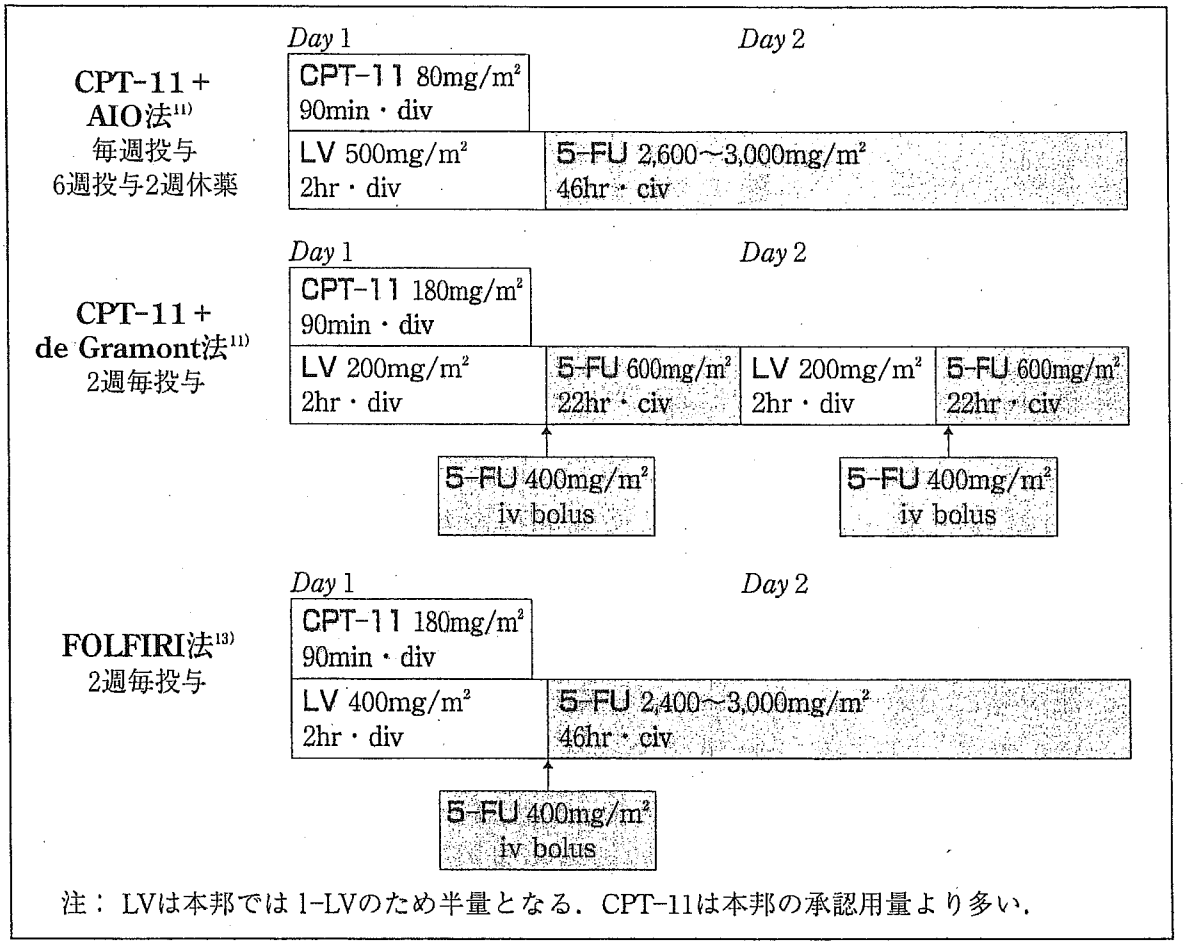

図2　5-FU/LV＋CPT-11療法

OHP/CTP-11（IROX法）のRCTを実施した（N9741試験）[12]．MST，PFS，奏効率のすべてでIFL法よりFOLFOX 4法が有意に良好な結果を示した．好中球減少，下痢などの重篤な有害事象もFOLFOX 4法で有意に少なく，唯一L-OHPの特徴である末梢神経障害が多かった．この結果をもって，2004年米国においてFOLFOX 4法は一次治療として切除不能・再発大腸癌の標準化学療法としての地位を確立した．

2）FOLFOX 6，FOLFIRI

続いて2004年にはTournigandらにより一次治療にFOLFIRI法（図2），二次治療にFOLFOX 6法を用いた群と，一次治療にFOLFOX 6法（図3），二次治療にFOLFIRI法を用いた群とのRCTが行われた（GERCOR試験）．それぞれの初回治療法の奏

