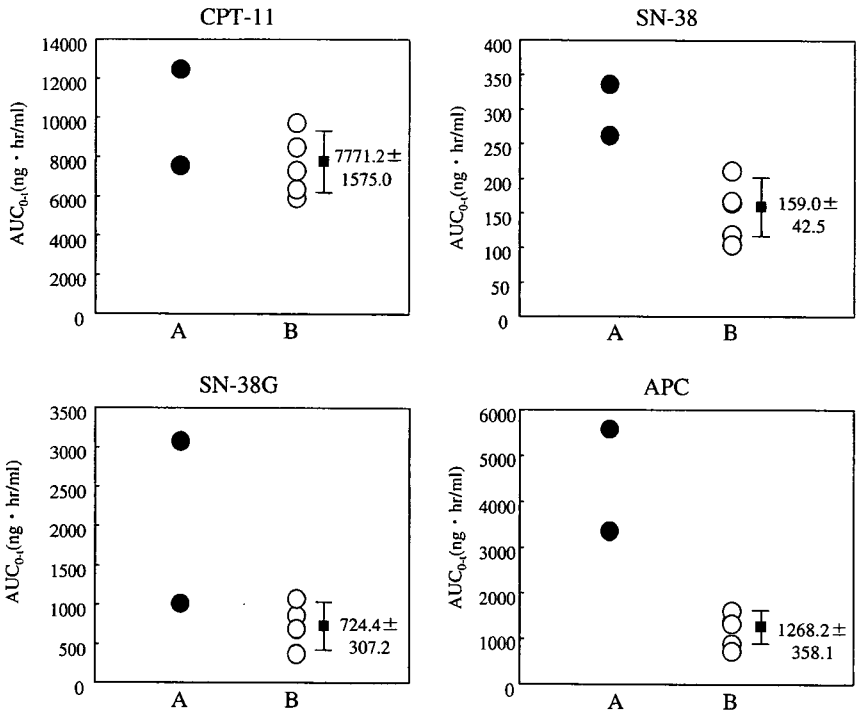


Fig. 2 Area under the concentration versus time curve for irinotecan and metabolites in patients with biliary drainage (*A*, $n = 2$) and without drainage (*B*, $n = 5$). The values are expressed as the mean ± SD

Table 5 Pharmacokinetic parameters after single administration of irinotecan at a dose of 100 mg/m^2 ($n = 7$)

		C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-t} (ngh/ml)	CL (l/h m^2)
Irinotecan	A	1,188.5, 1,997.6	1.6, 1.5	7.8, 8.2	7,762, 12,692	11.8, 7.1
	B	1,701.0 ± 348.3	1.5 ± 0.1	7.7 ± 0.9	7,771.2 ± 1,575.0	12.4 ± 2.5
SN-38	A	25.5, 26.2	2.1, 1.5	14.7, 9.9	268, 342	–
	B	17.5 ± 3.8	2.3 ± 0.8	30.2 ± 27.6	159.0 ± 42.5	
SN-38G	A	81.3, 207.2	3.6, 2.0	10.8, 12.5	1,063, 3,130	–
	B	78.8 ± 34.1	2.2 ± 0.2	21.6 ± 13.2	724.4 ± 307.2	
APC	A	309.2, 359.3	2.6, 5.5	7.0, 9.5	3,441, 5,673	–
	B	116.6 ± 39.7	3.0 ± 0.6	8.8 ± 0.7	1,268.2 ± 358.1	

A Patients with biliary drainage $n = 2$

B Patients without biliary drainage (parameters are represented as the mean ± SD) $n = 5$

episodes before onset. Although our study indicated that weekly irinotecan administration would be tolerable in patients with metastatic pancreatic cancer, careful observation is required during the treatment period, since pancreatic cancer patients tend to suffer various tumor-related complications and easily take a turn to the worse because of tumor progression.

There are two studies of single-agent irinotecan that assessed efficacy and toxicity against pancreatic cancer [14, 22]. Sakata et al. [14] studied irinotecan at a dose of 100 or 150 mg/m^2 administered weekly or bi-weekly to previously treated or untreated patients with pancreatic cancer in Japan. Although 57 of 61 enrolled patients were assessable, only 4 patients (7.0%) showed a PR. This study included 28 patients (49.1%) with poor performance status of 2–3 and 22 patients (38.6%) with prior chemotherapy, and no patient showed a PR in these patients with poor performance status or prior chemotherapy. Wagener et al. [22] demonstrated that irinotecan at a dose of 350 mg/m^2 administered every 3 weeks to chemo-naïve pancreatic cancer patients with performance status of ≤ 2, achieved a PR in 3 of 32 assessable patients (9.4%) with an median overall survival of 5.2 months. Although precise reason for the discrepant response rates between our study and the other two studies is unclear, patient background may be one possible explanation because only chemo-naïve patients with good performance status were entered into our study (89.2% of our patients had good KPS of ≥ 90).

For the purpose of the improvement on response rate and prognosis, several studies of combination therapy have been conducted in patients with pancreatic cancer. With regard to irinotecan with gemcitabine, an

encouraging activity, response rates between 20.0 and 24.7% and median overall survival between 5.7 and 7 months, have been reported in two phase II studies [11, 18]. However, survival benefit of this combination therapy was not shown in a phase III study [12], in which, 360 patients were randomized to treatment with a combination of gemcitabine 1,000 mg/m^2 followed by irinotecan 100 mg/m^2 given on days 1 and 8 of a 3-week cycle versus gemcitabine monotherapy. The response rate for the combination therapy was higher at 16.1% compared with 4.4% for gemcitabine alone, but there was no difference in median overall survival (6.3 vs. 6.6 months). However, several clinical studies have recently indicated that irinotecan-based chemotherapy seemed to be an effective treatment for advanced pancreatic cancer after gemcitabine failure: irinotecan–raltitrexed combination demonstrated overall response rate of 16% (3/19) in patients with gemcitabine-pretreated pancreatic cancer [21], and Cantore et al. [3] reported that irinotecan plus oxaliplatin showed response rate of 10% (3/30) with a clinical benefit response of 20% (6/30) for patients with advanced pancreatic cancer after gemcitabine failure.

Because biliary excretion is a major elimination pathway for irinotecan and its metabolites, we investigated the impact of biliary drainage on the pharmacokinetics for this agent. Our results suggested that patients with biliary drainage tended to have higher area under the concentration versus time curve of irinotecan and metabolites compared with patients without biliary drainage. Meyerhardt et al. [10] reported that modest elevation of bilirubin (1.0–1.5 mg/dl) is associated with increased grade 3 to 4 neutropenia in patients treated with irinotecan. The fact that the two patients with biliary drainage in the current study had slight elevation of baseline serum bilirubin level (1.4 and 1.7 mg/dl) might influence pharmacokinetics for irinotecan. Although no severe hematological or non-hematologic toxicities appeared in these two patients, careful observation may be required when treating patients with biliary drainage.

In conclusion, single-agent irinotecan showed a substantial antitumor activity for patients with metastatic pancreatic cancer, rendering a 27.0% response rate. The toxicity with this schedule appears manageable, though it must be monitored carefully.

Acknowledgments This article is dedicated to the memory of Dr. Okada, a principal investigator and Mr. Sahashi, who assisted for management of this study. We are grateful to Drs T. Taguchi, T. Hayakawa, K. Nagao, Y. Ohashi and M. Kurihara for their kind advice, Drs Y. Sakata, N. Moriyama, and M. Hiraoka for extramural review, and Miss T. Tomizawa and K. Ohno for good support. We also thank Messrs T. Asano and H. Takizawa for assistance in data management. This study was supported by Yakult Honsha and Daiichi Pharmaceutical, Japan.

References

1. Aoki K, Okada S, Moriyama N, Ishii H, Nose H, Yoshimori M, Kosuge T, Ozaki H, Wakao F, Takayasu K, Mukai K (1994) Accuracy of computed tomography in determining pancreatic cancer tumor size. Jpn J Clin Oncol 24:85–87
2. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15:2403–2413
3. Cantore M, Rabbi C, Fiorentini G, Oliani C, Zamagni D, Iacono C, Mambrini A, Del Freo A, Manni A (2004) Combined irinotecan and oxaliplatin in patients with advanced pretreated pancreatic cancer. Oncology 67:93–97
4. Carmichael J, Fink U, Russell RG, Spittle MF, Harris AL, Spiessi G, Blatter J (1996) Phase II study of gemcitabine in patients with advanced pancreatic cancer. Br J Cancer 73:101–105
5. Casper ES, Green MR, Kelsen DP, Heelan RT, Brown TD, Flombaum CD, Trochanowski B, Tarassoff PG (1994) Phase II trial of gemcitabine (2,2′-difluorodeoxycytidine) in patients with adenocarcinoma of the pancreas. Invest New Drugs 12:29–34
6. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, Kuriyama T, Ariyoshi Y, Negoro S, Masuda N, Nakajima S, Taguchi T (1992) A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. CPT-11 Lung Cancer Study Group. J Clin Oncol 10:16–20
7. http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei03/hyo5.html (accessed August 18, 2005)
8. Japan Society for Cancer Therapy: Criteria for the evaluation of the clinical effects of solid cancer chemotherapy (1993) J Jpn Soc Cancer Ther 28:101–130
9. Masuda N, Fukuoka M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Negoro S, Nishioka M, Nakagawa K, Takada M (1992) CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 10:1225–1229
10. Meyerhardt JA, Kwok A, Ratain MJ, McGovren JP, Fuchs CS (2004) Relationship of baseline serum bilirubin to efficacy and toxicity of single-agent irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 22:1439–1446
11. Rocha Lima CM, Savarese D, Bruckner H, Dudek A, Eckardt J, Hainsworth J, Yunus F, Lester E, Miller W, Saville W, Elfring GL, Locker PK, Compton LD, Miller LL, Green MR (2002) Irinotecan plus gemcitabine induces both radiographic and CA 19–9 tumor marker responses in patients with previously untreated advanced pancreatic cancer. J Clin Oncol 20:1182–1191
12. Rocha Lima CM, Green MR, Rotche R, Miller WH Jr, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, Miller LL (2004) Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. J Clin Oncol 22:3776–3783
13. Rothenberg ML, Moore MJ, Cripps MC, Andersen JS, Portenoy RK, Burris HA 3rd, Green MR, Tarassoff PG, Brown TD, Casper ES, Storniolo AM, Von Hoff DD (1996) A phase II trial of gemcitabine in patients with 5-FU-refractory pancreas cancer. Ann Oncol 7:347–353

