotherapy, surgery, or oral cavity. Neither did oral disease, self-oral care, dentures, untreated teeth, nor age affects outcomes.

OM incidence rate and severity by primary focus of cancer

Next we compared OM incidence rate with primary focus of cancer (Fig. 1). Among the total study population, 82 (36%) of the 227 patients did not experience any OM during the courses of the protocol treatment. Seventy-eight (76.5%) of 102 breast cancer patients had grades 1 to 3 OM during four courses of treatment, which was the highest OM incidence rate. Furthermore, 21 (67.7%) of 31 HNSCC patients, 17 (63%) of 27 colorectal cancer patients, 7 (57.8%) of 13 esophageal cancer patients, 21 (42.9%) of 49 malignant lymphoma patients, and 1 (20%) of 5 cancer of unknown primary patients developed OM, respectively. The grades 2 and 3 OM incidence rates were higher in patients with HNSCC and breast cancer at 26% (11 of 42) and 23% (40 of 142), respectively. However, overall, only three patients (two with HNSCC and one with breast cancer) developed severe OM (≥grade 3), which showed that the severity of OM induced by conventional chemotherapy is not serious.

OM incidence rate by regimens

Then we evaluated OM incidence rate by regimens and individual anticancer agents. The high-risk regimens for OM were TPF (85.7%), FOLFIRI (80%), CAF (78.8%),

Table 2 Correlation between OM incidence and prior therapy or oral condition

	OM Incidence (%) Yes/No	*p* value
Newly diagnosed cancer/recurrence	63.4/64.5	1.00
Prior history of cancer	64.1/57.9	0.62
Prior history of radiation	63.6/63.0	1.00
Prior history of chemotherapy	63.9/65.6	1.00
Prior history of surgery	58.6/70.7	0.07
Prior history of oral cavity surgery	63.3/75	0.54
Denture (full/partial/no)	100/52.9/65.1	0.34
Untreated teeth	63.9/66.7	1.0
Self oral care/by nurse	64.0/50.0	1.0
Oral wound before chemotherapy	64.0/62.05	1.0

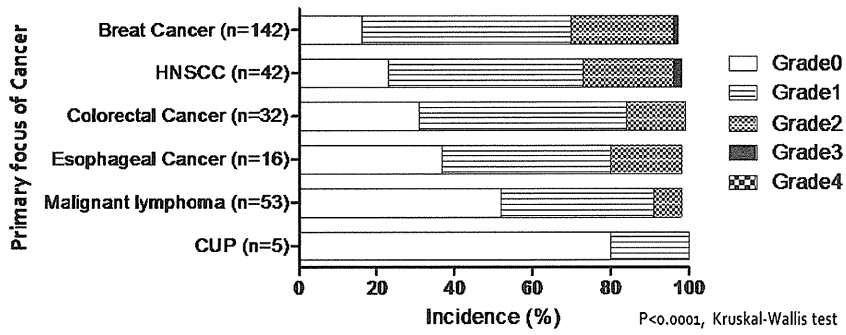

Fig. 1 OM incidence rate according to primary focus of cancer. OM was graded according to NCI CTCAE ver 3.0. *HNSCC* head and neck squamous cancer, *CUP* cancer of unknown primary

AC (70.6%), and FOLFOX (60%) in order (Fig. 2). Interestingly, AC regimen (doxorubicin hydrochloride and cyclophosphamide) for breast cancer showed the higher rate of OM, while CHOP regimen (cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone) for malignant lymphoma showed a much lower OM frequency (40.9%). These data indicated that 5-FU containing regimens and adriamycin–cyclophosphamide for breast cancer were high-risk regimens for OM onset.

OM incidence was related to the previous occurrence of OM

Next, the difference of OM incidence in patients with or without OM in previous chemotherapy through four cycles was investigated (Fig. 3). Patients who experienced OM in a previous cycle tended to develop OM again compared with patients without previous OM. These results imply that the increased incidence is not only related to drugs used in chemotherapy regimens but also to host factors.

OM was associated with gastrointestinal adverse events

We evaluated the correlation between gastrointestinal adverse events and OM incidence rate, to clarify whether OM influences a patient's quality of life. The patients with OM had higher incidence rate of gastrointestinal adverse events including anorexia (70.2% vs. 40.1%, $p<0.001$), diarrhea (32.5% vs. 16.3%, $p<0.001$), and dysphagia (29.1% vs. 9.9%, $p<0.001$) significantly than patients without OM. Patients who had gastrointestinal events were impaired in nutrition and performance status, which may worsen their quality of life.

Laboratory assessments

Laboratory data regarding neutrophil count, serum albumin level, and serum C-reactive protein (CRP) before treatment were assessed to have prognostic power on the onset of OM or not. All of them were found to be similar at day 1 of chemotherapy in patients with or without OM. Unexpectedly, serum CRP level under the lower limit of normal was the only factor found to be correlated with OM ($p=0.01$). However, the multivariate analysis showed no significant difference of CRP level in these two groups.

Overall survival at 2 years had no significant difference

Finally, the long-term follow-up data for disease outcomes were available for 257 patients. The median follow-up duration was 24 months. The 2-year overall survival rate was 74% in patients with OM, 84% in patients without OM

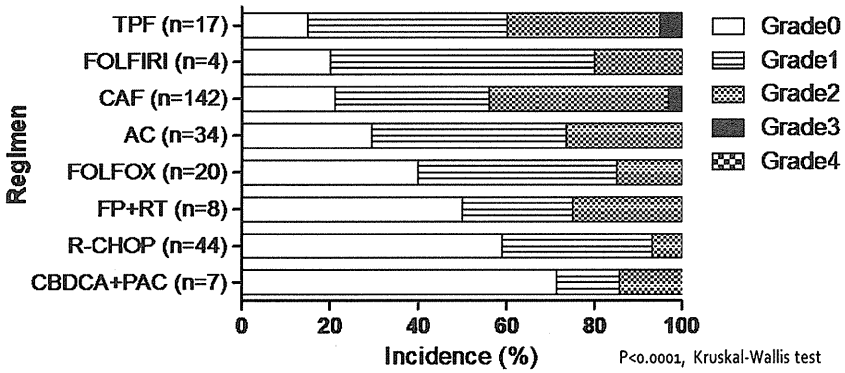

Fig. 2 OM incidence rate according to regimen. OM was graded according to NCI CTCAE ver 3.0. *TPF* docetaxel, cisplatin, 5-FU; *FOLFIRI* 5-FU, leucovorin, irinotecan (CPT11); *CAF* 5-FU, doxorubicin and cyclophosphamide; *AC* doxorubicin, cyclophosphamide; *FOLFOX* 5-FU, leucovorin, oxaliplatin, *FP+RT* 5-FU, cisplatin, radiation; *R-CHOP* rituximab, doxorubicin, vincistine, cyclophosphamide, prednisone; *CBDCA+PAC* carboplatin, paclitaxel

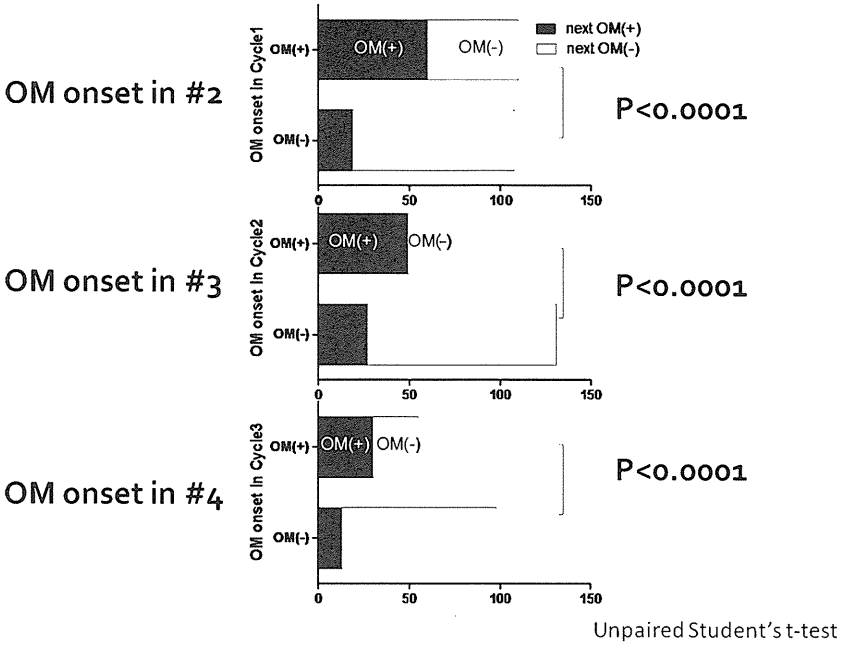

Fig. 3 OM incidence rate stratified by cycle number. *Black or white bar* shows the number of patients with OM or without OM, respectively

(Fig. 4). The Kaplan–Meier estimate curves for overall survival time showed no significant difference between the patients with OM and those without OM (p=0.651), with no median for the study reached.