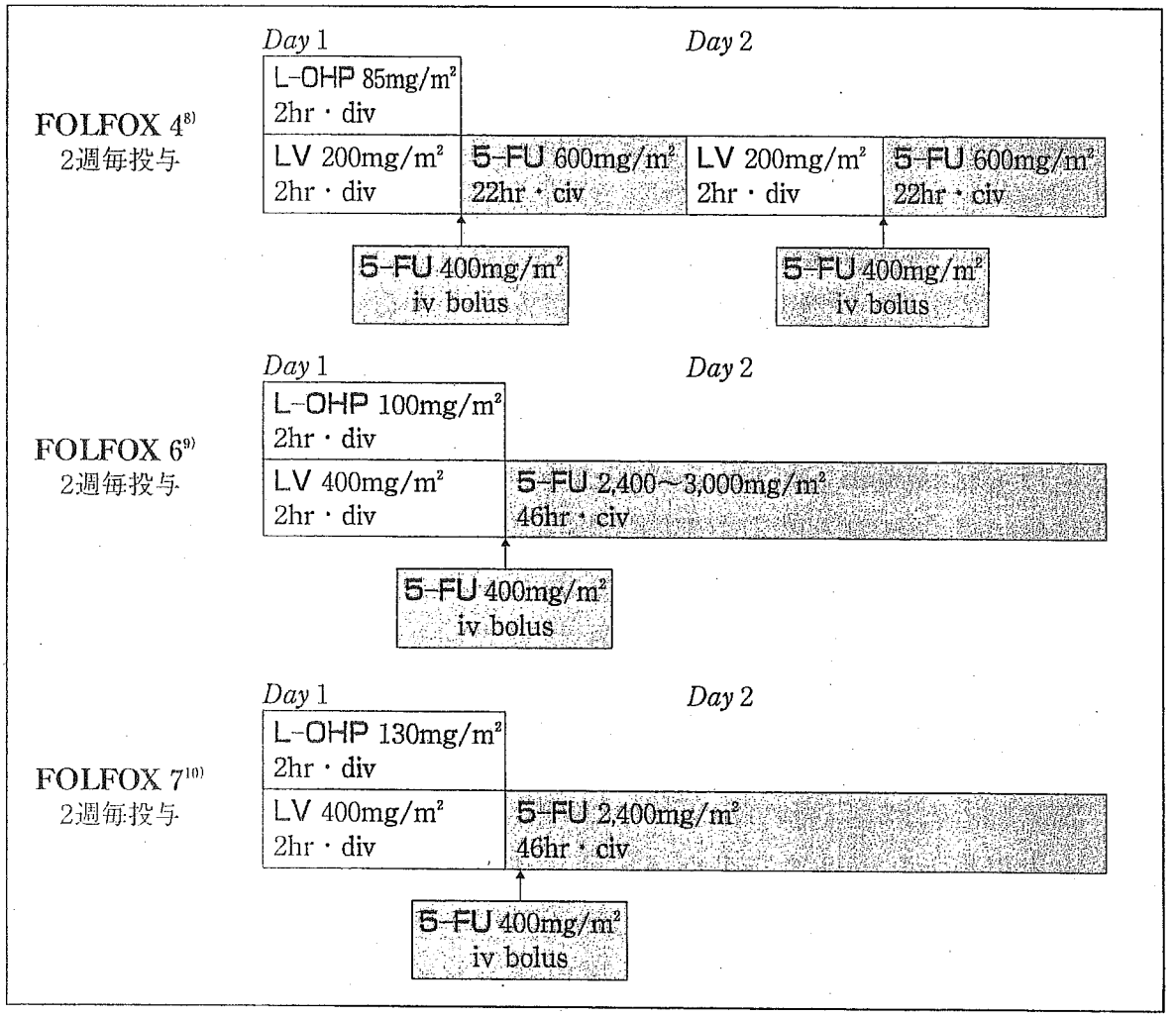

図3　おもな5-FU/LV＋L-OHP（FOLFOX）療法

効率（56％vs 54％），PFS（中央値：8.5カ月 vs 8.0カ月），全体のMST（21.5カ月，20.6カ月）は同等の成績が得られた[13]．また，上述のRCTを含むおもなRCTの検討において5-FU/LV，CTP-11，L-OHPの3種類の薬剤が全治療期間中に使用された症例の割合と全生存期間が相関することが明らかにされており，3剤を全治療経過中にうまく組み合わせることにより，20カ月を超えるMSTが得られるとされている[14]．

以上のような結果より，欧米においてはFOLFOX法，およびFOLFIRI法が切除不能・再発大腸癌の標準化学療法とされている．

本邦ではL-OHPは2005年4月よりinfusional 5-FU/LV療法

との併用において，L-OHP 85mg/m²を1日1回静脈内に2時間で点滴投与する方法で承認され，2005年7月現在，市販後調査，安全性確認試験が施行されている．

4．分子標的治療薬

細胞の癌化，増殖，浸潤，転移にかかわる分子機構が次第に解明され，それぞれのkey moleculeをtargetとした分子標的治療薬の開発が精力的に行われている．

1）Cetuximab

cetuximab（C-225）は大腸癌細胞で過剰発現することが知られるEGFR（epidermal growth factor receptor）を標的にしたIgG1モノクローナル抗体である．CPT-11に不応性となった大腸癌に対してCPT-11＋C-225併用療法群とCPT-11単剤群のRCTが行われ，奏効率，TTPとも有意差をもってCPT-11＋C-225併用療法群の優れていることが報告された（BOND試験）[15]．有害事象としてはアレルギー反応，にきび様の皮疹，肺臓炎などが報告されている．2004年1月に米国にて承認された．現在，FOLFIRI法やFOLFOX法との併用療法が検討されている．

2）Bevacizumab

bevacizumabは，腫瘍組織の血管新生に主要な役割を果たしているVEGF（vascular endothelial growth factor）に対する抗体である．一次治療としては5-FU/LV療法（RPMI法）にbevacizumabの併用効果を検討したRCTが行われた．奏効率はbevacizumab＋5-FU/LV療法で26.0％，5-FU/LV療法15.2％で有意差は認めなかったが，PFSでそれぞれ9.2カ月 vs 6.8カ月と有意にbevacizumab併用群で延長された[16]．IFL法vs IFL法＋bevacizumabの併用療法のRCTが行われ，奏効率（34.7％ vs 44.9％），MST（15.6カ月vs 20.3カ月）ともに，bevacizumab群で有意に優れていた．有害事象に関してはbevacizumab併用群で消化管穿孔，高血圧，静脈血栓症などが特徴的に認められたが，忍容性があるとされている[17]．この結果よりbevacizumabは2004

年2月米国で転移性大腸癌に対する治療薬として承認された．

二次治療としては，5-FU/CPT-11の既治例を対象としてbevacizumab＋FOLFOX 4併用療法 vs FOLFOX 4療法 vs bevacizumab単独療法のRCT（E3200試験）が行われ，2005年のASCOにてbevacizumab併用群が生命予後を有意に延長させることが明らかにされた[18]．また，CPT-11抵抗例に対してのbevacizumab＋cetuximab＋CPT-11併用療法 vs cetuximab＋bevacizumab併用療法の無作為化比較第Ⅱ相試験（BOND Ⅱ試験）が行われ，奏効率でそれぞれ37％ vs 23％，TTPで7.9カ月 vs 4.0カ月とbevacizumab併用による明らかな上乗せ効果が報告されている[19]．

以上のように，一次治療および二次治療においても分子標的治療薬の有用性が報告され，5-FU/LV，CPT-11，L-OHPに次ぐ大腸癌に対する第4のkey drugとなったが，これら抗体は薬剤費が高価であることが問題となっており，有効症例の治療前選別など，今後さらに検討する必要があるとされている．本邦では，現在bevacizumab，cetuximabの臨床試験が開始されている．

5．経口フッ化ピリミジン製剤

経口フッ化ピリミジン製剤はおもに本邦で開発され，汎用されてきた．欧米では最近まで転移性大腸癌に対する治療は前述のごとく静脈注射が中心であったが，利便性，経済性の点から経口剤の評価が行われてきている．このうちcapecitabineやUFTやS-1などが検討されている．

1）UFT

UFTは日本で開発されたdihydropyrimidine dehydrogenase（DPD）阻害薬の一つである．1990年代に欧米にて5-FU/LV療法とUFT/LV療法との大規模RCTが行われ，奏効率および生存率が両群で同等であること，UFT/LV群の副作用が有意に少ないことが報告されている．この結果より，欧州では切除不能・再発大腸癌に対する一次治療として承認された．また，日米ブリッ

ジング試験の結果，日本人における有効性と安全性に関して米国人との同等性が確認され[20]，本邦でも2003年に使用可能となった（**図4**）．

2）Capecitabine

capecitabineは消化管粘膜から吸収され肝臓のcarboxylesteraseによって5′-deoxy-5-fluorocytidineに，次に肝臓と腫瘍組織内のcytidine deaminaseで5′-deoxy-5-fluoropyrimidineに，さらに腫瘍組織内に豊富に存在するthymidine phosphorylaseで5-FUに変換され，抗腫瘍効果を発揮する薬剤である．5-FU/LV（Mayo法）療法とのRCTが行われ，PFS，MSTにおいては同等性が示され，毒性に関しては消化管毒性や好中球減少は有意に低率であったことより，欧米で切除不能・再発大腸癌に対する治療薬として承認された．本邦では現在，欧米と同じ用法，用量での臨床第Ⅱ相試験が終了したところであり，いまだ承認されていない．