14. Sakata Y, Shimada Y, Yoshino M, Kambe M, Futatsuki K, Nakao I, Ogawa N, Wakui A, Taguchi T (1994) A late phase II study of CPT-11, irinotecan hydrochloride, in patients with advanced pancreatic cancer (in Japanese). CPT-11 study group on gastrointestinal cancer. Jpn J Cancer Chemother 21:1039–1046
15. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL, Miller LL (2000) Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 343:905–914
16. Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, Kambe M, Taguchi T, Ogawa N (1993) Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. J Clin Oncol 11:909–913
17. Sparreboom A, de Bruijn P, de Jonge MJA, Loos WJ, Stoter G, Verweij J, Nooter K (1998) Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces. J Chromatogr B Biomed Sci Appl 712:225–235
18. Stathopoulos GP, Rigatos SK, Dimopoulos MA, Giannakakis T, Foutzilas G, Kouroussis C, Janninis D, Aravantinos G, Androulakis N, Agelaki S, Stathopoulos JG, Georgoulias V (2003) Treatment of pancreatic cancer with a combination of irinotecan (CPT-11) and gemcitabine: a multicenter phase II study by the Greek Cooperative Group for Pancreatic Cancer. Ann Oncol 14:388–394
19. Tanaka S, Yoshida Y, Suzuki W, Sudo K, Hakusui H (1994) A new HPLC method for the determination of irinotecan hydrochloride (CPT-11) and its metabolite, SN-38 in human plasma and urine (in Japanese). Jpn Pharmacol Ther 22:163–172
20. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205–216
21. Ulrich-Pur H, Raderer M, Verena Kornek G, Schull B, Schmid K, Haider K, Kwasny W, Depisch D, Schneeweiss B, Lang F, Scheithauer W (2003) Irinotecan plus raltitrexed vs raltitrexed alone in patients with gemcitabine-pretreated advanced pancreatic adenocarcinoma. Br J Cancer 88:1180–1184
22. Wagener DJ, Verdonk HE, Dirix LY, Catimel G, Siegenthaler P, Buitenhuis M, Mathieu-Boue A, Verweij J (1995) Phase II trial of CPT-11 in patients with advanced pancreatic cancer, an EORTC early clinical trials group study. Ann Oncol 6:129–132

Jpn J Clin Oncol 2007;37(2)96–101
doi:10.1093/jjco/hyl145

The Role of the Outpatient Clinic in Chemotherapy for Patients with Unresectable or Recurrent Gastric Cancer

Kentaro Yamazaki, Narikazu Boku, Kaoru Shibamoto, Hirofumi Yasui, Akira Fukutomi, Takayuki Yoshino, Shuichi Hironaka, Yusuke Onozawa, Yosuke Otake, Noriaki Hasuike, Hiroyuki Matsubayashi, Tetsuya Inui, Yuichiro Yamaguchi and Hiroyuki Ono

Division of Gastrointestinal Oncology and Endoscopy, Shizuoka Cancer Center, Sunto-gun, Shizuoka, Japan

Received July 5, 2006; accepted September 30, 2006; published online February 1, 2007

Background: Recently, outpatient chemotherapy centers have become popular in Japan. To clarify the actual conditions of outpatient clinics, we surveyed entire clinical courses of chemotherapy in patients with unresectable or recurrent gastric cancer.
Methods: From the medical records of 64 patients with unresectable or recurrent gastric cancer with no prior chemotherapy, we obtained data on overall survival, non-hospitalized survival, the number of and reasons for attendance at the outpatient clinic and hospitalization, and medical conditions at discharge.
Results: The median follow-up time was 520 days, the median survival time was 353 days, and the median non-hospitalized survival time was 282 days. Patients attended the outpatient clinic 1917 times in total; 145 (8%) of these were unplanned visits for accidental disease, disease progression, or toxicity. Patients were hospitalized 291 times in total: 110 (38%) of hospitalizations were unplanned or emergencies because of disease progression or toxicity. Patients were discharged 290 times in total; in 56 of these discharges (19%) unresolved medical problems remained, such as toxicity, total parenteral nutrition, or symptoms related to cancer. Three patients (5%) died from treatment-related leucopenia and thrombocytopenia.
Conclusions: Patients with unresectable and recurrent gastric cancer were treated at outpatient clinics for periods up to 80% longer than the entire clinical course of chemotherapy. However, there were some unplanned or emergency hospitalizations and some patients still experienced medical problems at discharge. The role of the outpatient clinic is very important to chemotherapy for patients with unresectable or recurrent gastric cancer.

Key words: gastric cancer – chemotherapy – outpatient clinic

INTRODUCTION

Gastric cancer is one of the leading causes of death in Japan and throughout the world. Recent progress in diagnostic procedures and surgical treatment has improved the curability of gastric cancer in the resectable stages. However, the prognosis of unresectable or recurrent gastric cancer still remains poor. Randomized trials have demonstrated that fluorouracil (5-FU)-based chemotherapy can improve survival and quality of life (QOL) in patients with unresectable or recurrent gastric cancer compared with best supportive care (1). Although several phase III trials have been conducted for patients with advanced gastric cancer in recent decades, no standard treatment has been established.

However, various novel anti-tumor agents have been developed recently, including irinotecan (CPT-11), oral pyrimidines, taxanes and molecular target agents. Many phase I and II trials have reported on the activities of these new agents, which are used either as single agents or as combination therapy. For the patient, hospitalization deteriorates daily activity, and non-hospitalized survival can thus represent one substantial improvement to QOL. Many of these new drugs, especially oral anti-tumor drugs, can be used in an outpatient setting and may therefore contribute to prolonging the non-hospitalized survival of patients with gastric cancer treated with chemotherapy. While in Japan

For reprints and all correspondence: Kentaro Yamazaki, Division of Gastrointestinal Oncology and Endoscopy, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan. E-mail: k.yamazaki@scchr.jp

most patients for chemotherapy have received in-hospital treatment, many hospitals have recently been establishing chemotherapy centers, where efforts are made to treat patients on an outpatient basis. The Japan Clinical Oncology Group (JCOG) has adopted non-hospitalized survival time as a secondary endpoint in JCOG9912 (Randomized phase III study of 5-FU continuous infusion versus CPT-11 plus cisplatin versus S-1 in advanced gastric cancer).

In gastric cancer, conditions of patients may deteriorate suddenly as a result of various complications such as peritoneal dissemination, which is usually undetectable by radiological imaging and sometimes causes bowel obstruction, hydronephrosis and obstructive jaundice. It is suggested that management of gastric cancer by chemotherapy at outpatient clinics may be more difficult than other non-digestive malignancies.

Few reports have documented the clinical course from the initiation of treatment to death in patients with gastric cancer treated with chemotherapy, and actual problems in outpatient clinics have scarcely been reported in detail. For example, it is not even known how long the non-hospitalized survival is and what kind of problems are encountered at outpatient clinics during chemotherapy and therefore we are left to conclude that the provision of chemotherapy over a full clinical course for cancer patients is still in its infancy in Japan. In this retrospective study, we surveyed the entire clinical course of patients with unresectable and recurrent gastric cancer treated with chemotherapy to investigate the actual conditions of outpatient clinics during cancer treatment, in order to improve the system in the near future.

PATIENTS AND METHODS

Patient Selection

From 199 patients with unresectable and recurrent gastric cancer receiving chemotherapy at the Shizuoka Cancer Center between September 2002 and March 2004, we selected patients who fulfilled the following eligibility criteria listed in JCOG9912: (i) histologically proven unresectable or recurrent adenocarcinoma of the stomach, except for patients whose unresectable cancer was limited to class V by cytological examination of the abdominal cavity or with no visible tumor; (ii) no prior chemotherapy; (iii) adequate oral intake without nutritional support; (iv) no severe peritoneal dissemination associated with massive ascites or remarkable findings detected by barium enema; (v) age between 20 and 75 years; (vi) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 2 or better; (vii) no massive pleural effusion; (viii) no other active malignancies; (ix) adequate bone marrow (white blood count 3000–12 000/μl, platelets ≥100 000/μl), renal (creatinine: ≤1.5 mg/dl), and hepatic functions (aspartate aminotransferase ≤99 IU/l, alanine aminotransferase ≤99 IU/l, bilirubin ≤2.0 mg/dl); (x) no other serious medical complications; (xi) no symptomatic brain metastasis; and (xii) written informed consent for chemotherapy.