Discussion

The effects of chemotherapy-induced OM had been underestimated until the first guidelines were published by Sonis et al. in 2004[10], updated by Peterson in 2008 [6]. However, a retrospective study of 599 patients who developed myelosuppression with chemotherapy showed that severe OM is associated with an increased risk of malnutrition or systemic infections, which can decrease the dose intensity of chemotherapy and may be life threatening [1].

In the present study, firstly we have shown that OM occurs more frequently than we expected in patients receiving conventional chemotherapy; 64% of the patients experienced OM. However, severe OM (grade 3 or more by CTCAE v.3.0) was rare. In previous retrospective studies, the frequency of severe OM of grade 3 or more was reported in the range from 1% to 13% in patients with non-Hodgkin lymphoma and breast, lung, or colorectal cancers who were treated with standard chemotherapy [4]. The previous data are consis-

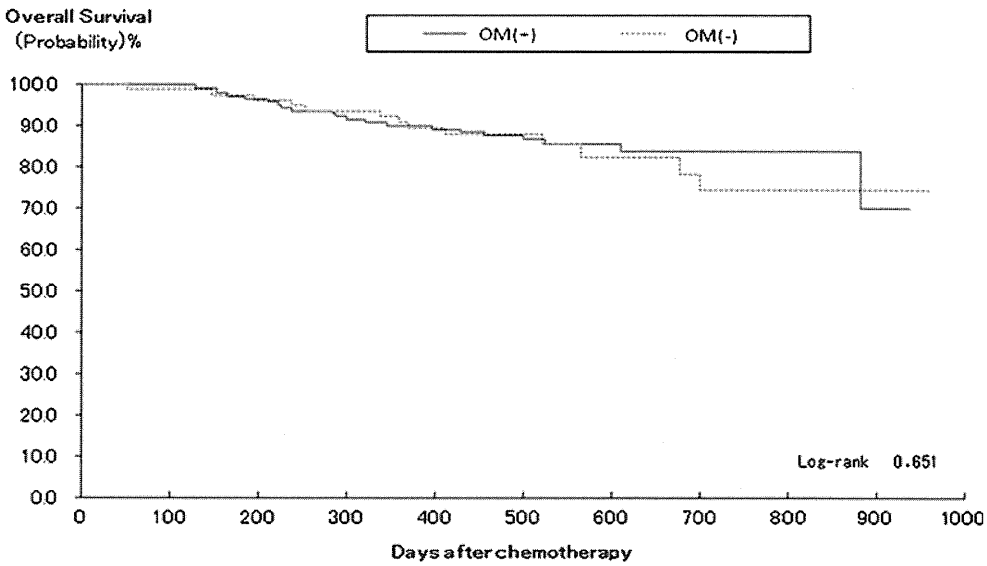

Fig. 4 Kaplan–Meier estimates of overall survival according to occurrence of OM

tent with our study; patients who developed severe OM in this study were from 0% to 4.8%.

However, low-grade OM occurred in at least 40% of patients with non-Hodgkin lymphoma and breast, head and neck, esophageal, colorectal cancers, or cancer of unknown primary site. Even patients who developed low-grade OM suffered from gastrointestinal adverse events including anorexia, diarrhea, and dysphagia. These adverse events impaired their quality of life and motivation for chemotherapy. This was among the first studies to show that low-grade OM can be accompanied with gastrointestinal adverse events.

Among the various chemotherapy regimens, those with higher risk for OM were 5-FU-containing regimens such as TPF, CAF, or FOLFIRI. 5FU has been reported to cause grade 3–4 OM with incidence of more than 15% [10]. The percentage of severe OM in this study was lower than the previous data. It might be due to careful oral care including dentists' check-up. However, 5-FU is of particular importance as the cause of chemotherapy-induced OM.

Another interesting point was the difference of OM incidence between R-CHOP regimen for non-Hodgkin lymphoma and AC regimen for breast cancer: 40.9% vs. 70.6%. It may be due to different patient backgrounds such as age and sex. In addition, the difference of supportive care should be considered as a cause of this difference. Sixteen milligrams of dexamethasone on day 1 and 8 mg on days 2–3 was used as an antiemetic agent with the AC regimen, while prednisolone 60 mg/m^2 on days 1–5 were used in R-CHOP regimen. This may imply that sufficient dose of prednisolone is more effective as an anti-mucositis agent than dexamethasone; however, in previous study, 40 mg/day prednisone had no difference in the degree of maximum mucositis expression and median duration of mucositis as compared with placebo group despite favoring shorter treatment interruptions in prednisone arm in prospective study [5]. Although we are in doubt regarding anti-OM effect by prednisolone, this is something we need to look at more closely in a next study.

Then we tried to find biomarkers for OM onset to choose patients for emerging preventing medicine, but we were unable to find one. Nevertheless, patients who experienced OM in previous cycle of chemotherapy showed a strong tendency for next OM onset. This is also the first evidence to show strong association of this tendency. To date, there is no significant predictive factor, so we have to wait to see OM onset after the administration of the first cycle of chemotherapy.

Finally, overall survival at 2 years showed no significant difference between patients with or without OM (Fig. 4). Relative dose intensity in patients with or without OM did not differ significantly because the grade of OM was mostly low. That was the reason why OM in this study did not affect survival.

This is a small and short-period study by a single institute, and the numbers of patients in different malignancies were variable. However, this is the first prospective and comprehensive study about OM induced by chemotherapy.

In conclusion, we conducted a prospective study to investigate OM incidence and severity with conventional chemotherapy. OM incidence was from 26% to 86%, varying according to regimens, but severe (grade 3 or more) OM was rare. Management of OM is important throughout chemotherapy not only for patients' quality of life but also on effective chemotherapy, because even low grade OM is associated with gastrointestinal AE. We suggest that preventive medicine should be used for high-risk patients of low-grade OM. Further study is warranted to investigate whether emerging agents for prevention of OM, such as palifermin, will be effective for high-risk patients: (1) patients with previous OM onset and (2) patients scheduled to undergo 5-FU-containing regimens and AC regimen for breast cancer.