3）S-1

S-1は日本で開発されたDPD阻害剤である．切除不能・再発大腸癌においての後期第Ⅱ相試験の結果では奏効率35.5％，MST 12カ月と，上記のUFT/LV療法，capecitabineとほぼ同等の成績が報告されている[21]が，承認はわが国のみであり5-FU/LV療法との比較試験はない．

UFT　300mg/m²/day
LV　75mg/body/day
1日3回に分けて（約8時間毎）
4週連日経口投与・1週間休薬

図4　UFT/LV療法

4）CPT-11，L-OHPとの併用

最近，これらの経口製剤とCPT-11やL-OHPとの併用療法の有望な成績が報告されつつあり，なかでもcapecitabine＋L-OHP併用療法（XELOX法）は第Ⅱ相試験においてRR 55％，PFS 7.7カ月，MST 19.5カ月と良好な成績を示している．2005年のASCOでは転移性結腸直腸癌患者に対する一次治療としてのcapecitabine＋L-OHP併用療法（CAPOX法）と5-FU/LV（AIO法）＋L-OHP併用療法（FUFOX法）を比較するRCTの報告がなされ，CAPOX法はFUFOX法とほぼ同等の効果と安全性を示すことが報告されている[22]．現在，XELOX法とFOLFOX 4法に対してbevacizumabをon-offする2×2デザインの4アームのRCTが進行中である，

おわりに

大腸癌に対する化学療法はこの10年で欧米を中心に劇的な変貌を遂げてきているが，わが国は海外から大きな遅れをとっている．現在ようやくFOLFIRI法，FOLFOX法が実施可能となったが，今後は分子標的薬の早期承認が切望される．

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(仁科智裕，兵頭一之介)

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Biological Markers as a Predictor for Response and Prognosis of Unresectable Gastric Cancer Patients Treated with Irinotecan and Cisplatin

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Background: Previously we reported that immunohistochemical examination of p53, bcl-2, glutathione *S*-transferase-π (GST-π), thymidylate synthase (TS) and vascular endothelial growth factor (VEGF) in biopsy samples was a useful method for predicting clinical outcome of gastric cancer patients treated with 5-fluorouracil and cisplatin. Here, we investigated if these biological markers can predict chemoresponse and survival of unresectable gastric cancer patients treated with irinotecan and cisplatin.

Methods: The subjects were 55 unresectable gastric cancer patients treated with irinotecan (70 mg/m^2, Days 1 and 15) and cisplatin (80 mg/m^2, Day 1). Expression of p53, bcl-2, VEGF was examined immunohistochemically in biopsy samples.

Results: The overall response rate and the median survival time were 55% (30/55) and 321 days, respectively. Thirty patients with intestinal-type adenocarcinoma survived longer than 25 patients with diffuse-type (median survival time: 446, 259 days, $P = 0.013$). The favorable phenotypes for chemoresponse were p53-negative, bcl-2-negative and VEGF-positive, which were in accordance with previous findings. The response rate was significantly correlated with the total number of these favorable phenotypes ($P = 0.043$). The 39 patients having 2 or 3 favorable phenotypes (p53-negative, bcl-2-negative and VEGF-positive) survived longer than the remaining 16 patients (median survival time: 444, 259 days, $P = 0.021$). In the Cox model, the number of the favorable phenotypes showed a tendency to correlate with survival after adjustment for potentially prognostic factors such as histological type or performance status ($P = 0.070$).

Conclusions: Immunohistochemical examination of biological markers may be useful in predicting the clinical outcome of unresectable gastric cancer patients treated with irinotecan and cisplatin.

Key words: gastric cancer – chemotherapy – p53 – bcl-2 – VEGF

INTRODUCTION

Cisplatin is an active agent against gastric cancer (1), and several chemotherapy regimens including cisplatin have been reported to show high response rates (2–5). Irinotecan, which inhibits DNA topoisomerase I, is also active against various malignancies including gastric cancer (6). Marked synergism, lack of cross-resistance, different mechanisms of action and relatively different profiles of adverse reactions between irinotecan and cisplatin have encouraged the combination of these agents, and it has shown promising results against gastric and lung cancers. In a phase II study for metastatic gastric cancer, the response rate was 59% and the median survival time was 322 days in 29 patients who had not received previous chemotherapy (7). However, in recent phase III studies, regimens of combined chemotherapy including cisplatin have failed to demonstrate a survival benefit compared

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with the single agent 5-fluorouracil (5-FU) (8–10), which had been developed more than 40 years ago (11). The severe toxicity of these cisplatin-containing regimens seems to be one of the reasons for no survival benefit despite the higher response rates as compared with 5-FU alone. It appears that we need to select an optimal regimen for each patient by predicting the chemotherapeutic efficacy. Recently, remarkable advances in the basic research have led to the identification of many biological markers indicative of sensitivity to some antineoplastic agents, some of which have been proved to have clinical impact.

Previously we reported that immunohistochemical examination of biological markers [p53, bcl-2, glutathione *S*-transferase-π (GST-π), thymidylate synthase (TS) and vascular endothelial growth factor (VEGF)] in biopsy samples was useful method for predicting the effects of chemotherapy. Our report showed that VEGF-positive, TS-negative, p53-negative, GST-π-negative, bcl-2-negative were favorable phenotypes in terms of chemoresponse, and the number of favorable phenotypes was a good indicator of both response and survival in patients with unresectable gastric cancer treated with 5-FU and cisplatin (12). Mutant p53 and bcl-2 proteins protect cancer cells from apoptosis induced by many antineoplastic agents and confer cytotoxic drug resistance (13–15). GST-π is an enzyme that plays an important role in cellular detoxification, and increases in this enzyme have been associated with resistance to antineoplastic agents such as CDDP (16–18). Because drug delivery is important for the sensitivity of tumors to antineoplastic agents, VEGF may contribute to chemoresponse through the promotion of angiogenesis and/or vascular permeability (19,20). In the present study, we investigated the relationship between immunohistochemical expression of VEGF, p53, bcl-2, GST-π and effects of chemotherapy in patients with unresectable gastric cancer treated with irinotecan and cisplatin. TS was not examined because 5-FU was not included in this regimen.