Treatment Schedule

All chemotherapy regimens were approved in clinical practice to treat patients with gastric cancer by the Clinical Practice Review Committee of the Shizuoka Cancer Center. All patients provided informed consent before chemotherapy was initiated and the chemotherapy continued until tumor progression, unacceptable toxicity, or patient's refusal to continue. For each patient, the chemotherapy regimen was selected according to the patient and physician's choice for the first-line treatment. Treatment was generally performed by the following schedule: (i) S-1 alone: S-1 (40 mg/m^2 per day, orally twice daily) on days 1–28 every 6 weeks (2,3); (ii) S-1 and cisplatin (CDDP): S-1 (40 mg/m^2 per day, orally twice daily) on days 1–21 and CDDP (70 mg/m^2, intravenously) on day 8 every 5 weeks (4); (iii) sequential methotrexate (MTX) and 5-fluorouracil (5-FU): weekly administration of MTX (100 mg/m^2, bolus) followed by 5-FU (600 mg/m^2, bolus) at 3-h intervals, calcium leucovorin (10 mg/m^2, orally or intravenously) administered six times every 6 h starting 24 h after MTX (5), (iv) CPT-11 and CDDP: CPT-11 (70 mg/m^2, intravenously) on days 1 and 15, and CDDP (80 mg/m^2, intravenously) on day 1 every 4 weeks (6); (v) 5-FU continuous infusion (5-FU c.i.): 5-FU (800 mg/m^2, continuous infusion) on days 1–5 every 4 weeks (7); (vi) weekly paclitaxel (w-PTX): weekly administration of PTX (80 mg/m^2, intravenously) for 3 weeks every 4 weeks (8); (vii) CPT-11 and mitomycin C (MMC): CPT-11 (150 mg/m^2, intravenously) and MMC (5 mg/m^2, bolus) every 2 weeks (9); (viii) 5-FU and isovorin (l-LV): weekly administration of 5-FU (600 mg/m^2, bolus) and l-LV (250 mg/m^2, 2-h infusion) for 6 weeks every 8 weeks (10); (ix) 5-FU and CDDP: 5-FU (800 mg/m^2, continuous infusion) on days 1–5 and CDDP (80 mg/m^2, intravenously) on day 1 every 4 weeks (7); (x) CDDP injected intraperitoneally (11); (xi) CPT-11 alone: CPT-11 (150 mg/m^2, intravenously) every 2 weeks (12,13); (xii) CDDP and etoposide (VP-16): CDDP (80 mg/m^2, intravenously) on day 1 and VP-16 (100 mg/m^2, intravenously) on day 1 every 3 weeks (14); (xiii) hepatic arterial infusion (HAI): 5-fluorouracil (333 mg/m^2) each week, epirubicin (30 mg/m^2) once every 4 weeks and mitomycin-C (2.7 mg/m^2) once every 2 weeks administered by HAI (15); (xiv) MMC alone: weekly administration of MMC (5 mg/m^2, bolus). Dose and schedule were modified according to each patient's medical condition and any toxicities observed in the previous courses.

Evaluation and Statistical Analysis

The overall survival time was calculated from the date of the first administration of chemotherapy of the first-line treatment to the date of death by any causes, or to the last date of confirmed survival. The non-hospitalized survival time was

estimated by subtracting the period of hospitalization by any causes from the overall survival time. We checked the number of and reasons for attendance at outpatient clinics and recorded all treatments, including supportive care, performed at each attendance. We also assessed the number of times patients were hospitalized, the reasons for hospitalization and medical conditions at discharge. We accumulated early death observed within 30 days after the last administration of chemotherapy to investigate the cause of death. Survival analysis was performed using the methods of Kaplan and Meier, by adopting all deaths from any cause as events.

RESULTS

Patient Characteristics

One hundred and ninety-nine patients received chemotherapy during the study period, of whom 135 patients were excluded from analysis and 64 patients fulfilled the eligibility criteria and were entered into the study. The reasons for exclusion were prior chemotherapy (70 patients), no visible tumor (13 patients), older than 76 years (13 patients), severe peritoneal dissemination (10 patients), inadequate oral intake (nine patients), other active malignancy (eight patients), serious medical complication (seven patients), PS 3 or 4 (four patients), and symptomatic brain metastasis (one patient). Table 1 shows the characteristics of the patients. The median age was 64 years (range 32–75 years); 30 patients were PS 0, 27 patients were PS 1, and seven patients were PS 2. One patient had no metastatic sites, 34 patients had one metastatic site, 22 patients had two metastatic sites, eight patients had three or more metastatic sites.

Treatment

Table 2 lists the chemotherapy regimens received over the entire clinical courses of the patients. The median number of regimens was two (range, 1–6), 72% of patients received second-line chemotherapy, and 39% of patients had three or more chemotherapy regimens. In 31 patients (48%), the first-line chemotherapy was started at the outpatient clinic. Oral administration of S-1 was the most frequently used in first-line chemotherapy (35 patients, 55%), and 21 (33%) patients who were given CDDP-containing regimens or continuous infusion of 5-FU required hospitalization. The most frequently used forms of second-line chemotherapy were w-PTX (26 patients, 57%) and a combination of CPT-11 and CDDP (11 patients, 24%).

Table 1. Patient characteristics

Age (years)		
	Median	64
	Range	(32–75)
Gender		
	Male	46
	Female	18
PS	0/1/2	30/27/7
Number of metastatic sites	0/1/2/3/4	1/34/21/6/2

PS, performance status.

Attendance at the Outpatient Clinic

Table 3 lists the number of and reasons for attendance at the outpatient clinic. The median number of visits to outpatient clinics was 29 visits per patient (range, 0–84). The total number of visits was 1917, of which 145 (8%) were unplanned, which were caused by accidental disease (50 visits), disease progression (46 visits), toxicity (45 visits), or for prescription (four visits). Supportive care was performed in outpatient clinics at 142 visits (7%) such as hydration (88 visits), transfusion (28 visits), abdominal paracentesis (eight visits), insertion of a central venous line (seven visits), and administration of granulocyte colony-stimulating factor (two visits).

Survival and Hospitalization

Although some patients were referred to other hospitals, we obtained the information concerning the reason and period

Table 2. Treatment

	1st line	2nd line	3rd line	≥4th line
	$n = 64$	$n = 46$	$n = 25$	$n = 10$*
S-1	35	5	–	3
S-1/CDDP	10	1	–	–
MTX/5-FU	7	–	–	3
CPT11 + CDDP	6	11	2	–
5-FUci	5	–	–	–
Weekly PTX	1	26	12	2
CPT11 + MMC	–	3	4	–
5-FU/l-LV	–	–	2	3
5-FU + CDDP	–	–	–	1
CDDPip	–	–	2	–
CPT-11	–	1	2	–
CDDP + VP-16	–	–	–	1
Hepatic arterial infusion	–	–	1	2
MMC	–	–	–	1

*Repetition (+).
S-1, tegafur-gimeracil-oteracil-potassium; CDDP, cisplatin; MTX, methotrexate; 5-FU, 5-fluorouracil; CPT11, irinotecan; ci, continuous infusion; PTX, paclitaxel; MMC, mitomycin; l-LV, l-leucovorin; ip, intra peritoneum; VP-16, etoposide.

Table 3. Attendance to outpatient clinic and providing supportive care

	Number	Median	(Range)
Total	1917	29	(0–84)
Planned	1772	25	(0–79)
Emergent	145	2	(0–10)
Accidental disease	50		
Disease progression	46		
Side effect	45		
Prescription	4		
Supportive treatment	142		
Hydration	88		
Transfusion	28		
Abdominal paracentesis	8		
Insertion of CV line	7		
Exchange of drainage	5		
G-CSF	2		
Wound care	2		
Enema	1		
Withdrawing of urine	1		

CV, central venous; G-CSF, granulocyte colony-stimulating factor.

of hospitalization, and the date and cause of death by making inquiries directly to these hospitals.

The median follow-up was 520 days (range, 309–871 days), and the median overall survival time was 353 days. The 1-year survival rate was 49%, while the 2-year survival rate was 26% (Fig. 1). The median non-hospitalized survival time was 282 days (range, 0–786 days) and the median total period of hospitalization for each patient was 59 days (range, 0–138 days) (Fig. 2). The median number of hospitalizations was four (range, 0–15) per patient and the median period of each hospitalization was six days (range, 1–96 days). The total number of hospitalizations was 291, of which 110 (38%) were unplanned and reasons for unplanned hospitalization were related to disease progression (85 hospitalizations), toxicity (14 hospitalizations), accidental disease (nine hospitalizations), or examination (two hospitalizations) (Table 4).

Medical Condition at Discharge

Patients were discharged 290 times (Table 5), 56 (19%) of which were associated with an unresolved medical problem needing intensive care or follow-up to be managed at the outpatient clinic. These included toxicity (14 discharges), total parenteral nutrition (14 discharges), symptoms of cancer (17 discharges), percutaneous endoscopic gastrostomy (seven discharges), and other problems (four discharges).

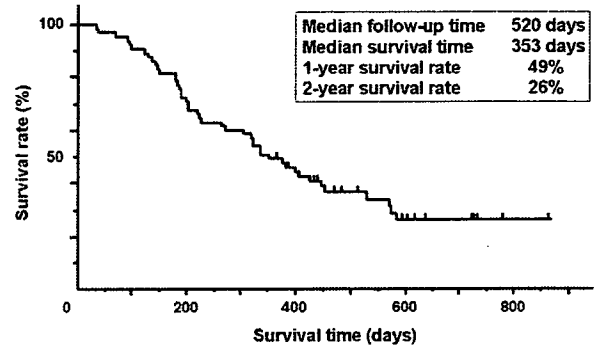


Figure 1. Overall survival.

Treatment-related Death

Fifteen patients (23%) died within 30 days after the last administration of chemotherapy. Of these 15 patients, three died of treatment-related death (TRD), and the other 12 early deaths within 30 days after last administration of chemotherapy occurred after confirming tumor progression. Two of three TRDs were treated at the outpatient clinic. One patient with PS 2 and massive ascites caused by peritoneal dissemination received w-PTX regimen as the second line setting. Vomiting appeared on day four after the sixth administration of chemotherapy and he entered the hospital for septic shock on the same day. Despite intensive supportive care he died on day five. Another patient with PS 1 and chronic renal failure received w-PTX regime as the third line setting. Although the only complication he had was grade 1 (NCI-CTC ver.2) nausea till day four, he entered hospital for grade 4 leucopenia on day five after the eighth administration of chemotherapy agent. While he recovered from leucopenia on day 11, grade 3 thrombocytopenia persisted. Bleeding from primary tumor occurred after confirming disease progression and he died of hypovolemic shock on day 26. The last patient died of pneumocystis carini

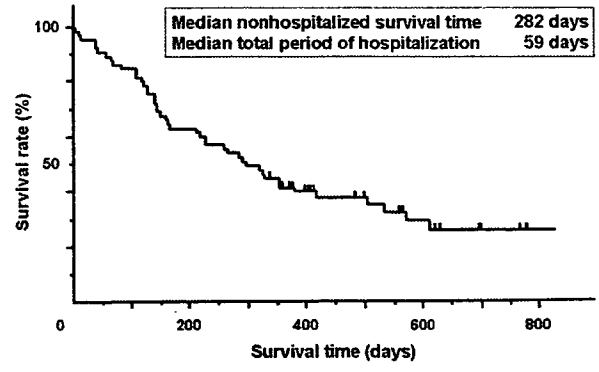


Figure 2. Non-hospitalized survival.