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References

1. Elting LS, Cooksley C, Chambers M, Cantor SB, Manzullo E, Rubenstein EB (2003) The burdens of cancer therapy. Clinical and economic outcomes of chemotherapy-induced mucositis. Cancer 98:1531–1539
2. Garden AS, Chambers MS (2007) Head and neck radiation and mucositis. Curr Opin Support Palliat Care 1:30–34
3. Jones JA, Avritscher EB, Cooksley CD, Michelet M, Bekele BN, Elting LS (2006) Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. Supportive Care Cancer 14:505–515
4. Keefe DM, Schubert MM, Elting LS, Sonis ST, Epstein JB, Raber-Durlacher JE, Migliorati CA, McGuire DB, Hutchins RD, Peterson DE (2007) Updated clinical practice guidelines for the prevention and treatment of mucositis. Cancer 109:820–831

5. Leborgne JH, Leborgne F, Zubizarreta E, Ortega B, Mezzera J (1998) Corticosteroids and radiation mucositis in head and neck cancer. A double-blind placebo-controlled randomized trial. Radiother Oncol 47:145–148
6. Peterson DE, Bensadoun RJ, Roila F (2008) Management of oral and gastrointestinal mucositis: ESMO clinical recommendations. Ann Oncol 19(2):122–125
7. Rubenstein EB, Peterson DE, Schubert M, Keefe D, McGuire D, Epstein J, Elting LS, Fox PC, Cooksley C, Sonis ST (2004) Clinical practice guidelines for the prevention and treatment of cancer therapy-induced oral and gastrointestinal mucositis. Cancer 100:2026–2046
8. Ruescher T, Sodeifi A, Scrivani S, Kaban L, Sonis S (1998) The impact of mucositis of alpha-hemolytic streptococcal infection in patients undergoing autologous bone marrow transplantation for hematologic malignancies. Cancer 82:2275–2281
9. Sonis S, Oster G, Fuchs HJ (2001) Oral mucositis and the clinical and economic outocomes of hematopoietic stem-cell transplantation. J Clin Oncol 19:2201–2205
10. Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M, Bekele BN, Raber-Durlacher J, Donnelly JP, Rubenstein EB (2004) Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. Cancer 100:1995–2025
11. Spielberger R, Stiff P, Gensinger W, Gentile T, Emmanouilides C (2004) Palifermin for oral mucositis after intensive therapy for hematologic cancers. N Eng J Med 351:2590–2598
12. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, Komaroff E, Nalysnyk L, Zilberberg MD (2003) Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. Radiother Oncol 66:253–262

Original Article

Circulating Endothelial Progenitors and CXCR4-Positive Circulating Endothelial Cells Are Predictive Markers for Bevacizumab

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BACKGROUND: Bevacizumab plus chemotherapy is a standard option in the treatment of metastatic colorectal cancer (mCRC). The aim of this study was to investigate the potential of circulating endothelial cell progenitors (CEPs) and phenotypical circulating endothelial cells (CECs) as surrogate markers of clinical outcome in mCRC patients to identify responders to bevacizumab in combination with chemotherapy. **METHODS:** A total of 69 patients with measurable mCRC were enrolled in this prospective study. Whole blood samples were analyzed before initiation of treatment and on days 4 and 14. Phenotypical CECs and CEPs were then isolated and enumerated by using flow cytometry. **RESULTS:** CEP levels of less than 0.04% on day 4 were significantly associated with longer progression-free survival (PFS) and overall survival (OS) ($P < .001$, $P = .002$, respectively) as compared with levels of 0.04% or more. In addition, CXCR4-positive CEC levels of less than 20% at baseline were significantly associated with longer PFS and OS as compared other indicators investigated ($P < .001$, $P = .002$, respectively). **CONCLUSIONS:** Levels of CEPs on day 4 and proportion of CXCR4-positive CECs at baseline were correlated with the prognosis of bevacizumab combination chemotherapy, suggesting that these surrogate markers may play a core role in the selection of candidates for bevacizumab treatment. ***Cancer* 2011;117:4026–32.** © *2011 American Cancer Society.*

KEYWORDS: circulating endothelial progenitors, CXCR4-positive circulating endothelial cells, bevacizumab, metastatic colorectal cancer, chemotherapy.

Antiangiogenic agents such as bevacizumab that target the vascular endothelial growth factor (VEGF) pathway have shown promise in the treatment of a variety of malignancies.[1] However, clinical biomarkers are needed for quantitative evaluation of the effect of bevacizumab.

VEGF is known to promote the mobilization of bone-marrow–derived circulating endothelial progenitors (CEPs) and survival by activating antiapoptotic pathways in circulating endothelial cells (CECs),[2–4] which may subsequently differentiate into mature endothelial cells.[5,6] Recently, CEPs were reported to be involved in tumor angiogenesis in tumor implantation models[7–10] and in clinical studies.[11,12] According to several clinical reports, baseline CEC levels in cancer patients have shown higher values compared with those in healthy controls and were correlated with response and outcome.[13–15]

The aim of this study was to investigate the potential of CEPs and phenotypical CECs as surrogate markers of clinical outcome in metastatic colorectal cancer (mCRC) patients to identify responders to chemotherapy with bevacizumab.

MATERIALS AND METHODS

Patients

Principal inclusion criteria were measurable mCRC and commencement of a new systemic therapy. Other inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate organ function, and

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radiographic evidence of disease progression as defined by the Response Evaluation Criteria in Solid Tumors (RECIST). All patients were enrolled on protocols approved by the institutional review board at the Cancer Institute Hospital in The Japanese Foundation for Cancer Research. Written informed consent was obtained from all patients.

Assessment of Biomarkers

Whole blood samples were collected and analyzed at the following times: before initiation of treatment (baseline), immediately after completion of 1 course (day 4), and before commencement of a second cycle (day 14). Blood samples were drawn into 8.5-mL evacuated tubes (BD Biosciences, Franklin Lakes, NJ).

Mononuclear cells isolated by density gradient centrifugation were analyzed using the method established by Duda DG et al.[16] Briefly, Ficoll gradient was used to isolate peripheral blood mononuclear cells (PBMC) and remove red cells and platelets before incubation with antibodies. The following directly conjugated monoclonal antibodies were used for detection of CECs and CEPs by 4-color flow cytometry in peripheral blood: anti-CD31-FITC (BD Pharmingen, San Diego, Calif), anti-CD133-PE (Miltenyi Biotec, Auburn, Calif), anti-CD34-APC (BD Pharmingen), and anti-CD45-PerCP/Cy5.5 (BD Pharmingen). The proportions of CECs (CD31-positive and CD45 negative fractions) and CEPs (CD31-positive, CD34 highly positive, CD133-positive, and CD45 dimly positive fractions) were calculated as percentages of the total number of mononuclear cells after evaluation of at least 50,000 cellular events. Phenotypical CECs expressing VEGFR1, VEGFR2, Tie-2, or CXCR4 were also analyzed. The proportions of these CEC phenotypes were calculated as percentages of the total number of CECs.

Observation of CECs and CEPs

For morphological and immunohistological observation of CECs and CEPs, a small portion of mononuclear cells was fractionated into CXCR4-positive CECs or CEPs by using FACSVantage (Becton Dickinson, Franklin Lakes, NJ). The nuclei of the isolated live CECs and CEPs were stained with DRAQ5 (Alexis, now part of Enzo Life Sciences, Farmingdale, NY) and then observed by confocal laser scanning microscopy (FV1000; Olympus, Center Valley, Penn).

Table 1. Characteristics of Patients Treated With FOLFOX Plus Bevacizumab

Characteristics	Regimen
N=69	FOLFOX+bevacizumab
Median age (range)	61 (27-73)
Sex men/women	38/31
Primary site rectum/colon	24/45
Prior colorectomy +/-	6/63
Metastatic site	
Liver	37
Lung	36
LN	28
Local recurrence	5
Peritoneum	17
Bone	3
Chemotherapy +/-	14/55
5-FU	7
Other	7
CR/PR/SD/PD	2/46/15/6

CR indicates complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Statistical Analysis

Kaplan-Meier survival plots were generated based on CEC levels at each time point of blood sampling, and the curves were compared by using the log-rank test. The Cox proportional hazards regression model was used to determine univariate and multivariate hazard ratios for progression-free survival (PFS) and overall survival (OS).

RESULTS

Patient Characteristics

A total of 69 patients were enrolled. Patient characteristics at baseline are summarized in Table 1. Among 69 patients treated with FOLFOX4 plus bevacizumab assessable for response, we observed complete response in 2 (3%), partial response in 46 (67%), stable disease in 15 (22%), and progressive disease (PD) in 6 (8%) during treatment. Overall response rate was 70%.