PATIENTS AND METHODS

Study Population

A total of 55 gastric cancer patients treated with irinotecan and cisplatin were retrospectively included in this exploratory analysis; the study subjects included nine patients entered into a phase II study of combination chemotherapy (7), which is one of experimental arms of ongoing randomized phase III trial in Japan (JCOG 9912), and 46 patients consecutively selected to be suitable for combination chemotherapy using the same regimen in clinical practice at the National Cancer Center Hospital East and its affiliated institutions. All the patients fulfilled the recruitment criteria used in JCOG 9912 except (viii): in brief, (i) histological confirmation of gastric cancer; (ii) Eastern Clinical Oncology Group scale performance status (PS) of 2 or better; (iii) age of 75 years or younger; (iv) no previous chemotherapy; (v) adequate bone marrow, liver and renal function; (vi) no other active malignancy; (vii) no severe medical complication; and (viii) primary tumors from which it was possible to obtain a sufficient amount of cancerous tissue for examining biological markers before chemotherapy.

Treatment Schedule

The treatment schedule of the combination of irinotecan and cisplatin and dose modification were the same as in the phase II study (7), briefly, drip infusion of irinotecan (70 mg/m^2, day 1 and 15) and cisplatin (80 mg/m^2, day 1) with adequate hydration. This treatment was repeated every 4 weeks until disease progression, patient refusal or unacceptable adverse reactions.

Evaluation of the Effects of Chemotherapy

Responses to chemotherapy in measurable lesions were evaluated by the standard World Health Organization response criteria (21). For primary lesions, responses were evaluated according to the criteria proposed by the Japanese Research Society for Gastric Cancer (22) using either gastroscopy or barium gastrography. Overall response was defined as the sum of the number of complete and partial responses. All patients were followed for at least 1 year after the initiation of chemotherapy, and survival time was defined as the period from the date of initiation of chemotherapy to the date of death due to any cause or the date of last confirmation of survival.

Immunohistochemical Examination

Immunohistochemical staining was performed in the same way as in our previous study (12). All immunohistochemical examinations were performed on tissue sections of formalin-fixed and paraffin-embedded biopsy specimens from primary tumors. Serial 3 μm thick slices were cut, deparaffinized in xylene and dehydrated with a graded series of ethanol solutions, then immersed in methanol containing 0.3% H_2O_2 for 20 min to inhibit endogenous peroxidase activity. The sections stained for p53 and bcl-2 were heated to 95°C by microwave irradiation for 10 min in phosphate-buffered saline (PBS) and 10 mM citrate buffer, respectively. The sections stained for VEGF were treated with 0.05% pepsin in 0.01 N HCl for 20 min at room temperature. After blocking with 10% normal swine serum in PBS (blocking buffer) for 60 min at room temperature, all sections were incubated overnight at room temperature with the primary antibodies diluted in blocking buffer to the following concentrations: anti-p53 antibody (Nichirei, Tokyo, Japan), 1:20 000; anti-bcl-2 antibody (DAKO, Glostrup, Denmark), 1:40; anti-GST-π antibody (MBL, Nagoya, Japan), 1:24 000; anti-VEGF antibody (Santa Cruz Biochemistry, CA, USA), 1:500. The sections were washed with PBS and then incubated with biotinylated second antibody diluted to 1:200 for 1 h. After washing with PBS, the sections were incubated with ABC reagent (Vector Laboratories, CA, USA), and the color was developed in a reaction

mixture containing 2% 3-3′-diaminobenzidine and 0.3% hydrogen peroxide in Tris buffer. The sections were then counterstained with hematoxylin or methyl green. The two investigators, F.N. and N.B., who were blinded to clinical outcome, assessed immunohistochemical staining independently. The intensity of staining of p53 and GST-π was graded as (++) when strong, as (+) when faint and as (−) when no staining was visible. For bcl-2, the intensity of staining was graded as (++) when stronger than that in the case of lymphocytes, as (+) when equal and as (−) when weaker than that in the case of lymphocytes. The staining of VEGF was graded as (+) when the intensity of staining in the case of the cancer cells was stronger than that in the case of stromal cells, as (±) when equal and as (−) when weaker. For all markers, cases were defined as positive when >20% of all cancer cells in each section showed (++) or (+).

Statistical Analysis

Chi-squared test was applied for comparisons between the expression of biological markers and the chemoresponse. Mantel test was applied for comparisons between the chemoresponse and the number of favorable phenotypes. Survival curve was constructed using Kaplan–Meier method and compared using log-rank test. Prognostic importance of the number of favorable phenotypes was analysed using the Cox regression model, which included tumor extension, histological and macroscopic tumor type, performance status, and age as covariates. These covariates were selected because they were recognized as important variables to predict survival in the previous study (12). Statistic analysis was performed by JMP ver. 4.0.5J software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patient Backgrounds and Chemotherapeutic Effects

Clinicopathological features are listed in Table 1. The overall response rate was 55% (30/55), and the median survival time (MST) was 321 days. While the response rate did not differ between the patients with intestinal-type and diffuse-type (47%, 56%, $P = 0.843$), the former survived much longer than the latter (MST: 446, 259 days, $P = 0.013$).

Biological Marker Expression

Positive staining of p53 was observed in the nuclei of the cancer cells, whereas that of bcl-2 was observed in the cytoplasm. Staining of VEGF was observed in both cancer and stromal cells. The positive rates of p53, bcl-2, VEGF were 44% (24/55), 18% (10/55) and 64% (35/55), respectively. The clinicopathological features did not differ between the expression positive patients and the negative patients for p53, bcl-2 and VEGF, respectively. However, since GST-π-positive patients had a significantly better performance status than GST-π-negative patients, we excluded GST-π from the further investigation.

Table 1. Clinicopathological features of the subjects

Clinicopathological features	No. of patients (%)
Sex	
Male	37 (67)
Female	18 (33)
Performance status	
0	34 (62)
1, 2	21 (38)
Age (years)	
>60	27 (49)
≤60	28 (51)
Macroscopic type	
Non-scirrhous	20 (36)
Scirrhous	35 (64)
Histological type	
Intestinal	30 (55)
Diffuse	25 (45)
Degree of tumor extent	
Locally advanced	20 (36)
Metastatic	35 (64)

Table 2. Expression of biological markers and antitumor response

Marker	CR + PR (%)	NC + PD (%)	Total	*P*-value
p53 (−)	20 (65)	11 (35)	31	0.0915
p53 (+)	10 (42)	14 (58)	24	
bcl-2 (−)	25 (56)	20 (44)	45	0.9999
bcl-2 (+)	5 (50)	5 (50)	10	
VEGF (+)	22 (63)	13 (37)	35	0.1015
VEGF (−)	8 (40)	12 (60)	20	

CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

Biological Marker Expression and Response

Higher response rates were observed in patients with p53 negative, bcl-2 negative and VEGF positive, respectively (Table 2). These relationships were in agreement with the previous findings (12). We, therefore, designated p53-negative, bcl-2-negative and VEGF-positive as favorable phenotypes for chemoresponse.