Table 4. Number and period of hospitalization

	Number	Median number	(Range)	Median period (days)	(Range)
Total	291	4	(0–15)	6	(1–96)
Planned	181	–		5	(1–65)
Emergent	110	–		16	(1–96)
Disease progression	85	–		21	(2–96)
Toxicity	14	–		9	(1–22)
Accidental disease	9	–		8	(2–22)
Examination	2	–		4	(3–5)

Table 5. Medical condition at discharge

	Number
Total	290
No problem	193
Death	41
Problems unresolved	56
Toxicity	14
TPN	14
Symptoms of cancer	17
PEG	7
Mental	3
PTBD	2
HOT	1
Stent in bile duct	1
Unresolved accidental disease	1

TPN, total parenteral nutrition; PEG, percutaneous endoscopic gastronomy; PTBD, percutaneous transhepatic bile duct drainage; HOT, home oxygen therapy.

pneumonia caused by grade 4 leucopenia after 1st administration of CDDP and VP-16 initiated during hospitalization.

DISCUSSION

In the recent randomized studies investigating the effects of single agent 5-FU therapy or the combination therapy of 5-FU plus CDDP, docetaxel and 5-FU plus CDDP, 5-FU and doxorubicin plus MMC or etoposide and leucovorin plus 5FU, it was reported that the median survival time was 7–9 months, the 1-year survival rate was 28–40% and 2-year survival rate was 7–18% (7,16–19).

For the single agent therapy of S-1, a novel oral derivative of 5-FU, the median survival time of 207 days, and 1- and 2-year survival rates of 36 and 14%, respectively, were reported in a Japanese phase II study (2,3). Furthermore a Japanese phase I/II study of S-1 combined with CDDP reported a median survival time of 383 days, and 1- and 2-year survival rates of 52 and 10%, respectively (4). However, a Japanese phase II study on CPT-11 combined with CDDP showed a median survival time of 322 days (6). In our study, the median survival time was 353 days, and 1- and 2-year survival rates were 49 and 26%, respectively. Although our survival data were obtained by retrospective analysis, our clinical outcomes seem to be equal or exceed those reported in previous studies.

In our study, the median non-hospitalized survival time was 282 days and median overall survival time was 352 days. We found no reports referring to non-hospitalized survival of patients with gastric cancer and it is difficult to compare our results with those of other researchers. In our hospital, we use various supportive systems to help patients remain at home and to care for patients from the initiation of chemotherapy to the terminal stage.

The incidence of TRD is 1–5% in some phase III studies (7,16). Three TRDs caused by leucopenia and thrombocytopenia occurred in our study (5%). Of two patients who were treated at the outpatient clinic, one patient entered hospital quickly after symptoms appeared. Another patient recovered from the leucopenia immediately after hospitalization so we do not consider that chemotherapy at the outpatient clinic caused delay of supportive care and that TRD might have been avoided if the patients had been treated in hospital. The number of early deaths within 30 days after the last administration of chemotherapy in our series seems high. The median number of chemotherapy regimens was two, and many patients received three or more chemotherapy regimens. Some of them were initiated despite poor medical conditions. We thus hypothesize that the risk of TRD increases according to the number of regimens received. Moreover, the indications for chemotherapy, especially in the subsequent treatment lines, should be decided more carefully to promote the safety of chemotherapy.

Most patients undergoing chemotherapy visit the hospital usually once every week or two. The median number of visits to the outpatient clinic was 29 and the median survival time was about 1 year. However, because of toxicity or disease progression, the patients' medical conditions sometimes changed between planned visits. We found that 8% of the total number of visits to the outpatient clinic were unplanned and that 7% of all visits required supportive care. We made an effort to prolong non-hospitalized survival by providing home nutrition and other supporting systems. This situation might make the incidence of unplanned attendance at the outpatient clinic look high, but we believe these are important in providing chemotherapy for patients with gastric cancer.

The incidence of unplanned or emergency hospitalization was 38% of the total number of hospitalizations. The main reason for hospitalization was worsening of patient's medical conditions caused by disease progression. Gastric cancer sometimes causes impaired oral intake, ileus, ascites, hydronephrosis and other severe complications. These serious complications can not be managed at an outpatient

clinic, and therefore the median duration of emergency hospitalization (16 days) was longer than that of planned hospitalization (five days). These data suggest the importance of establishing a system by which patients are accepted quickly for unplanned or emergency hospitalization in order to ensure safety of chemotherapy.

As mentioned above, we made an effort to prolong non-hospitalized survival by providing various support systems and 19% of the total number of discharges had associated problems such as toxicity, total parenteral nutrition at home, symptoms of cancer and percutaneous endoscopic gastrostomy. Although we helped patients adapt to these problems before discharge, our data suggest that these problems could also be managed or resolved at an outpatient clinic.

In conclusion, chemotherapy for patients with unresectable recurrent gastric cancer can be performed safely with support in hospitals. Japanese hospitals should not only establish outpatient chemotherapy centers but also a system to quickly provide emergency care during chemotherapy. We expect that the support system for providing chemotherapy safely will become more popular in Japan, and contribute to patients' QOL in the near future.

Conflict of interest statement

None declared.

References

1. Murad AM, Santiago FF, Petroianu A, Rocha PR, Rodrigues MA, Rausch M. Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. *Cancer* 1993;72:37–41.
2. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T. Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. *Eur J Cancer* 1998;34:1715–20.
3. Koizumi W, Kurihara M, Nakano S, Hasegawa K. Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 Cooperative Gastric Cancer Study Group. *Oncology* 2000;58:191–7.
4. Koizumi W, Tanabe S, Saigenji K, Ohtsu A, Boku N, Nagashima F, et al. Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer. *Br J Cancer* 2003;89:2207–12.
5. Yamao T, Shimada Y, Shirao K, Ohtsu A, Ikeda N, Hyodo I, et al. Phase II study of sequential methotrexate and 5-fluorouracil chemotherapy against peritoneally disseminated gastric cancer with malignant ascites: a report from the Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group. JCOG 9603 Trial. *Jpn J Clin Oncol* 2004;34:316–22.
6. Boku N, Ohtsu A, Shimada Y, Shirao K, Seki S, Saito H, et al. Phase II study of a combination of irinotecan and cisplatin against metastatic gastric cancer. *J Clin Oncol* 1999;17:319–23.
7. Ohtsu A, Shimada Y, Shirao K, Boku N, Hyodo I, Saito H, et al. Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: The Japan Clinical Oncology Group Study (JCOG9205). *J Clin Oncol* 2003;21:54–9.
8. Rosenberg P, Andersson H, Boman K, Ridderheim M, Sorbe B, Puistola U, et al. Randomized trial of single agent paclitaxel given weekly versus every three weeks and with peroral versus intravenous steroid premedication to patients with ovarian cancer previously treated with platinum. *Acta Oncol* 2002;41:418–24.
9. Yamao T, Shirao K, Matsumura Y, Muro K, Yamada Y, Goto M, et al. Phase I–II study of irinotecan combined with mitomycin-C in patients with advanced gastric cancer. *Ann Oncol* 2001;12:1729–35.
10. Petrelli N, Douglass HO Jr, Herrera L, Russell D, Stablein DM, Bruckner HW, et al. The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. Gastrointestinal Tumor Study Group. *J Clin Oncol* 1989;7:1419–26.
11. Smith EM, Jayson GC. The current and future management of malignant ascites. *Clinical Oncology* 2003;15:59–72.
12. Taguchi T, Wakui A, Hasegawa K, Niitani H, Furue H, Ohta K, et al. Phase I clinical study of CPT-11. Research group of CPT-11. *Gan To Kagaku Ryoho* 1990;17:115–20.
13. Futatsuki K, Wakui A, Nakao I, Sakata Y, Kambe M, Shimada Y, et al. Late phase II study of irinotecan hydrochloride (CPT-11) in advanced gastric cancer. CPT-11 Gastrointestinal Cancer Study Group. *Gan To Kagaku Ryoho* 1994;21:1033–8.
14. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 2002;346:85–91.
15. Kumada T, Arai Y, Itoh K, Takayasu Y, Nakamura K, Ariyoshi Y, et al. Phase II study of combined administration of 5-fluorouracil, epirubicin and mitomycin-C by hepatic artery infusion in patients with liver metastases of gastric cancer. *Oncology* 1999;57:216–23.
16. Moiseyenko VM, Ajani JA, Tjulandin SA, Majlis A, Constenla M, Boni C, et al. Final results of a randomized controlled phase III trial (TAX 325) comparing docetaxel (T) combined with cisplatin (C) and 5-fluorouracil (F) to CF in patients (pts) with metastatic gastric adenocarcinoma (MGC). ASCO Annual Meeting 2005; Abstract No. 4002.
17. Kim NK, Park YS, Heo DS, Suh C, Kim SY, Park KC, et al. A phase III randomized study of 5-fluorouracil and cisplatin versus 5-fluorouracil, doxorubicin, and mitomycin C versus 5-fluorouracil alone in the treatment of advanced gastric cancer. *Cancer* 1993;71:3813–8.
18. Cullinan SA, Moertel CG, Wieand HS, O'Connell MJ, Poon MA, Krook JE, et al. Controlled evaluation of three drug combination regimens versus fluorouracil alone for the therapy of advanced gastric cancer. *J Clin Oncol* 1994;12:412–6.
19. Vanhoefer U, Rougier P, Wilke H, Ducreux MP, Lacave AJ, van Cutsem E, et al. Final results of a randomized phase III trial of sequential high-dose methotrexate, fluorouracil, and doxorubicin versus etoposide, leucovorin, and fluorouracil versus infusional fluorouracil and cisplatin in advanced gastric cancer: A trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Group. *J Clin Oncol* 2000;18:2648–57.

found in *H. pylori* gastritis.[10,11] With immunohistochemistry results with CD79a and CD138, we hypothesize that some of these crystal-laden 'histiocytes' are plasma cells that have failed to secrete immunoglobulin. Overproduction of immunoglobulins has been known to be a possible mechanism of crystal formation in CSH and has been described in plasma cell granuloma or post-transplantation plasmacytosis.[1] Although an additional or associated mechanism is uncertain at present, overproduction of immunoglobulin caused by *H. pylori* infection is a plausible aetiology. To the best of our knowledge, the present case is the second documented case of gastric CSH and the first case of localized CSH associated with polyclonal plasma cell proliferation probably caused by *H. pylori* infection.