Relation Between CEP Levels and Outcome

Univariate Cox regression analysis revealed that CEP levels on day 4 were significantly associated with PFS in 30 of the 69 patients in the training set. To identify the level of CEPs that most clearly distinguished patients responsive to FOLFOX with bevacizumab, thresholds of 0.01%-0.20% of the total number of PBMCs on day 4 were systematically correlated with PFS. Median PFS in patients with levels above or below each threshold differed at 0.04% CEPs of the total number of PBMCs, reaching a plateau at approximately that level. At this level, the Cox

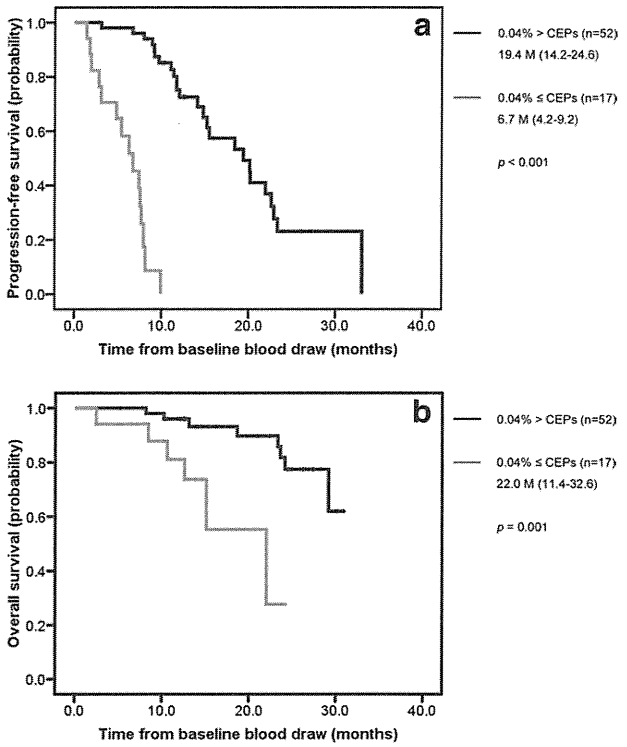

Figure 1. Depicted are (a) Kaplan-Meier plots of progression-free survival (PFS) and (b) Kaplan-Meier plots of overall survival (OS).

proportional-hazard ratio signifying the difference between slow and rapid progression of disease also reached a peak. Therefore, a cutoff of 0.04% CEPs was chosen to distinguish patients. The Kaplan-Meier 0.04% CEP counts were available on day 4 for 30 of the 69 patients in the training set and for 39 of the 69 patients in the validation set. Because the 2 sets of data were nearly identical, they were combined to estimate PFS and OS for the entire study population. Patients with 0.04% or more CEPs on day 4 had a shorter median PFS (6.7 months; 95% CI, 4.2-9.2 months) than those with less than 0.04% CEPs on day 4 (19.4 months; 95% CI, 14.2-24.6 months) ($P < .001$) (Fig. 1a). Patients with 0.04% or more CEPs on day 4 had a shorter median OS (22 months; 95% CI, 11.4-32.6 months) than those with less than 0.04% CEPs on day 4 ($P = .001$) (Fig. 1b).

Relation Between CEC Phenotype and Efficacy

Levels of CXCR4 in patients with PD were significantly higher than in those with no PD. Other phenotypes showed no differences between patients with PD and those without (Fig. 2).

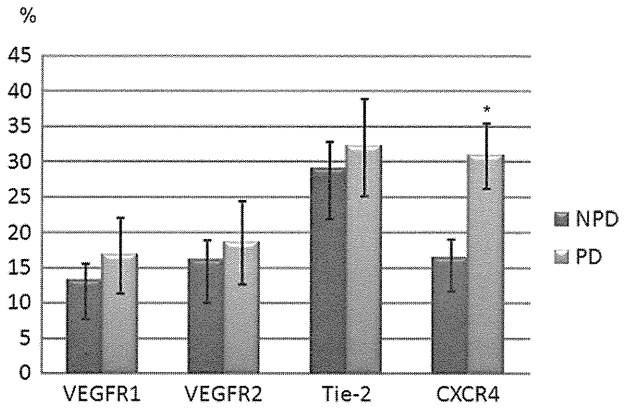

Figure 2. The relation is shown between levels of CEC phenotypes at baseline and bevacizumab efficacy in bevacizumab combination chemotherapy. PD indicates progressive disease; NPD, nonprogression disease. Results are expressed as mean ± standard error of the mean (SE). *$P < .05$

Relation Between CEC Phenotype and Outcome

According to univariate Cox regression analysis, CEC levels at baseline were significantly associated with PFS. To explore the predictive potential of CEC phenotypes at baseline, we analyzed the relation between baseline levels of CEC phenotypes and PFS. Univariate Cox regression analysis revealed that CXCR4-positive CEC levels at baseline were significantly associated with PFS. On the other hand, no correlation was observed between baseline VEGFR1-positive, VEGFR2-positive, or Tie-2–positive CEC levels and PFS. To identify the level of CXCR-positive CECs that most clearly distinguished patients responsive to FOLFOX with bevacizumab, thresholds of 1% to 45% of the total number of CECs at baseline were systematically correlated with PFS. Median PFS in patients with levels of above or below each threshold differed at 20% CXCR4-positive CECs. At this level, the Cox proportional-hazards ratio signifying the difference between slow and rapid progression of disease also reached a plateau. Therefore, a distinguishing cutoff of 20% CXCR4-positive CECs was chosen. The Kaplan-Meier CXCR4-positive CEC count was available at baseline for 30 of the 69 patients in the training set and for 39 of the 69 patients in the validation set. No significant difference was observed in either PFS or OS in either set. Because the 2 sets of data were nearly identical, they were combined to estimate PFS and OS for the entire study population. Patients with 20% or more CXCR4-positive CECs at baseline had a shorter median PFS (6.7 months; 95% CI, 4.1-9.3 months) than those with less than 20% CXCR4-positive

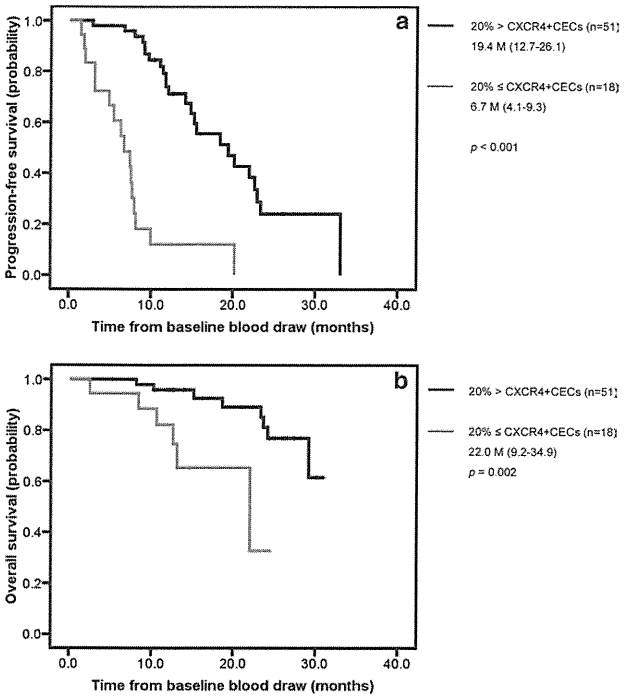

Figure 3. Depicted are Kaplan-Meier plots of (a) progression-free survival (PFS) and (b) overall survival (OS).

CECs at baseline (19.4 months; 95% CI, 12.7-26.1 months) ($P < .001$) (Fig. 3a). Patients with 20% or more CXCR4-positive CECs at baseline had a shorter median OS (22 months; 95% CI, 9.2-34.9 months) than those with less than 20% CXCR4-positive CECs at baseline ($P = .002$) (Fig. 3b).

Univariate and multivariate Cox proportional hazards regression was performed to assess the association between factors of interest and PFS or OS. According to the univariate Cox regression analysis, liver metastasis, lung metastasis, CEP levels on day 4, and CXCR4-positive CEC levels at baseline were associated with PFS; furthermore, peritoneal metastasis, CEP levels on day 4, and CXCR4-positive CEC levels at baseline were associated with OS (Table 2). To evaluate the independent predictive effect of these markers, multivariate Cox regression analysis was carried out (Table 3). Levels of CEP on day 4 and CXCR4-positive CEC levels at baseline were the strongest predictors.