Biological Marker Expression and Survival

As a single factor, the patients with favorable phenotypes survived slightly longer than the patients without such

Table 3. Number of favorable phenotypes and antitumor response

No. of favorable phenotypes*	Antitumor response		
	CR + PR (%)	NC + PD (%)	Total
3	13 (72)	5 (28)	18
2	11 (52)	10 (48)	21
1 or 0	6 (38)	10 (62)	16

CR, complete response; PR, partial response; NC, no change; PD, progressive disease.
*Favorable phenotypes include p53-negative, bcl-2-negative and VEGF-positive.

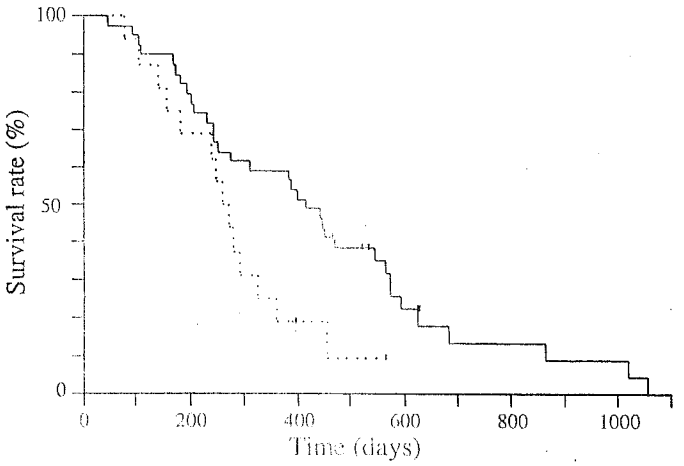

Figure 1. Survival of patients with or without favorable phenotypes. The solid line and dotted line represent patients with or without 2 or 3 favorable phenotypes, respectively ($P = 0.021$).

favorable phenotypes, but they were not significant (p53, $P = 0.504$; bcl-2, $P = 0.402$; VEGF, $P = 0.479$).

Combination of Biological Markers

The total number of these favorable phenotypes demonstrated a significant association with the response rate ($P = 0.043$, Table 3). Thirty-nine patients with 2 or 3 favorable phenotypes survived longer than the remaining 16 patients with statistical significance (MST: 444, 259 days, $P = 0.021$, Fig. 1). Table 4 shows relationships between the number of favorable phenotypes and clinicopathological features which were recognized as important prognostic factors in the previous study. Twenty-five (64%) of the 39 patients with 2 or 3 favorable phenotypes had intestinal-type adenocarcinoma histologically, whereas 5 (31%) of the 16 patients with 1 or 0 had intestinal-type ($P = 0.053$). There was no difference in other clinicopathological features between the two groups.

Multivariate Analysis

The covariates in the Cox model were set to the same as those in our previous study to improve the comparability between the previous and present results (Table 5). In the Cox model, the number of the favorable phenotypes showed a tendency to correlate with survival; the prognosis in patients having only <2 favorable phenotypes was poorer compared with that in patients having 2 or 3 favorable phenotypes (hazard ratio: 1.43, $P = 0.070$). Among the covariates in the model, histological type and performance status were significantly correlated with survival. Tumor extension, macroscopic type and age were not significant in the present study.

Table 4. Clinicopathological features and number of favorable phenotypes

Clinicopathological features	No. of favorable phenotypes		
	2 or 3 (%)	1 or 0 (%)	*P*-value
Age (years); median (range)	60 (26–74)	51 (26–68)	
Sex			
Male/female	26/13 (67/33)	11/5 (69/31)	0.9999
Performance status			
0/1, 2	23/16 (59/41)	11/5 (69/31)	0.7056
Macroscopic type			
Non-scirrhous/scirrhous	17/22 (44/56)	3/13 (19/81)	0.1238
Histological type			
Intestinal/diffuse	25/14 (64/36)	5/11 (31/69)	0.0537
Tumor extent			
Locally advanced/metastatic	13/26 (33/66)	7/9 (44/56)	0.2501

DISCUSSION

Our results support the hypothesis that immunohistochemical examination of biological markers may be useful in predicting the clinical outcome for unresectable gastric cancer patients receiving chemotherapy. The number of favorable phenotypes (p53-negative, bcl-2-negative and VEGF-positive) indicates chemotherapeutic effects. We are aware of no published reports that describe relation between biological markers and therapeutic effects in gastric cancer patients treated with irinotecan and cisplatin. Our results also confirm the results of the previous report that suggested the utility of combination of biological markers (12).

Up to the present, a few biological mechanisms have been implicated in determining the sensitivity to antineoplastic agents. Yeh et al. (23) reported that overexpression of p53 was not associated with resistance of gastric cancer to 5-FU-based systemic chemotherapy, whereas Nakata et al. (24) reported that p53 protein overexpression could serve as a predictor of the response to chemotherapy in gastric cancer. Thus, the correlation between some biological markers and chemoresponse is still controversial in cases of gastric cancer.

In the present study, patients who are either p53-negative, bcl-2-negative or VEGF-positive showed only a slightly higher response rate than the others. A single biological marker seems to have a small impact in predicting chemosensitivity, as shown in our previous study. Nakata et al. (25) investigated the relationship between bcl-2 and bax proteins and effect

Table 5. Cox proportional regression analysis for survival

Variable	Categories	*P*-value	Hazard rate ratio (95% CI)
No. of favorable phenotypes	2–3 versus 0–1	0.0704	1.433 (0.969–2.104)
Histological type	Intestinal versus diffuse	0.0066	1.695 (1.159–2.487)
Tumor extension	Locally advanced versus metastatic	0.1029	1.346 (0.941–1.942)
Performance status	0 versus 1 and 2	0.0208	1.514 (1.065–2.173)
Macroscopic type	Non-scirrhous versus scirrhous	0.6078	0.914 (0.651–1.293)
Age (years)	≤60 versus >60	0.7098	0.942 (0.684–1.287)

CI, confidence interval.

of chemotherapy in gastric cancer patients, and reported that among the bax-positive cases patients with bcl-2-positive tumors were significantly more resistant to 5-FU and had a worse prognosis than bcl-2-negative cases. Some other reports have also described the utility of combination of a couple of biological markers (26,27).

In the present study, the number of favorable phenotypes showed a clear correlation with response rates. Moreover, patients with 2 or 3 favorable phenotypes survived significantly longer than those with 1 or 0. From these results, the number of favorable phenotypes may be a good predictor of therapeutic effects in gastric cancer patients treated with irinotecan and cisplatin. Patients with intestinal-type adenocarcinoma survived longer than those with the diffuse-type, though the reasons for this difference are not clear. Yonemura et al. (28) reported a close relationship between VEGF-C expression, lymphatic spread and prognosis after surgery in gastric cancer patients. During the last few years, it was revealed that VEGF-A plays a role of prime importance in angiogenesis (29,30). The majority of subtypes of VEGF may explain the difference in chemoresponse.