M Joo[1,2]
J E Kwak[1]
S H Chang[1,2]
H Kim[1]
J G Chi[1]
Y-S Moon[3]
K-M Kim[4]

[1]*Department of Pathology,* [2]*Clinical Research Centre, and* [3]*Internal Medicine, Ilsanpaik Hospital, Inje University College of Medicine, Koyang,* [4]*Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea*

1. Jones D, Bhatia V, Krausz T, Pinkus GS. Crystal-storing histiocytosis: a disorder occurring in plasmacytic tumors expressing immunoglobulin kappa light chain. *Hum. Pathol.* 1999; **30**; 1441–1448.
2. Lebeau A, Zeindl-Eberhart E, Müller EC *et al.* Generalized crystal-storing histiocytosis associated with monoclonal gammopathy: molecular analysis of a disorder with rapid clinical course and review of the literature. *Blood* 2002; **100**; 1817–1827.
3. Ionescu DN, Pierson DM, Qing G, Li M, Colby TV, Leslie KO. Pulmonary crystal-storing histiocytoma. *Arch. Pathol. Lab. Med.* 2005; **129**; 1159–1163.
4. Hirota S, Miyamoto M, Kasugai T, Kitamura Y, Morimura Y. Crystalline light-chain deposition and amyloidosis in the thyroid gland and kidneys of a patient with myeloma. *Arch. Pathol. Lab. Med.* 1990; **114**; 429–431.
5. Llobet M, Castro P, Barcelo C, Trull JM, Campo E, Bernado L. Massive crystal-storing histiocytosis associated with low-grade malignant B-cell lymphoma of MALT-type of the parotid gland. *Diagn. Cytopathol.* 1997; **17**; 148–152.
6. Stirling JW, Henderson DW, Rozenbilds MA, Skinner JM, Filipic M. Crystalloidal paraprotein deposits in the cornea: an ultrastructural study of two new cases with tubular crystalloids that contain IgG kappa light chains and IgG gamma heavy chains. *Ultrastruct. Pathol.* 1997; **21**; 337–344.
7. Papla B, Spólnik P, Rzenno E *et al.* Generalized crystal-storing histiocytosis as a presentation of multiple myeloma: a case with a possible pro-aggregation defect in the immunoglobulin heavy chain. *Virchows Arch.* 2004; **445**; 83–89.
8. Bosman C, Camassei FD, Boldrini R *et al.* Solitary crystal-storing histiocytosis of the tongue in a patient with rheumatoid arthritis and polyclonal hypergammaglobulinemia. *Arch. Pathol. Lab. Med.* 1998; **122**; 920–924.
9. Ensari A, Savas B, Okcu Heper A, Kuzu I, Idilman R. An unusual presentation of *Helicobacter pylori* infection: so-called 'Russell body gastritis'. *Virchows Arch.* 2005; **446**; 463–466.
10. Scullin DC Jr, Shelburne JD, Cohen HJ. Pseudo-Gaucher cells in multiple myeloma. *Am. J. Med.* 1979; **67**; 347–352.
11. Kapadia SB, Enzinger FM, Heffner DK, Hyams VJ, Frizzera G. Crystal-storing histiocytosis associated with lymphoplasmacytic neoplasms. Report of three cases mimicking adult rhabdomyoma. *Am. J. Surg. Pathol.* 1993; **17**; 461–467.

API2-MALT1 chimeric transcript-positive gastroduodenal MALT lymphoma with subsequent development of adenocarcinoma as a collision tumour over a clinical course of 7 years

DOI: 10.1111/j.1365-2559.2007.02718.x

Sir: Both gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type (MALT lymphoma)[1] and gastric adenocarcinoma have a strong association with *Helicobacter pylori* infection in their pathogenesis and development,[2,3] but their collision in the stomach is rare. Furthermore, synchronous *API2-MALT1*+ MALT lymphoma and adenocarcinoma of the stomach independent of *H. pylori* infection has not been previously reported. t(11;18)(q21;q21) translocation occurs specifically in MALT lymphoma and this translocation generates a functional *API2-MALT1* fusion product which activates nuclear factor NF-κB.[4] Most cases of *API2-MALT1*+ gastric MALT lymphoma do not respond to *H. pylori* eradication.[5,6] We report a unique case of *API2-MALT1*+, *H. pylori*-negative, advanced stage, gastroduodenal MALT lymphoma in which two lesions of overt gastric adenocarcinoma developed 7 years after the initial diagnosis of MALT lymphoma.

A 63-year-old Japanese woman presented with weight loss of 14 kg over 6 months. Upper gastrointestinal endoscopy disclosed multiple erosions and oedematous mucosa throughout the stomach and a biopsy specimen revealed gastric MALT lymphoma spreading from the stomach to the duodenal bulb and second portion (Figure 1a,b). Although tissue culture and a ^{13}C urea breath test for *H. pylori* infection were negative, she received eradication therapy 2 years

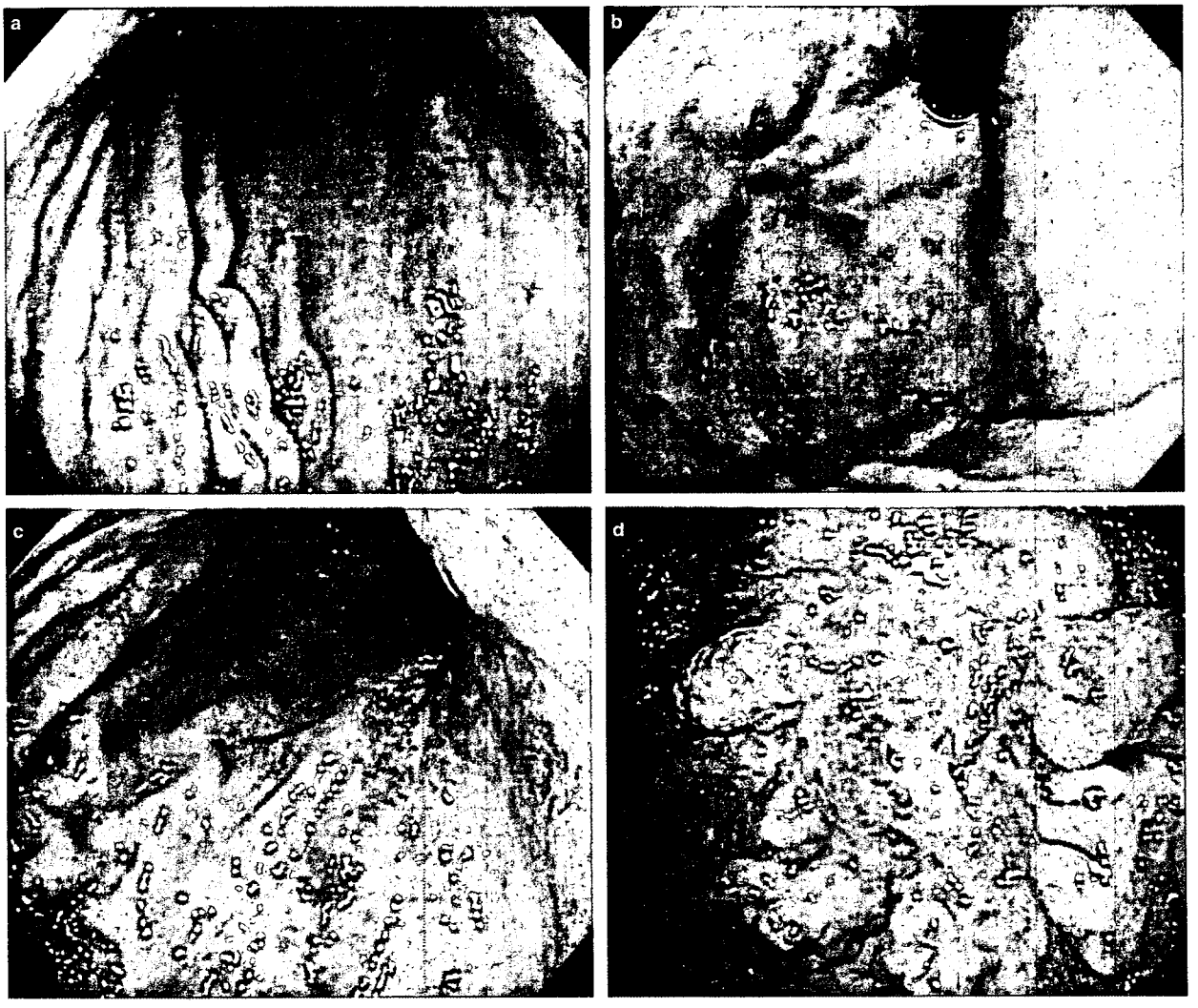


Figure 1. Upper gastrointestinal endoscopy. **a,b,** Upper gastrointestinal endoscopy based on the initial diagnosis shows multiple erosions and oedematous mucosa. **c,d,** Five years after eradication therapy, i.e. 7 years after initial diagnosis, upper gastrointestinal endoscopy reveals two lesions, Borrmann 3 gastric carcinomas, on the posterior wall of the middle gastric body (no. 1) and on the greater curvature of the upper gastric body (no. 2). Both are located within the area involving the mucosa-associated lymphoid tissue lymphoma.

after the initial diagnosis, resulting in no improvement in MALT lymphoma, and was thereafter followed without more therapy at her request. At this time, the patient was also evaluated as being at clinical stage IV with bone marrow involvement. *API2-MALT1* chimeric transcripts were detected by reverse transcriptase-polymerase chain reaction and nucleotide sequencing[7] in tissue samples obtained by biopsy and resection of the gastric MALT lymphoma. Five years after *H. pylori* eradication therapy, i.e. 7 years after the initial diagnosis, upper gastrointestinal endoscopy revealed two lesions of Borrmann 3 gastric adenocarcinoma on the posterior wall of the middle gastric body no. (1) and the greater curvature of the upper gastric body (no. 2) (Figure 1c,d, respectively). The lesions were located within the area involved by the MALT lymphoma, thereby constituting contiguous/collision tumours. Gastrectomy was performed, but the patient eventually died of peritonitis carcinomatosa 2 months after surgery. Seven years had passed since the initial diagnosis of MALT lymphoma. No changes had been seen in the size, stage or clinical features of the MALT lymphoma over the 7-year follow-up period.