Table 2. Independent Predictive Factors by Univariate Cox Regression Analysis for Progression-Free Survival and Overall Survival

Parameter	No. of Patients	HR	95% CI	*P*	X^2
PFS					
CEP	69	7.01	3.5-14.05	<.001	<.001
CXCR4+CEC	69	22.96	8.52-61.87	<.001	<.001
Liver metastasis	69	2.71	1.36-5.38	.004	.003
Lung metastasis	69	2.44	1.22-4.90	.012	.009
OS					
CEP	69	5.45	1.71-17.4	.004	.002
CXCR4+CEC	69	5.26	1.64-16.9	.005	.002
Peritoneal metastasis	69	3.46	1.16-10.33	.026	.018

HR indicates hazard ratio; CI, confidence interval; PFS, progression-free survival; CEP, circulating endothelial progenitor; CEC, circulation endothelial cell; OS, overall survival.

DISCUSSION

Some authors have suggested that CECs are a predictive marker of clinical outcome in cancer patients treated with bevacizumab-based chemotherapy. In breast cancer, most studies[14,17,18] have reported that high CEC levels at baseline indicate a better outcome than low CEC levels. On the other hand, in colorectal cancer, low CEC levels at baseline were reported to indicate a better outcome than high CEC levels.[19,20] These results suggest vascular formation differs according to tumor origin. However, these differences in results between these 2 types of cancer may have resulted from differences in the measurement protocols used. A number of methods and protocols are used to evaluate and count CECs. Two widely used protocols involve the use of flow cytometry. Duda et al[16] reported a cytometry protocol for phenotypic identification and enumeration of CECs and CEPs using 4 surface markers: CD31, CD34, CD133, and CD45. This procedure is believed to allow detection of 0.1% to 6.0% of viable CECs and 0.01% to 0.20% of CEPs from among a blood mononuclear cell population and is mainly used in colorectal cancer. Mancuso et al[21] reported a protocol for the phenotypic identification and enumeration of CECs and CEPs involving 6-color flow cytometry, nuclear staining with Syto16 (Molecular Probes, Eugene, Ore) and 7-AAD (Flow Labs, Irvine, UK) and a panel of monoclonal antibodies, including CD45, CD133, CD31, and CD146. This protocol has been mainly used in breast cancer. In this study, we selected the protocol of Duda et al.

Willet et al[19] reported that CEP levels decreased on day 3 after initiation of bevacizumab with chemoradiation in rectal cancer patients. On the basis of this earlier report, we decided, in this study, to collect samples at 3 days (day 4) after initiation of chemotherapy with bevacizumab. We

Table 3. Independent Predictive Factors by Multivariate Cox Regression Analysis for Progression-Free Survival and Overall Survival

		HR	95% CI	*P*	Model χ^2
PFS					
No. of patients	69				<.001
CEP		27.71	9.51-80.72	<.001	
Liver metastasis		2.95	1.46-5.95	.002	
No. of Patients	69				<.001
CXCR4+CEC		15.71	6.31-39.13	<.001	
Liver metastasis		2.71	1.33-5.55	.006	
Bone metastasis		0.09	0.02-0.48	.005	
OS					
No. of patients	69				<.001
CEP		8.90	2.48-31.93	.001	
Peritoneal metastasis		5.49	1.71-17.66	.004	
No. of Patients	69				<.001
CXCR4+CEC		6.14	1.85-20.41	.003	
Peritoneal metastasis		9.85	2.59-37.43	.001	

HR indicates hazard ratio; CI, confidence interval; PFS, progression-free survival; CEP, circulating endothelial progenitor; CEC, circulation endothelial cell; OS, overall survival.

found that bevacizumab combination therapy resulted in a marked and significant decrease in CEP levels on day 4 in comparison with those at the other time points selected. Levels of CEP on day 4 were the strongest predictor of PFS and OS. These results suggest that bevacizumab inhibits bone marrow-dependent tumor vasculogenesis by reducing endothelial progenitor cells mobilizing from bone marrow into the peripheral blood and reducing the proliferation of CEPs. Based on these results, we believe that if CEP levels do not decrease immediately after initiation of bevacizumab, then the patient must be considered unresponsive, and that it would not be beneficial to continue.

These results support the view of Ronzoni et al[20] that low CECs at baseline are indicative of longer PFS. Ronzoni reported that low levels of total CECs at baseline were correlated with improved PFS, but not significantly so. However, analysis of resting CEC levels at baseline revealed a significant correlation with improved PFS, indicating the potential of phenotypical subgroups of CECs as biological markers. Torrisi et al[18] reported that VEGFR-1–positive CEC levels showed a significant increase with bevacizumab-combination treatment. To explore the predictive potential of CEC phenotypes that express markers such as VEGFR1, VEGFR2, Tie-2, and CXCR4 at baseline, we analyzed the relation between baseline levels of CEC phenotypes and bevacizumab efficacy. We found that a lower ratio of CXCR4-positive CECs at baseline may indicate a beneficial effect for bevacizumab treatment. Xu et al[22] reported that bevacizumab upregulated stromal cell-derived factor 1alpha (SDF-1alpha) and its receptor, CXCR4, and that higher SDF-1alpha plasma levels during bevacizumab treatment were significantly associated with distant metastasis at 3 years. Siegel et al[23] reported that SDF-1 levels decreased from baseline in all patients after 8 weeks of bevacizumab, with an increase noted at time of progression. Their results suggest that SDF-1 is a resistance factor for bevacizumab, with SDF-1 inducing CXCR4-positive CECs in peripheral blood. Several studies[24,25] reported that the SDF-1/CXCR4 axis may contribute to functional vascular establishment and that the antiangiogenic effects of the blockade of CXCR4 are related to a reduction in the establishment of tumor endothelium independent of VEGF inhibition. Therefore, we confirmed differentiation by pathology between CEPs and CXCR4-positive CECs. Live CEPs sorted by flow cytometry were observed by using confocal microscopy, and cell surface expression of CD31 and CD34 was confirmed (Fig. 4a). Similarly, live CXCR4-positive CECs were also observed. The nuclear/cytoplasm ratio of CEPs was higher than that of CXCR4-positive CECs (Fig. 4b). The cell nuclei of the CEPs were mononuclear, but those of CXCR4-positive CECs were lobulated. These results indicate that the CEPs and CXCR4-positive CECs were different populations and that the CEPs were more immature than the CXCR4-postive CECs. Our findings suggest that activation of CXCR4-positive CECs may be responsible for