The median survival time of the phase II study of irinotecan and cisplatin was 322 days, and other phase III studies for patients with metastatic gastric cancer generally showed median survival time of 7–11 months (8–10,31). And the overall response rate and survival in the present study were very similar to the results of phase II study of irinotecan and cisplatin. From these points, the overall survival time of patients with intestinal-type and 2 or 3 favorable phenotypes was remarkably long (data was not shown). Although survival elongation dose not always need high response rate, we already reported usefulness of biological markers and we have a tendency that the number of favorable phenotypes correlates to survival in the present study.

Investigating biological markers with consecutive patients eligible for enrollment criteria based on JCOG9912, as the present study is retrospective and some selection biases may not be excluded, we cannot confirm a utility of combinations of biological markers. We think a utility of biological markers should be confirmed in a large-scale study prospectively. The Japan Clinical Oncology Group had already initiated a three-arm randomized trial comparing 5-FU alone with S-1 alone and with irinotecan and cisplatin (JCOG9912). We are planning to investigate biological markers in this phase III trial.

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ORIGINAL ARTICLE

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A phase I study of doxifluridine combined with weekly paclitaxel for metastatic gastric cancer

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Abstract *Purpose*: Based on the synergistic effect in preclinical studies, a phase I clinical trial for the combination of paclitaxel and doxifluridine (an intermetabolite of capecitabine) was performed to determine the recommended dose for the treatment of patients with metastatic gastric cancer. *Methods*: The dose of paclitaxel was increased from 60 mg/m^2 at level 1 to 90 mg/m^2 at level 5. It was administered as a 1-h infusion on days 1 and 8. The dose of doxifluridine was fixed at 600 mg/m^2 per day up to level 3, and escalated to 800 mg/m^2 per day at levels 4 and 5. It was administered orally for 2 weeks. The treatment was repeated every 3 weeks. *Results*: A total of 28 patients were enrolled. No dose-limiting toxicity (DLT) was observed at levels 1 and 2 (paclitaxel 70 mg/m^2). A DLT of grade 4 neutropenia lasting for more than 4 days was observed in one patient at level 3 (paclitaxel 80 mg/m^2). In addition, the first five of six patients in this group experienced grade 3 neutropenia during the first treatment cycle. A further six patients were added in order to confirm the safety of this dosage level, and no more DLTs except for grade 3 nausea in one patient were observed in the second cohort. No DLT was seen in three patients at level 4 (paclitaxel 80 mg/m^2). DLTs (grade 3 neuropathy in one patient and a treatment delay of the second cycle for more than 1 week due to grade 3 neutropenia in another) were observed in two out of six patients at level 5 (paclitaxel 90 mg/m^2), and this dose level was determined as the maximum tolerated dose. The tumor response rate was 42% (95% confidence interval 20–67%) in 19 patients with measurable lesions. *Conclusions*: The recommended dose was determined as 80 mg/m^2 of paclitaxel (days 1 and 8) and 800 mg/m^2 of doxifluridine (days 1–14) every 3 weeks. The results of this phase I study are encouraging and a phase II trial is thus warranted.

Keywords Doxifluridine · Thymidine phosphorylase · Taxane · Gastric cancer · Clinical trial

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Introduction

The incidence of gastric carcinoma is still high in Asia and it remains one of the leading causes of death [13, 28]. The prognosis for patients with unresectable or metastatic gastric carcinoma is poor, but chemotherapy confers a benefit when compared with best supportive care alone [9, 23]. In the past over 20 years, several anticancer drugs such as 5-fluorouracil (5-FU), cisplatin, methotrexate, doxorubicin, epirubicin, mitomycin, and etoposide, have been studied either alone or in combination as treatments for this disease. However, no new combination has yet emerged that is superior to 5-FU alone or to 5-FU plus cisplatin in terms of overall survival [13, 22, 31]. There is a pressing need for the evaluation of new agents such as the oral fluoropyrimidines and taxanes.

Paclitaxel promotes microtubule assembly and then exhibits its antitumor effect by arresting the cell cycle in the G_2/M phase. This mechanism of action is different from conventional anticancer drugs, and it has therefore been suggested that combination therapy with other anticancer drugs may be clinically effective [17]. The efficacy of paclitaxel has previously been confirmed clinically in various tumors including gastric cancer [1, 5, 10, 18, 19, 21, 33]. Furthermore, some promising regimens of paclitaxel combined with 5-FU/leucovorin/cisplatin, or with 5-FU/cisplatin have been reported in advanced gastric cancer [11, 14].

Preclinical studies have shown that paclitaxel induces thymidine phosphorylase (dThdPase) specifically in various human tumor tissues [26]. The oral fluoropyrimidine capecitabine and its intermetabolite doxifluridine are prodrugs that are converted to 5-FU by dThdPase in tumor tissues [6, 12]. A synergistic effect on inhibition of tumor growth has been reported when these agents are combined with paclitaxel [26]. Modest activity of capecitabine and doxifluridine has been reported in the treatment of advanced gastric cancer [7, 15, 20, 32]. Doxifluridine was approved for use in the treatment of advanced gastric cancer in 1987 in Japan, but capecitabine is still under investigation for this disease.

Thus, we conducted a phase I clinical trial in order to study the feasibility of paclitaxel/doxifluridine combined therapy. The tumor response was also investigated.

Patients and methods

Patients

All patients had to fulfill the following eligibility criteria: (1) histological confirmation of gastric adenocarcinoma; (2) inoperable metastatic disease or recurrent metastatic disease after surgery; (3) measurable or evaluable lesions; (4) aged from 20 to 75 years; (5) performance status (PS) ≤ 2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) a maximum of one prior chemotherapy other than paclitaxel or doxifluridine for advanced disease (prior chemotherapy for advanced disease must have been completed at least 4 weeks prior to enrollment); (7) adequate bone marrow function (absolute granulocyte count $\geq 1500/mm^3$ and platelet count $\geq 100{,}000/mm^3$; (8) adequate liver function (serum bilirubin < 1.5 mg/dl and serum transaminase < 100 U/l); (9) adequate renal function (serum creatinine < 1.2 mg/dl); (10) no other severe medical conditions; (11) no other active malignancies; (12) no pregnant or lactating patients; (13) no peripheral neuropathy; and (14) provision of written informed consent.

This study was approved by the Institutional Review Board of the National Shikoku Cancer Center.

Dose-limiting toxicity and maximum tolerated dose

Dose-limiting toxicities (DLTs) were determined during the first treatment cycle. The definitions of DLTs were as follows: (1) grade 4 neutropenia lasting for at least 4 days, or grade 3 or 4 neutropenia with fever, (2) grade 4 thrombocytopenia, (3) grade 3 non-hematological toxicity, and (4) treatment delay of more than 2 weeks following the last administration of doxifluridine. The maximum tolerated dose (MTD) was defined as the dose level at which two of the three to six treated patients experienced DLT, and the recommended dose (RD) was determined at one level below.