The resected stomach was opened along the lesser curvature. Figure 2 shows the macroscopic

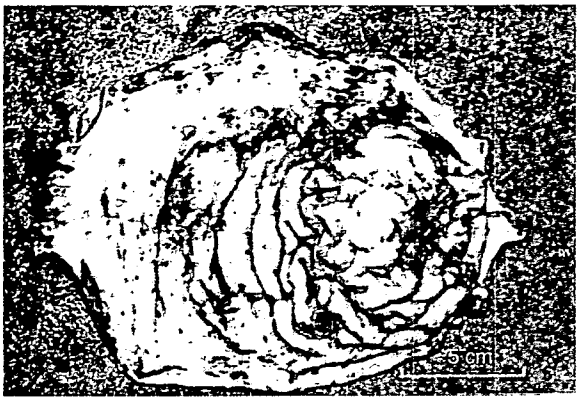


Figure 2. Macroscopic findings of the resected stomach. Multiple erosions and oedematous mucosa are evident in the whole stomach. Irregularly shaped depressions are indicated by green arrows.

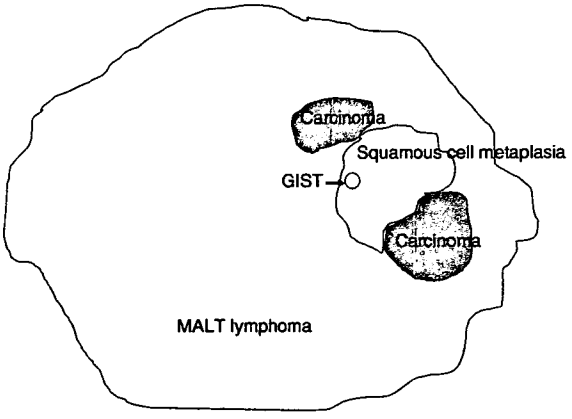


Figure 3. Schematic illustration of Figure 2 based on the histological findings. The brown area shows involvement of the mucosa-associated lymphoid tissue (MALT) lymphoma. The two depressed, red lesions represent gastric carcinoma surrounded by the MALT lymphoma. The whitish mucosa between the two gastric carcinomas represents squamous cell metaplasia, under which MALT lymphoma tissue has invaded. The gastrointestinal stromal tumour (GIST), located at the subserosal portion, is shown as a blue circle.

findings. Multiple erosions and oedematous mucosa were evident throughout the stomach. Irregularly shaped depressions measuring 25 × 20 mm and 25 × 20 mm were seen in the fornix and gastric body, as indicated by arrows in the figures. The mucosa between the two lesions was whitish. Figure 3 is a histological schematic illustration of the resected stomach. The brown area indicates MALT lymphoma involvement. Two depressed, red gastric carcinoma lesions were surrounded by the MALT lymphoma. The whitish mucosa between the two

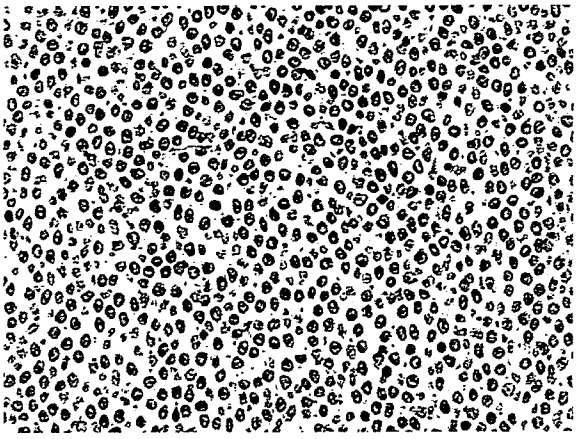


Figure 4. Histology of mucosa-associated lymphoid tissue (MALT) lymphoma. The MALT lymphoma is largely composed of low-grade B-cell lymphoma cells. (H&E.)

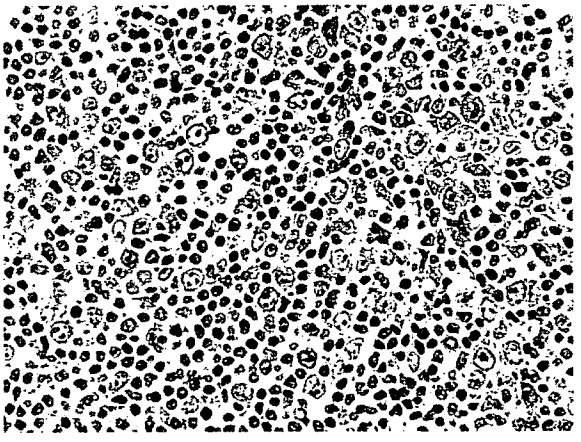


Figure 5. Histology of mucosa-associated lymphoid tissue lymphoma. Diffuse large-cell lymphoma tissue can be seen in the duodenum. (H&E.)

gastric carcinomas represents squamous cell metaplasia, beneath which invasion by the MALT lymphoma was evident. A gastrointestinal stromal tumour (GIST), which was located at the subserosal portion of the stomach, is shown as a blue circle (see below for details).

Histology revealed that invasion by the MALT lymphoma was primarily limited to the submucosal tissue with partial invasion into the subserosal tissue. This lymphomatous proliferation was predominantly occupied by low-grade MALT lymphoma tissue, consisting of centrocyte-like cells (Figure 4) accompanied by small, scattered clusters of blasts in the duodenal portion (Figure 5). Therefore, the postoperative

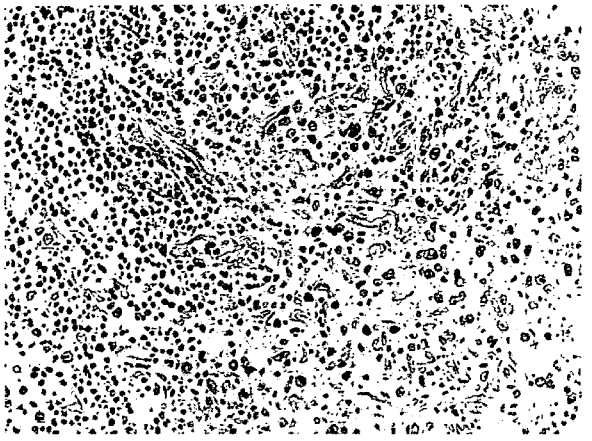


Figure 6. Histology of the collision portion of gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The gastric carcinoma is composed of poorly differentiated adenocarcinoma or signet-ring cell carcinoma, in the right lower part. The MALT lymphoma is evident in the left upper part. (H&E.)

histological diagnosis was low-grade MALT lymphoma with a high-grade diffuse large B-cell lymphoma component. The tumour cells were immunophenotypically positive for CD20, CD79a and Bcl-2, but not for CD3, CD5, CD10, CD23, CD45RO or cyclin D1, and *API2-MALT1* chimeric transcript was detected in fresh tissue. Intestinal metaplasia and atrophic changes in the mucosa adjacent to the MALT lymphoma were also evident. Microlymphomas were not seen. Lymph node dissection was limited to the lymph nodes surrounding the gastric wall, due to peritoneal dissemination. The regional lymph nodes surrounding the gastric wall were all involved by the MALT lymphoma. On the other hand, involvement of signet-ring cell carcinoma was limited to the lymph nodes in the gastric wall.

Both gastric carcinomas were poorly differentiated adenocarcinoma of diffuse type and/or signet-ring cell carcinoma extending to the serosa and were located within the area involved by the MALT lymphoma with no evidence of normal tissue between the two, indicating synchronous contiguous/collision tumours (Figure 6). Epstein–Barr virus (EBV) infection was not detected by *in situ* hybridization for EBV-encoded non-polyadenylated RNA in either MALT lymphoma or adenocarcinoma tissue.

An intramural nodule, measuring 15 × 10 mm, was eventually found as a synchronous independent tumour at the subserosal portion of the stomach (Figure 3), which showed a uniform proliferation of spindle cells with few mitoses or necrosis. Based on positivity of CD117/KIT, but not of S100 protein, desmin or smooth muscle action, the diagnosis of this nodule was GIST.

Most cases of gastric *API2-MALT1*+ MALT lymphoma are characterized by unresponsiveness to antibacterial treatment against *H. pylori*, and the pathogenesis of this tumour is independent of infection with this microorganism.[5,8,9] The *API2-MALT1* chimeric transcript is also exclusively largely detected in low-grade MALT lymphomas.[10,11] Furthermore, thus far, this transcript has shown few additional gene alterations, indicating that fusion-positive tumours are genetically more stable than negative ones,[11,12] although there is an exceptional case report of *API2-MALT1*+ MALT lymphoma with secondary cytogenetic abnormalities.[13] However, the natural history and actual prognosis of *API2-MALT1*+ MALT lymphoma have yet to be clarified due to the lack of any long-term follow-up observations. Notably in the present patient, gastric MALT lymphoma did not affect the prognosis during a 7-year clinical course, in spite of advanced clinical stage and the presence of a diffuse large B-cell lymphoma component. This is in keeping with previous reports that the prognosis of patients with double primary gastric lymphoma and adenocarcinoma is more closely associated with the adenocarcinoma than the lymphoma.[14,15] As far as the present case is concerned, the *API2-MALT1*+ MALT lymphoma provided no evidence of a relationship with *H. pylori* infection and preceded the development of gastric carcinoma.