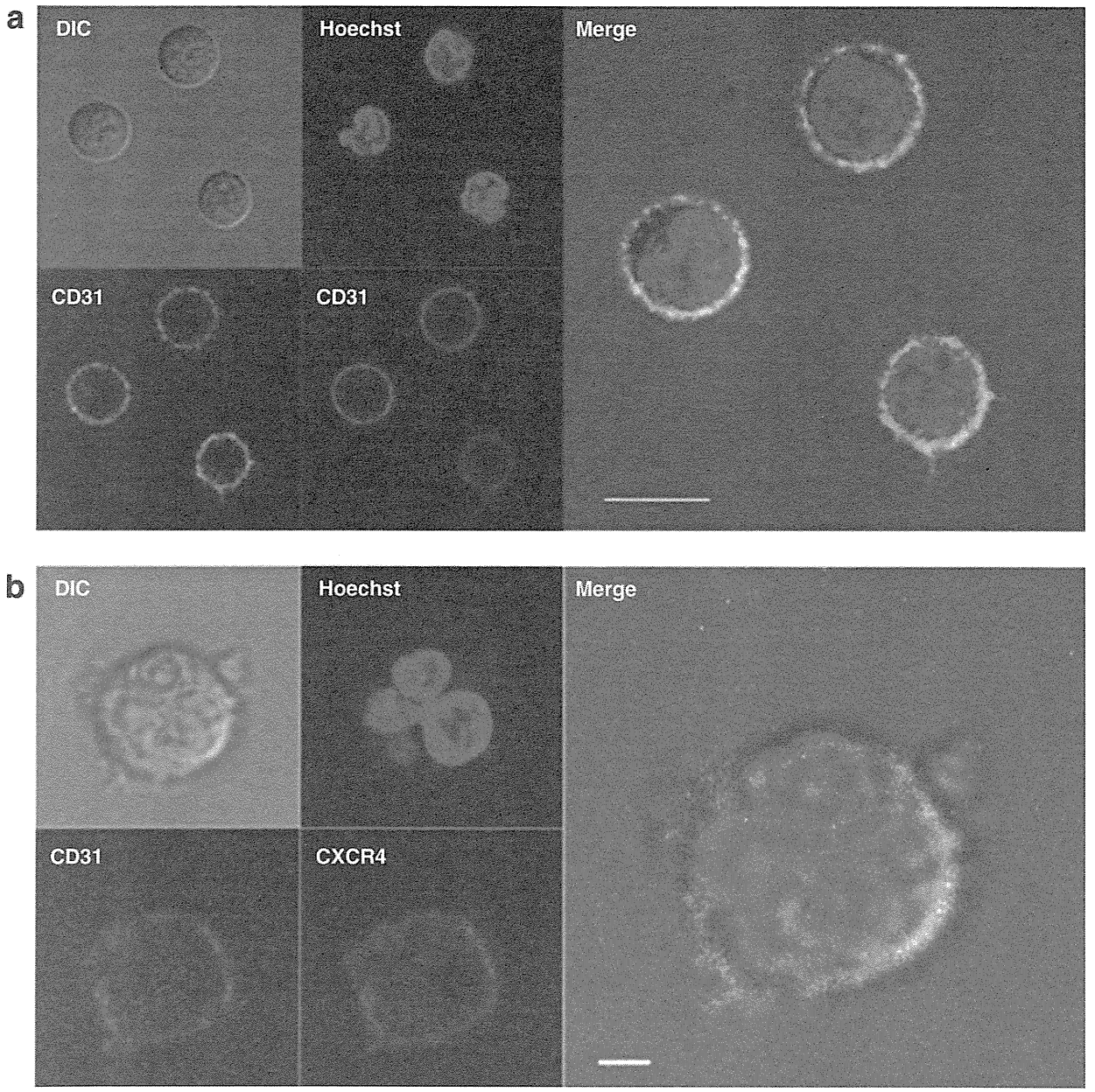

Figure 4. (a) CEPs and (b) CXCR4 + CECs were sorted by flow cytometry as described in the Materials and Methods section and analyzed by confocal microscopy. DIC indicates differential interferences contrast; bar, 5 μm.

angiogenesis occurring in cases where the VEGF antibody, bevacizumab, has proved ineffective. However, this also suggests that resistance to the antiangiogenic effects of bevacizumab may be neutralized by administration of SDF-1/CXCR4.

In conclusion, CEP levels on day 4 and proportions of CXCR4-positive CECs at baseline showed a correlation with prognosis in bevacizumab combination chemotherapy. This indicates the potential of these surrogate markers in the selection of candidates for bevacizumab treatment. Further research in the form of large-scale clinical trials is needed, however, to confirm these results.

CONFLICT OF INTEREST DISCLOSURES

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REFERENCES

1. Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol.* 2007;25:1539-1544.
2. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* 1997;275:964-967.
3. Larrivee B, Lane DR, Pollet I, et al. Vascular endothelial growth factor receptor-2 induces survival of hematopoietic progenitor cells. *J Biol Chem.* 2003;78:22006-22013.
4. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest.* 2000;105:71-77.
5. Asahara T, Takahashi T, Masuda H, et al. VEGF contributes to potential neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 1999;18: 3964-3972.
6. Raffi S, Heissig B, Hattori K. Efficient mobilization and recruitment of marrow-derived endothelial and hematopoietic stem cells by adenoviral vectors expressing angiogenic factors. *Gene Ther.* 2002;9:631-641.
7. Monestiroli S, Mancuso P, Burlini A, et al. Kinetics and viability of circulating endothelial cells as surrogate angiogenesis marker in an animal model of human lymphoma. *Cancer Res.* 2001;1:4341-4344.
8. Bertolini F, Paul S, Mancuso P, et al. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. *Cancer Res.* 2003;63:4342-4346.
9. Schuch G, Heymach JV, Nomi M, et al. Endostatin inhibits the vascular endothelial growth factor-induced mobilization of endothelial progenitor cells. *Cancer Res.* 2003;63:8345-8350.
10. Capillo M, Mancuso P, Gobbi A, et al. Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. *Clin Cancer Res.* 2003;9:377-382.
11. Buckstein R, Kerbel RS, Shaked Y, et al. High-dose celecoxib and metronomic "low-dose" cyclophosphamide is an effective and safe therapy in patients with relapsed and refractory aggressive and safe therapy in patients with relapsed and refractory aggressive histology non-Hodgkin's lymphoma. *Clin Cancer Res.* 2006;12:5190-5198.
12. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med.* 2004;10:649.
13. Furstenberger G, von Moos R, Lucas R, et al. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. *Br J Cancer.* 2006;4:524-531.
14. Dellapasqua S, Bertolini F, Bagnardi V, et al. Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. *J Clin Oncol.* 2008;26: 4899-4905.
15. Mancuso P, Colleni M, Calleri A, et al. Circulating endothelial-cell kinetics and viability predict survival in breast cancer patients receiving metronomic chemotherapy. *Blood.* 2006;108:452-459.
16. Duda DG, Cohen KS, Scadden DT, Jain RK. A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood. *Nat Protoc.* 2007;2:805-810.
17. Calleri A, Bono A, Bagnardi V, et al. Predictive potential of angiogenic growth factors and circulating endothelial cells in breast cancer patients receiving metronomic chemotherapy plus bevacizumab. *Clin Cancer Res.* 2009;15:7652-7657.
18. Torrisi R, Bagnardi V, Cardillo A, et al. Preoperative bevacizumab combined with letrozole and chemotherapy in locally advanced ER- and/or PgR-positive breast cancer: clinical and biological activity. *Br J Cancer.* 2008;99:1564-1571.
19. Willett CG, Duda DG, di Tomaso E, et al. Efficacy, safety, biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. *J Clin Oncol.* 2009;27:3020-3026.
20. Ronzoni M, Manzoni M, Mariucci S, et al. Circulating endothelial cells and endothelia progenitors as predictive markers of clinical response to bevacizumab-based first-line treatment in advanced colorectal cancer patients [published online ahead of print May 23, 2010]. *Ann Oncol.* 2010;21: 2382-2389.
21. Mancuso P, Antoniotti P, Quarna J, et al. Validation of a standardized method for enumerating circulating endothelial cells and progenitors: flow cytometry and molecular and ultrastructural analyses. *Clin Cancer Res.* 2009;15:267-273.
22. Xu L, Duda DG, Tomaso E, et al. Direct evidence that bevacizumab, an anti-VEGF antibody, up-regulates SDF-1α, CXCR4, CXCL6, and neuropilin 1 in tumors from patients with rectal cancer. *Cancer Res.* 2009;69:7905.
23. Siegel AB, Cohen EI, Ocean A, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol.* 2008;26: 2992.
24. Salvucci O, Yao L, Villalba S, et al. Regulation of endogenous chemokine stromal-derived factor-1. *Blood.* 2002;99: 2703-2711.
25. Guleng B, Tateshi K, Ohta M, et al. Blockade of the stromal cell-derived factor-1/CXCR4 axis attenuates in vivo tumor growth by inhibiting angiogenesis in a vascular endothelial growth factor-independent manner. *Cancer Res.* 2005; 65:5864-5871.