Baseline evaluation included a complete medical history, physical examination, complete blood cell count, serum chemistry, urinary analysis, ECG, gastroscopy, gastrography, abdominal CT scan, and chest radiography. Blood, chemistry, urinary analyses, and subjective/objective symptoms for toxicity were monitored on a weekly basis during the treatment. Blood cell counts were determined at least every 2 days if hematological toxicities of grade 3 or more were seen in the first treatment cycle. When patients received the subsequent treatment cycle, they had to fulfill the previous eligibility criteria (7), (8), and (9), and their non-hematological toxicities had to recover to grade 1.

Toxicities were evaluated according to the National Cancer Institute common toxicity criteria (version 2.0).

Dosage and administration

The previous reports of phase I clinical trials studying the weekly administration of paclitaxel as a single agent in breast and ovarian cancer revealed that the RD was 80–100 mg/m^2 [16, 27]. We set the starting dose of paclitaxel (Taxol; Bristol-Myers Squibb Company, Tokyo, Japan) at 60 mg/m^2 and the dose was escalated by 10 mg/m^2 for each dose level up to dose level 3. Paclitaxel dissolved in 500 ml of an isotonic sodium chloride solution was administered on days 1 and 8 as an intravenous (i.v.) drip injection over 60 min following the short premedication (dexamethasone sodium phosphate 20 mg i.v. drip, diphenhydramine hydrochloride 50 mg orally, and ranitidine hydrochloride 50 mg i.v. 30 min before paclitaxel administration). Because 600–800 mg/m^2 per day of doxifluridine (Fulturon; Chugai Pharmaceutical Company, Tokyo, Japan) was considered the dose for patients with gastric cancer and this dose had been approved as the single-agent RD in Japan [20, 33], we fixed doxifluridine at the dose of 600 mg/m^2 per day and administered it orally at regular intervals four times a day (after each meal and before sleep) for 14 days. If the MTD did not reach level 3, the dose of each drug in the subsequent level was escalated in tandem by 10 mg/m^2 of paclitaxel and by 200 mg/m^2 of doxifluridine as shown in Table 1.

This treatment was repeated every 3 weeks (one cycle each) until disease progression or unacceptable toxicity was seen. The first cycle of the treatment was performed in the in-patient setting in our center. If the patient experienced DLT followed by no disease progression, the subsequent cycle was started at the next lower level after complete recovery from the toxic effects of the previous cycle.

Tumor response

Tumor response was evaluated every 6 weeks by means of CT scan. Measurable lesions were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) [30].

Table 1 Dose level, number of patients enrolled, and DLT

Level	Paclitaxel (mg/m^2)	Doxifluridine (mg/m^2)	No. of patients	DLT
1	60	600	4	None
2	70	600	3	None
3	80	600	12	One grade 4 neutropenia lasting more than 4 days; one grade 3 nausea
4	80	800	3	None
5	90	800	6	One grade 3 neuropathy; one treatment delay due to neutropenia

Results

A total of 28 patients were enrolled, with 4 patients dosed at level 1, 3 at level 2, 12 at level 3, 3 at level 4, and 6 at level 5 from September 2001 to January 2003 (Table 1). Because one patient dosed at level 1 developed a grade 1 hypersensitivity reaction during the first treatment cycle and refused further treatment, a replacement patient was added to this dosage group. The patient characteristics are shown in Table 2. Of the 28 patients, 21 exhibited a good PS (0 or 1), and 22 had had a prior chemotherapy. The most frequent prior chemotherapy was 5-FU (17 patients). Nine patients had differentiated histological gastric adenocarcinoma, and the remainder had the undifferentiated type. The major metastatic sites were peritoneum, lymph nodes and liver.

The adverse events in the first cycle are summarized in Table 3. The most frequently observed toxicity was neutropenia. DLTs were not observed at levels 1 and 2, but a DLT (grade 4 neutropenia which continued for more than 4 days) was observed in the second patient at level 3. Then three patients were added to this dosage group. No DLT was observed in these additional patients. However, grade 3 neutropenia was observed in five patients (83%) in the first treatment cycle at this dose level. In addition, a 1-week postponement of the second cycle was needed due to the neutropenia in one patient and grade 4 neutropenia developed in another patient in the second cycle. Therefore, an additional six patients were enrolled in order to confirm the safety of this dose level. No DLT except for grade 3 nausea in one patient was observed in this second cohort, and we moved to the next dosage level. At level 4, grade 3 neutropenia was observed in two of three patients. However, no DLT was seen in this cohort. DLT (more than a 1-week treatment delay due to grade 3 neutropenia) was observed in the third patient at level 5. Three patients were added to this level. DLT (grade 3 peripheral neuropathy) was observed in the sixth patient. Grade 2 neuropathy appeared following the first administration of paclitaxel on day 1 and increased to grade 3 immediately after the second administration on day 8. The treatment was continued up to three cycles at the next lower dosage level, although grade 1 or 2 peripheral neuropathy developed during every cycle. From these results, level 5 was determined as the MTD and level 4 (paclitaxel 80 mg/m^2, doxifluridine 800 mg/m^2) was set as the RD. The lowest neutrophil counts in the first cycle at each dosage level are shown in Table 4. The medians of the lowest absolute neutrophil counts were graded as grade 3 neutropenia in levels 3, 4 and 5. Their values were apparently lower than those in levels 1 and 2. The period of recovery to grade 1 was around a week in levels 3, 4, and 5. It was also longer than that in levels 1 and 2.

The main toxicity of this combined therapy was myelotoxicity, neutropenia in particular. Grade 3 or 4 neutropenia was observed in 0 of 12 cycles (0%) at level 1, 1 of 20 cycles (5%) at level 2, 14 of 76 cycles (18%) at level 3, 3 of 13 cycles (23%) at level 4, and 3 of 15 cycles (20%) at level 5. Non-hematological toxicities of greater than grade 3 were observed in four patients during all treatment cycles. Two of these were the DLT. One of the remaining two patients showed grade 3 diarrhea in the fourth cycle at level 4, and the other patient showed grade 3 peripheral neuropathy after five cycles at level 5. A total of seven patients needed dose reduction during all treatment cycles. Four patients with DLT (Table 1) and two patients with grade 3 diarrhea and grade 3 peripheral neuropathy, respectively, were included. The other was the patient who showed grade 4 neutropenia in the second cycle at level 3. Peripheral neuropathy of grade 1 or 2 occurred in 2 of 12 patients at level 3, 1 of 3 patients at level 4, and 3 of 6 patients at level 5. It tended to be more severe following repeated administration of paclitaxel and seemed cumulative. Hand-foot syndrome