In one summary of the literature on synchronous lymphoma and adenocarcinoma of the stomach,[16] the author found that *H. pylori* infection was detected in 78% of 32 patients. The majority of lymphomas were low grade (75%) and were larger than the carcinoma (81%). The majority of carcinomas (65.6%) were at an early stage. The author[16] also suggested that lymphoma might develop before carcinoma or the presence of MALT lymphoma might increase the risk of developing carcinoma. Three cases of metachronous gastric MALT lymphoma with a rearrangement of *API2-MALT1* and early gastric carcinoma have recently been reported by Copie-Bergman *et al.*[17] These cases had a good prognosis after detection of subsequent carcinoma and resection of the stomach. The present case has provided additional evidence for their assertion that prolonged residual gastric MALT lymphoma could constitute an additional risk factor for the development of gastric carcinoma, but it is unique in revealing that the latter rapidly progressed into advanced-stage disease and was the greatest influence on prognosis. Further investigation is required to clarify the possibility that gastric

glandular epithelium presenting within an *API2-MALT1+* MALT lymphoma might be prone to neoplastic transformation.

The findings in this case suggest that the prognosis of *API2-MALT1+* gastric MALT lymphoma is relatively good, even if the clinical stage is advanced. However, if follow-up is selected as the treatment strategy for this particular lymphoma, the physician should be aware of the possibility of the development of concomitant gastric carcinoma.

T Isaka
T Nakamura[1]
M Tajika[1]
H Kawai[1]
H Imaoka
Y Okamoto
M Aoki
H Inoue
K Takahashi
N Mizuno
A Sawaki
K Yamao
M Seto[2]
T Yokoi[3]
Y Yatabe[3]
S Nakamura[4]

Department of Gastroenterology,
[1]*Department of Endoscopy,*
[2]*Division of Molecular Medicine and*
[3]*Department of Pathology and Molecular Diagnostics, Aichi Cancer Centre Hospital, and*
[4]*Department of Pathology and Clinical Laboratories, Nagoya University Hospital, Nagoya, Japan*

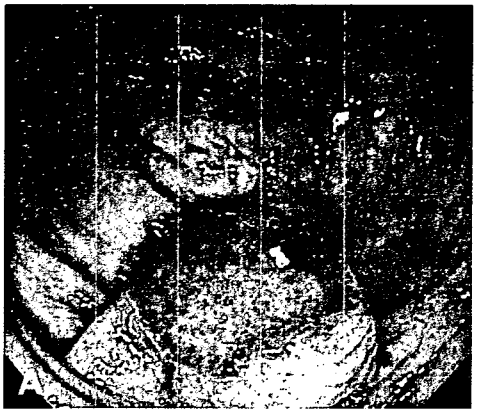
1. Harris NL, Jaffe ES, Diebold J *et al.* The World Health Organization classification of neoplastic diseases of the haematopoietic and lymphoid tissues: report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Histopathology* 2000; **36**; 69–86.
2. Wotherspoon AC, Doglioni C, Diss TC *et al.* Regression of primary low-grade B-cell gastric lymphoma of mucosa- associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 1993; **342**; 575–577.
3. Uemura N, Okamoto S, Yamamoto S *et al. Helicobacter pylori* infection and the development of gastric cancer. *N. Engl. J. Med.* 2001; **345**; 784–789.
4. Lucas PC, Yonezumi M, Inohara N *et al.* Bcl10 and MALT1, independent targets of chromosomal translocation in MALT lymphoma, cooperate in a novel NF-kappaB signaling pathway. *J. Biol. Chem.* 2001; **21**; 21.
5. Sugiyama T, Asaka M, Nakamura T *et al.* API2-MALT1 chimeric transcript is a predictive marker for the responsiveness of *H. pylori* eradication treatment in low-grade gastric MALT lymphoma. *Gastroenterology* 2001; **120**; 1884–1885.
6. Liu H, Ye H, Ruskone-Fourmestraux A *et al.* t(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. *Gastroenterology* 2002; **122**; 1286–1294.
7. Motegi M, Yonezumi M, Suzuki H *et al.* API2-MALT1 chimeric transcripts involved in mucosa-associated lymphoid tissue type lymphoma predict heterogeneous products. *Am. J. Pathol.* 2000; **156**; 807–812.
8. Nakamura T, Nakamura S, Yonezumi M *et al. Helicobacter pylori* and the t(11;18)(q21;q21) translocation in gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type. *Jpn J. Cancer Res.* 2000; **91**; 301–309.
9. Nakamura T, Nakamura S, Yokoi T *et al.* Clinicopathologic comparison between the API2-MALT1 chimeric transcript-positive and -negative gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type. *Jpn J. Cancer Res.* 2002; **93**; 677–684.
10. Ott G, Katzenberger T, Greiner A *et al.* The t(11;18)(q21;q21) chromosome translocation is a frequent and specific aberration in low-grade but not high-grade malignant non-Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT–) type. *Cancer Res.* 1997; **57**; 3944–3948.
11. Starostik P, Patzner J, Greiner A *et al.* Gastric marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. *Blood* 2002; **99**; 3–9.
12. Remstein ED, James CD, Kurtin PJ. Incidence and subtype specificity of API2-MALT1 fusion translocations in extranodal, nodal, and splenic marginal zone lymphomas. *Am. J. Pathol.* 2000; **156**; 1183–1188.
13. Hosaka S, Akamatsu T, Nakamura S *et al.* Mucosa-associated lymphoid tissue (MALT) lymphoma of the rectum with chromosomal translocation of the t(11;18)(q21;q21) and an additional aberration of trisomy 3. *Am. J. Gastroenterol.* 1999; 94: 1951–1954.
14. Nakamura K, Ueyama T, Yao T *et al.* Pathology and prognosis of gastric carcinoma. Findings in 10,000 patients who underwent primary gastrectomy. *Cancer* 1992; **70**; 1030–1037.
15. Nakamura S, Aoyagi K, Iwanaga S *et al.* Synchronous and metachronous primary gastric lymphoma and adenocarcinoma: a clinicopathological study of 12 patients. *Cancer* 1997; **79**; 1077–1085.
16. Chan AO, Chu KM, Yuen ST *et al.* Synchronous gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma in association with *Helicobacter pylori* infection: comparing reported cases between the East and West. *Am. J. Gastroenterol.* 2001; **96**; 1922–1924.
17. Copie-Bergman C, Locher C, Levy M *et al.* Metachronous gastric MALT lymphoma and early gastric cancer: is residual lymphoma a risk factor for the development of gastric carcinoma? *Ann. Oncol.* 2005; **16**; 1232–1236.

Two cases of hepatoid adenocarcinoma of the intestine in association with inflammatory bowel disease

DOI: 10.1111/j.1365-2559.2007.02715.x

Sir: Hepatoid adenocarcinoma (HAC) is a distinctive type of extrahepatic adenocarcinoma that shows

A case of colonic morule with colitis cystica profunda



C

A 48-year-old man was referred to our institute for evaluation of a positive fecal occult blood test. Colonoscopy with a magnifying videoscope revealed a submucosal tumor with a sharply demarcated central depression in the ascending colon (**A**). After the area was sprayed with 0.2% indigo carmine, the magnified view of the depressed lesion showed a pitted pattern that was difficult to fit into the classification proposed by Kudo et al (**B**, orig. mag. ×50). The pit pattern basically was composed of tubular or roundish pits that were irregular in shape, size, and arrangement. Histologic examination of the biopsy specimen led us to suspect a well-differentiated adenocarcinoma. EUS demonstrated a nonhomogenous, well-circumscribed, hypoechoic lesion including a calcified component in the third hyperechoic layer of the 5-layer structure of the colonic wall. We speculated that early colon cancer developed on a submucosal tumor; however, we could not exclude invasive neoplasia, because an abdominal CT scan showed regional lymph node enlargement. Therefore, a right hemicolectomy was performed. The histologic diagnosis of the resected

specimen was colonic morule in tubular adenoma with severe atypia on a colitis cystica profunda (**C**, H&E, orig. mag. ×4; **D**, H&E, orig. mag. ×40). There was no cancerous involvement in the regional lymph nodes.

DISCLOSURE

None of the authors have any disclosures to make.

Masahiro Tajika, MD, Tsuneya Nakamura, MD, Hiroki Kawai, MD, Akira Sawaki, MD, Nobumasa Mizuno, MD, Kuniyuki Takahashi, MD, Takio Yokoi, MD, Yasushi Yatabe, MD, Takashi Hirai, MD, Kenji Yamao, MD, *Departments of Endoscopy, Gastroenterology, and Gastroenterological Surgery, Division of Pathology and Molecular Diagnosis, Aichi Cancer Center Hospital, Nagoya City, Japan*
Tadashi Kato, MD, *Department of Internal Medicine, Sannomaru Hospital, Nagoya City, Japan*
doi:10.1016/j.gie.2006.06.021

Commentary

A morule is a term familiar to us if we think back to our embryology classes and recall one of the early cleavage stages of the zygote (*L morum*, mulberry). Morules are associated with aberrant β-catenin expression and have been reported with both benign and malignant neoplasia, including carcinoma of the lung, uterus, thyroid, colon, and others as well as with nonneoplastic tissue such as pregnancy-related endometrium. Although somewhat controversial, it is believed that, at least in the colon, morules are cell clusters with a basal or stem cell phenotype and that they may have less proliferative and invasive potential than other cancer cells do. Histologically, a morule contains biotin-rich intranuclear inclusions referred to as "optically clear nuclei" (different from the cell nucleus) and that they are composed of cytologically bland cells in a syncytial-like arrangement with a solid or a fenestrated pattern. Colitis cystica profunda is another rare disease, characterized by submucosal mucin-filled cysts. Colitis cystica profunda has been associated with a variety of diseases, including adenocarcinoma of the colon, rectal prolapse, and solitary rectal ulcer. Taken together, the findings in this case teach us that there are a limited number of symptoms and signs the body can exhibit and, therefore, many disease processes from the congenital to the acquired and the inflammatory to the neoplastic may share presentations and appearances. Thankfully, today we have a wide range of techniques that enable us to unravel some of complexities of what we see and to echo Aristotle that "In all things of nature there is something of the marvelous."