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Phase I study of dasatinib (BMS-354825) in Japanese patients with solid tumors

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Dasatinib is a potent oral inhibitor of tyrosine kinases including the SRC family kinases, which are activated in tumors, and implicated in invasion and bone metastasis. This phase I dose-escalation study assessed safety, tolerability, maximum tolerated dose (MTD), antitumor activity, pharmacokinetics and pharmacodynamics in Japanese patients with refractory, advanced solid tumors. Dasatinib was administered once daily at 100, 150 and 200 mg/day. Sixteen patients were treated with dasatinib in the following doses: 100 mg (nine patients), 150 mg (three patients) and 200 mg (four patients). The most frequent adverse events (AE; ≥50%) were anorexia, fatigue, pleural effusion, anemia, constipation, diarrhea, vomiting and increased aspartate aminotransferase (AST). The most frequent AE of grade ≥3 (≥10%) were anemia, decreased lymphocyte count, fatigue and increased blood magnesium. Dose-limiting toxicities were observed in two patients: grade 2 pleural effusion and bronchial wall thickening at the 100-mg level and grade 3 dyspnea at the 200-mg level. In addition, grade 2 pleural effusion was observed in all four patients treated with 200 mg. Therefore, 150 mg was determined to be the MTD. The pharmacokinetic parameters were comparable among the dose levels. As a pharmacodynamic study, markers of bone metabolism were assessed. Bone resorption markers, NTx and TRACP-5b, showed a decrease of 46.3% and 22.2%, respectively. No objective responses were observed, but three patients had stable disease that lasted for over 6 months. In this study population, the safety profile of dasatinib was generally acceptable and 150 mg of dasatinib administered once daily was determined to be the MTD. (*Cancer Sci* 2011; 102: 2058–2064)

SRC family kinases (SFK) such as SRC, YES, LCK and FYN are non-receptor tyrosine kinases that have important roles in cell proliferation, motility, adhesion and survival.[1,2] SRC family kinases regulate signals from membrane-associated growth factor receptors such as epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R) and vascular endothelial growth factor receptor (VEGFR).[3] SRC was the first proto-oncogene identified and is upregulated in various tumors[1] through epigenetic processes. SRC and other SFK are associated with epidermal-to-mesenchymal transformation (EMT), VEGF overexpression, a propensity for metastases and shorter survival. SRC is also essential for osteoclast function,[4,5] and SRC overexpression accelerates bone metastases.[6]

Dasatinib (BMS) is a multi-target tyrosine kinase inhibitor that inhibits LCK, SRC, YES, BCR-ABL, KIT and platelet-derived growth factor receptor (PDGFR) *in vitro* with IC_{50} of 0.40, 0.50, 0.50, <1.0, 5.0 and 28, respectively.[7] Dasatinib showed antitumor efficacy for several types of solid cancer both *in vitro* and *in vivo*.[8,9] With better inhibitory activity for abl kinase than imatinib, dasatinib has proven clinical efficacy in patients with imatinib-resistant chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL),[10] and has been approved in many countries including Japan.

In the present study, we conducted a phase I study of dasatinib in Japanese patients with advanced solid tumors that were refractory to standard therapies or for which no effective standard therapy existed. The primary objective of the present study was to establish the maximum tolerated dose (MTD) of dasatinib with once daily administration.

Materials and Methods

Patients and eligibility criteria. This was a multi-center, open-label, phase I, dose-escalation study to assess the safety, tolerability, pharmacokinetics, pharmacodynamics and preliminary efficacy of dasatinib in patients with refractory solid tumors. The study was conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice, and in accordance with the ethical principles that are in the current Declaration of Helsinki, and was approved by the institutional review board at each of the participating institutions. All patients provided written informed consent.

Eligible patients were aged 20 years or older with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, and had a solid cancer, verified cytologically or histologically, that was refractory to conventional therapy, or for which there was no established therapy. Patients were eligible if they had adequate bone marrow function (neutrophil count ≥2000/mm^3, platelets ≥125 000/mm^3, hemoglobin ≥9.0 g/dL), liver function (total bilirubin, aspartate aminotransferase [AST], alanine aminotransferase [ALT] ≤ two times the upper limit of normal), renal function (creatinine ≤1.5 times the upper limit of normal) and serum potassium, magnesium and corrected calcium within the range of grade 0–1 of the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0. Patients should not have received chemotherapy, immunotherapy or radiotherapy within 4 weeks before the start of therapy (6 weeks if nitrosourea or mitomycin C and 2 weeks if endocrine therapy).

Patients were excluded if they had symptomatic brain metastasis, pleural effusion, prolonged QT syndrome or QTc prolongation of more than 450 ms, grade II or III atrioventricular block, heart rate of <50 b.p.m., uncontrolled hypertension, history of a significant bleeding disorder, vasculitis, gastrointestinal bleeding within 6 months, recent ischemic heart disease or drug allergy. Patients who were pregnant or breastfeeding or those who were of childbearing potential but unwilling or unable to use adequate contraception were also excluded. Prohibited medications included those known to increase the risk of Torsades

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de Pointes, irreversible inhibitors of platelet function and drugs that increase intra-gastric pH.

Drug administration. Dasatinib was administered orally once daily after breakfast. The initial dose was 100 mg, increased by increments of 50 mg up to a maximum dose of 250 mg. Treatment for 4 weeks (28 days) was defined as one treatment course. Initially, three patients were treated at a given dose level. If no dose-limiting toxicity (DLT) was observed, the dose would be escalated to the next higher level. If a DLT was observed in one of the three patients treated during the first course at that particular dose level, three additional patients would be enrolled and treated at the same dose level. If no further DLT was observed during the first course in these additional patients, the dose could be escalated to the next higher dose level. When two or more (≥33%) DLT were observed at the same dose level, there would be no further dose escalation.

Study treatment continued until progressive disease, death, withdrawal of consent or unacceptable toxicity was observed. All patients were followed for a minimum of 4 weeks after the last dose of study therapy.

Evaluation of toxicity. Toxicity was graded according to the CTCAE v3.0. Physical examination and laboratory assessment were performed regularly. Electrocardiograms were performed before the start of therapy and at 1 and 4 h after administration on days 1, 14 and 28 of the first cycle, and on days 14 and 28 of subsequent cycles.

The DLT was defined as grade 4 neutropenia for five consecutive days or longer, febrile neutropenia, grade 4 thrombocytopenia or bleeding requiring platelet transfusion, grade 3–4 nausea, vomiting or diarrhea despite adequate prophylaxis and therapy, other non-hematological toxicities of grade 3 or more except anorexia or fatigue, QTc interval of 530 ms or longer, and adverse events (AE) that caused interruption of administration for more than 14 days or dose reduction of two levels or more, during the first course.

Dose delays and modifications were defined as follows: with grade 3–4 neutropenia or febrile neutropenia, dosing was interrupted until recovery to grade 1 was achieved, and the dose was reduced when grade 4 neutropenia continued for 5 days or longer or with febrile neutropenia; with grade 3–4 thrombocytopenia, dosing was interrupted until platelets recovered to 100 000/mm^3 or more; with bleeding, dosing was interrupted; with fluid retention or pleural effusion, diuretics or the use of pleural drainage was commenced, and with severe or recurrent effusion, dosing was interrupted and was restarted at a reduced dose; with a grade 3 non-hematological toxicity except anorexia or fatigue, dosing was interrupted until recovery to grade 1 was achieved; and with a grade 4 non-hematological toxicity except anorexia or fatigue, dosing was interrupted until recovery to grade 1 was achieved and was restarted at a reduced dose.

Objective response evaluation. The tumor response was evaluated using Response Criteria in Solid Tumors (RECIST) ver. 1.0[11] in patients with measurable lesions. Assessment was performed at the fourth week of the first cycle, and every 4–8 weeks thereafter.

Pharmacokinetic studies. Blood samples for pharmacokinetic study were collected before dosing, and at 30 min, 1, 1.5, 2, 3, 4, 6, 12 and 24 h on days 1, 14 and 28 of the first cycle. Samples were analyzed to determine the concentration of dasatinib and its metabolite (BMS-582691). Pharmacokinetic (PK) parameters were calculated using a non-compartmental method, including C_{max}, AUC(0–t), AUC(TAU), T_{max}, t1/2, accumulation index, LCo and Vz/F for dasatinib, and C_{max}, AUC(0–t) and T_{max} for BMS-582691.

Pharmacodynamic studies. Because a lot of evidence shows that SRC is essential to osteoclast activity[4] and dasatinib suppresses osteoclast activity *in vivo*,[12] we opted to measure the bone metabolic markers of osteolast activity as the phamacodynamic marker of dasatinib acitivity. Blood and urine samples for the study were collected during screening, and on days 14 and 28 of the first cycle. Samples were analyzed for urine type 1 collagen N-telopeptide (NTx) and serum tartrate-resistant acid phosphatase (TRACP-5b).