Table 2 Characteristics of patients

Age (years)	
Median	63
Range	44–75
Sex	
Male/female	16/12
Performance status (ECOG)	
0/1/2	10/11/7
Prior therapy	
Gastrectomy	20
Chemotherapy (5-FU)	22 (17)
Histological type	
Differentiated	9
Undifferentiated	19
Sites of metastasis	
Liver	6
Abdominal lymph nodes	17
Lung	5
Peritoneum	19
Spleen	2

Table 3 Adverse reactions during the first treatment cycle

Toxicity	Level 1 ($n=4$)		Level 2 ($n=3$)		Level 3 ($n=6$)				Level 4 ($n=3$)		Level 5 ($n=6$)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Hematological												
Leukopenia	2	0	2	1	5	1	1	2	2	0	5	0
Neutropenia	3	0	2	1	1	5[a]	3	2	0	3	3	2[a]
Lymphocytopenia	4	0	1	1	1	0	1	1	2	0	6	0
Anemia	3	1	3	0	4	1	5	0	0	2	4	1
Thrombocytopenia	2	0	0	0	0	0	2	0	0	0	1	0
Non-hematological												
Hypersensitivity reaction	1	0	0	0	0	0	0	0	0	0	0	0
Infection	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue	2	0	0	0	1	0	1	0	3	0	0	0
Nausea	2	0	0	0	0	0	1	1[a]	0	0	2	0
Vomiting	0	0	0	0	0	0	1	0	0	0	0	0
Anorexia	1	0	0	0	1	0	0	0	2	0	1	0
Diarrhea	0	0	0	0	2	0	1	0	2	0	0	0
Stomatitis	1	0	0	0	0	0	0	0	0	0	1	0
Alopecia	1	–	0	–	3	–	2	–	2	–	5	–
Edema	0	0	0	0	0	0	0	0	0	0	0	0
Hand-foot syndrome	0	0	0	0	0	0	0	0	0	0	0	0
Rash	1	0	0	0	0	0	1	0	0	0	0	0
Palpitations	0	0	0	0	0	0	1	0	1	0	0	0
Peripheral neuropathy	0	0	0	0	0	0	0	0	1	0	0	1[a]

[a]DLT was observed in one patient

Table 4 Lowest absolute neutrophil count (LNC) during the first treatment cycle

Level	LNC (per mm^3)		Time to LNC (days)		Recovery from LNC (days)	
	Median	Range	Median	Range	Median	Range
1	1860	1287–1884	14	12–22	2	2–6
2	1700	558–1768	14	4–17	3	2–10
3	904	200–1672	14	6–16	8	4–15
4	867	738–996	14	14	8	7–8
5	902	801–1824	14	13–17	6	6–21

was not observed in any patient during any treatment cycle. There was no treatment-related death.

The median numbers of administration cycles were 2 (range 1–8) for level 1, 6 (range 3–11) for level 2, 6 (range 2–13) for level 3, 4 (range 3–6) for level 4, and 3 (range 2–5) cycles for level 5.

An objective tumor response was not observed in the patients at dosage levels 1 and 4. Three patients showed a partial response (PR) at level 2. Two patients showed a complete response (CR) and one patient showed a PR at level 3. Two patients showed a PR at level 5. The overall response rate in all 19 patients with measurable lesions was 42% (95% confidence interval 20–67%). The response rate in pretreated patients was 43% (6/14) and that in chemonaive patients was 40% (2/5). The ascites disappeared in two of three patients without measurable lesions at level 3.

Discussion

Based on the results of phase I and II clinical trials of paclitaxel, the RD was set at 210 mg/m^2 over a 3-week dosing schedule in Japan, and a relatively high tumor response rate of 23% has been reported for advanced gastric cancer [29, 33]. In addition, paclitaxel yielded the same response rate in the second-line setting (23%) as in the first line setting (24%), and non-cross resistance with other anticancer drugs was suggested. In terms of the toxicity, leukopenia and neutropenia of higher than grade 3 were observed in 28% and 58% of patients, respectively. These results are supported by other studies [1, 5]. In recent years, the concept of dose-dense therapy whereby the interval between administrations is shortened to reduce the time for regrowth of neoplastic cells has been proposed [8]. Several clinical studies involving weekly dose-dense therapy of paclitaxel have been performed in lung, breast, and ovarian cancer [16, 24, 27]. Following a phase I clinical trial in 60 patients with advanced cancer who had been treated previously with systemic chemotherapy other than taxanes, the RD was 80 mg/m^2 of paclitaxel weekly, and grade 3 or higher toxicities were rarely observed [16]. In addition, a recent randomized trial comparing two administration methods of paclitaxel with the same dose intensity (conventional 3-week regimen of 200 mg/m^2 and weekly regimen of 67 mg/m^2) in patients with recurrent ovarian cancer revealed equal response rates and overall survival, and reduced toxicities with the weekly schedule [24]. Thus, weekly dosing of paclitaxel has been confirmed to have equal efficacy and lower toxicity than the conventional dosing regimen.

Capecitabine is still under investigation for advanced gastric cancer in Japan. Instead, the immediate precursor to capecitabine, doxifluridine, has been approved for the treatment of advanced gastric cancer. In preclinical evaluations, the therapeutic index of doxifluridine has been shown to be much more than that of 5-FU [4]. In clinical studies, high oral bioavailability of doxifluridine has been noted, and it has shown prominent antitumor activity in patients with breast, colorectal, and gastric cancers [2, 7, 20]. The DLT of doxifluridine is diarrhea. Recently, it has been reported that several anticancer drugs, including paclitaxel, upregulate the expression of dThdPase specifically in tumor tissues and that paclitaxel in combination with doxifluridine shows the synergistic activity in several human cancer xenograft models [26]. Furthermore, the major toxicities of paclitaxel and doxifluridine do not overlap. Therefore, we adopted a weekly dosing regimen of paclitaxel in combination with doxifluridine.

Neutropenia was the most frequently observed toxicity with this combination therapy, and was dose-limiting. However, no neutropenic fever was seen in this study. At dose level 3, one patient experienced a DLT (grade 4 neutropenia for more than 4 days), and five of six patients, including the patient with DLT, exhibited grade 3 or more neutropenia in the first treatment cycle. Based on these results, the investigators and the independent efficacy and safety committee considered it appropriate to stop dose loading at level 3. However, the MTD was not achieved at this level. Then six patients were added to confirm the safety of this dose level. No DLT except for a patient with grade 3 nausea was observed in this additional cohort. Thus, dose escalation was reopened and no grade 4 neutropenia was observed at level 4. Non-hematological toxicity was generally mild. Peripheral sensory neuropathy, one of DLTs of paclitaxel, was well tolerated up to level 4, and diarrhea, a DLT of doxifluridine, was not severe in the initial few cycles at all levels. At level 4, there was only one patient who needed dose reduction due to diarrhea after four treatment cycles. The median numbers of cycles administered were 6 (range 2–13) at level 3 and 4 (range 3–6) at level 4. The main reason for stopping the treatment was disease progression at levels 3 and 4. From