Results

Patient characteristics. A total of 16 patients, all of whom were treated with dasatinib, were enrolled. Patient disposition is shown in Table 1. Nine patients were treated with 100 mg, three with 150 mg and four with 200 mg once daily. All patients discontinued the study medication due to disease progression (9/16, 56%), study drug toxicity (5/16, 31%) or the patient's request (2/16, 13%).

Baseline patient characteristics are shown in Table 2. The median age was 54 years (range, 33–65), male/female ratio was 5/11 and baseline ECOG PS was 0 or 1 in all patients. All patients had experienced prior treatments including chemotherapy (14/16, 88%), surgery (14/16, 88%), radiation therapy (9/16, 56%), hormonal or immunotherapy (4/16, 25%) and the use of other agents (2/16, 13%). The median number of previous chemotherapy regimens was five (range, 2–11). The primary tumors were breast cancer in four, colon or rectal cancer in six, soft tissue sarcoma in two (one gastrointestinal stromal tumor), renal cell carcinoma (RCC) in one, head and neck cancer in one, thymoma in one and ovarian cancer in one.

Toxicities. Four patients discontinued study therapy so early that they were not evaluable for DLT. The reasons for their early discontinuation were disease progression in one, QT prolongation in one (which was later shown to be due to an electrocardiogram error) and patient request in two (these two patients refused to continue treatment after grade 1 fatigue and anorexia in one, and grade 3 fatigue and grade 2 dyspnea in the other). In total, 12 patients were evaluable for DLT and two DLT were observed in two patients. One DLT at the 100-mg level was reported as a grade 2 non-hematological toxicity (blood lactate dehydrogenase increase, cell marker [KL-6] increase, bronchial wall thickening and pleural effusion), due to which the investigator decided to discontinue the study. The other was a grade 3 non-hematological toxicity (dyspnea and general physical health deterioration) at the 200-mg level. In addition, pleural effusion of grade 2 or more was observed in four patients treated with 200 mg. These results indicated that 200 mg once daily was not acceptable. Therefore, 150 mg once daily was determined to be the MTD in this study.

A summary of overall safety is shown in Table 3. The most frequent AE (≥30%) were anorexia (69%), fatigue (69%), pleural effusion (63%), anemia (63%), constipation (56%), diarrhea (56%), vomiting (50%), aspartate aminotransferase increase

Table 1. Patient disposition

	Dasatinib			Total
	100 mg†	150 mg	200 mg†	
No. patients enrolled	9	3	4	16 (100)
No. patients treated	9	3	4	16 (100)
No. patients discontinued	9	3	4	16 (100)
Disease progression	5	2	2	9 (56)
Study drug toxicity	3	1	1	5 (31)
Discontinuation at the patient's request	1	0	1	2 (13)

†Three patients in the 100 mg cohort and one patient in the 200 mg cohort discontinued dasatinib early and were not evaluable for dose-limiting toxicity (DLT). One DLT was observed in the 100 mg cohort and one in the 200 mg cohort.

Table 2. Baseline patient characteristics

	Dasatinib			Total (%)
	100 mg	150 mg	200 mg	
Gender				
Male	2	2	1	5 (31)
Female	7	1	3	11 (69)
Age (years)				
Median	58.0	55.0	47.5	54.0
Min, Max	33, 65	39, 63	33, 53	33, 65
Age (years)				
<65	7	3	4	14 (88)
≥65	2			2 (13)
Performance status (ECOG)				
0	7	1	2	10 (63)
1	2	2	2	6 (38)
Tumor type				
Breast cancer	3		1	4 (25)
Colon cancer	1	2	1	4 (25)
Rectal cancer	1	1		2 (13)
Hypopharyngeal cancer	1			1 (6)
Renal cell carcinoma	1			1 (6)
Leiomyosarcoma	1			1 (6)
Gastrointestinal stromal tumor	1			1 (6)
Thymoma			1	1 (6)
Ovarian cancer			1	1 (6)

ECOG, Eastern Cooperative Oncology Group.

(50%), nausea (44%), headache (38%), rash (38%), dyspnea (31%), alanine aminotransferase increase (31%) and blood calcium decrease (31%). Grade 1 QTc prolongation was seen in two patients. The most frequent AE of grade 3 or more (≥10%) were anemia (19%), lymphocyte count decrease (19%), fatigue (13%) and blood magnesium increase (13%). There were no deaths within 30 days after completion of administration of the study drug. Three patients (19%) experienced serious AE: perianal abscess; open fracture; and pleural effusion (one case each). Five patients (31%) discontinued study therapy due to AE: pleural effusion (two cases); QT prolongation; exertional dyspnea; cancer pain; and anemia.

Efficacy. Antitumor efficacy is shown in Table 4. Sixteen patients were treated and six patients (38%) were not evaluable for an objective response. No patient achieved a complete or partial response. Five patients (31%) had stable disease and three patients (RCC, colon cancer and thymoma) experienced stable disease for 6 months or longer, one at each dose level. Five patients (31%) had disease progression.

Pharmacokinetics. A summary of the PK parameters of dasatinib is shown in Table 5. Dasatinib was rapidly absorbed after oral administration. Dasatinib was detectable in plasma 30 min after oral administration and its concentration reached C_{max} at a median T_{max} of 0.5–3.3 h (Fig. 1). The dasatinib C_{max} values were comparable across all dose levels and study days, with moderate to large variability (41–127% as CV%). The dasatinib AUC values were also comparable across all dose levels and study days, with moderate to large variability (33–114% as CV%), and were slightly increased with the doses administered on days 1 and 14.

The mean t1/2 of dasatinib ranged between 4.36 and 8.33 h and was similar among study days. The mean CLo ranged between 141.6 and 649.2 L/h and the mean Vz/F ranged between 606 and 9113 L, with large variability. No remarkable drug accumulation was observed after repeated dosing. The accumulation index values ranged between 0.48 and 2.30 across study days.

Pharmacodynamics. Markers of bone metabolism were measured during the first course (Table 6). The dose had no obvious effect on NTx and TRACP-5b. Mean NTx and TRACP-5b levels after multiple administrations in all patients decreased over time, with moderate to large variability. On the day before the first administration (baseline), and on days 14 and 28, the mean (SD) values across the dose levels were 66.79 (45.18), 48.00 (43.24) and 32.64 (18.07) nanomolar bone collagen equivalents/millimolar creatinine (nmol BCE/mmol Cr) for NTx, respectively, 5.13 (1.89), 4.13 (1.25) and 3.40 (0.61) U/L for TRACP-5b. Of the 13 and 12 patients who had NTx and TRACP-5b levels assessed at baseline and during the study, 12 and 10 patients experienced a decrease in each marker, respectively (Fig. 2). The maximum percentage decreases in the levels of NTx and TRACP-5 were 46.3% (−17.3–86.5, $n = 13$) and 22.2% (−16.7–51.6, $n = 12$), respectively.

Discussion

In the present study, with once daily, continuous dosing, the DLT of dasatinib were grade 2 pleural effusion, bronchial wall thickening and laboratory abnormalities at 100 mg once daily,

Table 3. Adverse events

	All (%) $n = 16$		100 mg $n = 9$		150 mg $n = 3$		200 mg $n = 4$	
	All grades	Grade ≧3	All grades	Grade ≧3	All grades	Grade ≧3	All grades	Grade ≧3
Anorexia	11 (69)	2 (13)	5		2		4	2
Fatigue	11 (69)		6		1		4	
Anemia	10 (63)	3 (19)	4		2		4	3
Pleural effusion	10 (63)	1 (6)	5		1		4	1
Constipation	9 (56)		5		2		2	
Diarrhea	9 (56)	1 (6)	3		2		4	1
Vomiting	8 (50)		4		2		2	
AST increase	8 (50)		4		2		2	
Nausea	7 (44)		5				2	
Headache	6 (38)		3		1		2	
Rash	6 (38)		2		2		2	
Dyspnea	5 (31)	1 (6)	1				4	1
ALT increase	5 (31)		3		1		1	
Blood calcium decrease	5 (31)	1 (6)	2		1		2	1
Magnesium increase	2 (13)	2 (13)	1	1			1	1

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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