Lawrence J. Brandt, MD
Associate Editor for Focal Points

Thymidylate Synthase Gene Expression in Primary Tumors Predicts Activity of S-1-based Chemotherapy for Advanced Gastric Cancer

Hiroya Takiuchi, Shin-ichiro Kawabe, Masahiro Gotoh, Ken-ichi Katsu

ABSTRACT

Purpose: To evaluate the association between dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) levels in primary gastric tumors and clinical response to S-1 or S-1 plus irinotecan in patients with unresectable advanced gastric cancer, and to investigate the molecular mechanism of augmented antitumor activity of the combination using human gastric cancer xenografts with high TS activity.

Materials and Methods: TS mRNA expression and DPD mRNA expression were measured by reverse transcription polymerase chain reaction in initial primary cancer biopsy specimens in 29 patients with advanced gastric cancer who had received S-1 alone (n=18) or in combination with irinotecan (n=11). In an experimental study, antitumor effects of S-1, irinotecan, and the combination were assessed in mice bearing human gastric tumors with high TS expression (4-1-ST and AZ-521 tumors) and low TS expression (SC-2 tumors), and activities of 5-fluorouracil–metabolizing enzymes were measured.

Results: In the clinical study, a strong statistical association between high TS expression and clinical resistance to S-1 alone was found (P = .009). In the experimental studies, S-1 plus irinotecan showed augmented antitumor activity against tumors with high TS activity (P < .01) compared with either agent alone. A potential mechanism for this effect was suggested by the significant reduction in TS activity observed following irinotecan administration in tumors with high TS activity.

Conclusion: This study suggests that, via down-regulation of TS by irinotecan treatment, combination chemotherapy with S-1 and irinotecan could be effective in gastric cancer patients with high TS levels.

Gastrointest Cancer Res 1:172–177.

H. Takiuchi, MD and S. Kawabe, MD: Cancer Chemotherapy Center, Osaka Medical College Hospital, Osaka, Japan

M. Gotoh, MD and K. Katsu, MD: Department of Gastroenterology, Osaka Medical College Hospital, Osaka, Japan

Tumors expressing high levels of thymidylate synthase (TS), the rate-limiting enzyme of de novo DNA synthesis, have poor sensitivity to fluoropyrimidine-based chemotherapy,[1-4] and tumor levels of dihydropyrimidine dehydrogenase (DPD), a rate-limiting enzyme of 5-fluorouracil (5-FU) catabolism, have been reported to inversely correlate with sensitivity to 5-FU–based chemotherapy.[5-9] These observations have led to attempts to predict efficacy of 5-FU treatment by assessing TS and DPD levels in gastrointestinal tumors.[5, 8-11]

S-1 is a new oral fluoropyrimidine with high activity in gastric and colorectal cancer.[12-14] The drug contains the potent DPD inhibitor 5-chloro-2, 4-dihydroxypyrimidine (gimeracil, CDHP) and a potassium oxonate (oteracil potassium, Oxo) component that inhibits the phosphorylation of 5-FU, together with the 5-FU prodrug tegafur. The inclusion of CDHP as a component of S-1 is expected to reduce the effect of level of DPD expression on tumor response to fluoropyrimidine treatment. However, as with other fluoropyrimidines, tumors that express high levels of TS mRNA are expected to be relatively resistant to S-1. Ichikawa et al[15] reported a positive correlation between TS mRNA expression and expression of topoisomerase I, and Danenberg et al[16] have reported cases of metastatic colorectal cancer in which the topoisomerase I inhibitor irinotecan was effective in tumors with high levels of TS mRNA. Such findings suggest that the use of irinotecan in combination with S-1 might overcome tumor fluoropyrimidine resistance on the basis of high TS levels, and that selection of S-1 monotherapy or combined therapy could be made on the basis of tumor TS mRNA levels.

In the current study, we analyzed TS mRNA and DPD mRNA levels in initial biopsy samples from patients with un-

Address correspondence to: Hiroya Takiuchi, MD, Cancer Chemotherapy Center, Osaka Medical College Hospital, 2-7 Daigaku-machi, Takatsuki, Osaka, 569-8686, Japan. Phone: +81-72-683-1221; Fax: +81-72-684-6778; E-mail: in2028@poh.osaka-med.ac.jp

resectable advanced gastric cancer who had been treated with S-1 monotherapy or S-1 plus irinotecan to determine the association of expression levels with response to treatment. We further evaluated the antitumor activity of S-1 plus irinotecan using human gastric cancer xenografts with high or low TS activity in nude mice and investigated the molecular mechanism of the augmented activity seen with the combination.

MATERIALS AND METHODS

Clinical Study

Patient Characteristics and Treatment Outcome

The study population consisted of all 29 patients with unresectable advanced gastric cancer who were treated with first-line S-1 alone (n=18) or S-1 plus irinotecan (n=11) from January 2000 to December 2001 at the Second Department of Internal Medicine, Osaka Medical College (Osaka, Japan). This study was approved by the Institutional Review Board of Osaka Medical College, and all patients gave written informed consent. To be eligible, patients had to have a histologically confirmed diagnosis of gastric cancer; age of 20 to 75 years; performance status of 0, 1, or 2 on the Zubrod scale (Eastern Cooperative Oncology Group); no prior chemotherapy regimens before entry; adequate hematologic, hepatic, and renal function; and estimated survival of at least 3 months. Clinical characteristics of the patients are shown in Table 1.

Oral S-1 was given at 80 to 120 mg/day in two doses for 28 days, followed by a 14-day rest period for 1 course. S-1/irinotecan treatment consisted of S-1 at the same dose for 21 days followed by a 14-day rest period and irinotecan 80 mg/m^2 IV on days 1 and 15 for 1 course.[17] Objective response was determined by World Health Organization (WHO) criteria, and primary lesions were assessed by Japanese Research Society for Gastric Cancer criteria (originally established by WHO). Response was defined as complete or partial response; nonresponse was defined as no change or progressive disease.

Among 18 patients who received S-1 alone, 6 (33.3%) had partial response and 12 were nonresponders (8 with no change, 4 with progressive disease). Among 11 patients receiving S-1/irinotecan, 8 (72.7%) had partial response and 3 were nonresponders (all with no change).

Table 1. Main clinical characteristics of patients.

	S-1 alone (n=18)		S-1 + irinotecan (n=11)	
Sex				
Male	12	(67%)	6	(55%)
Female	6	(32%)	5	(45%)
PS				
0–1	14	(78%)	8	(73%)
2	4	(22%)	3	(27%)
Type				
Type 1	1	(5%)	0	(0%)
Type 2	4	(22%)	0	(0%)
Type 3	4	(22%)	2	(18%)
Type 4	9	(50%)	9	(82%)
Histology				
Differentiated	10	(56%)	2	(18%)
Undifferentiated	8	(44%)	9	(82%)
Response				
Responder	6	(33%)	8	(73%)
Nonresponder	12	(67%)	3	(27%)

[AUTHOR: Please explaing "types" in a footnote.]

Determination of TS mRNA and DPD mRNA in Tumor Specimens

Tumor samples were obtained from primary gastric tumors at the time of initial endoscopy. Immediately after biopsy, the tumor biopsy specimens were fresh frozen in liquid nitrogen until the time of RNA extraction. Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using a previously description method, the reliability and validity of which have been reported in detail by Ishikawa et al.[18] Total RNA for each sample was isolated using the RNeasy mini kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturer's instructions. Reverse transcription using 10 g of total RNA was performed in a total volume of 100 L containing 250 pmol oligo $(dT)_{18}$, 80 units of Rnasin (Promega, Madison, WI, USA), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, and 0.5 mM deoxynucleotide triphosphates solution. Initially, RNA and oligo $(dT)_{18}$ were heated to 70°C for 10 minutes and immediately chilled on ice; the remaining reagents were added and then incubated for 15 minutes at 30°C and 60 minutes at 42°C.

cDNA for genes of interest and an internal reference gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were quantified using a fluorescence-based real-time detection method (ABI PRISM 7700 Sequence Detection System [TaqMan]; Perkin-Elmer Applied Biosystems, Foster City, CA, USA) as described previously.[19]

The PCR reaction mixture consisted of 600 nM of each primer, 200 nM probe, 2.5 units AmpliTaq Gold Polymerase, 200 μM each of dATP, dCTP, and dGTP, 400 μM dUTP, 5.5 mM MgCl2, and 1 X TaqMan Buffer A containing a reference dye, to a final volume of 25 μL (all reagents Perkin-Elmer Applied Biosystems). Primer and probe sequences were those previously described.[20,21] Cycling conditions were 50°C for 10 seconds, 95°C for 10 minutes, followed by 46 cycles at 95°C for 15 seconds and 60°C for 1 minute. TaqMan analyses yield values expressed as ratios between two absolute measurements (gene of interest/internal reference gene).

Statistical Analysis

The Mann-Whitney U test was used to compare the responders and nonresponders in terms of the related gene expressions. To evaluate the association of DPD or TS mRNA with response, DPD or TS mRNA was categorized into low or high values with the cutoff value determined by receiver operating characteristic (ROC) analysis. Association with response was analyzed by two-sided Fisher's exact test.

Experimental Study

The antitumor effects of S-1 and irinotecan

were evaluated in human gastric cancer xenografts with high TS activity (4-1-ST and AZ-521) and low TS activity (SC-2) in nude mice. Human gastric tumors AZ-521, 4-1-ST, and SC-2 were obtained from Dai-Nippon Pharmaceutical Co., Ltd. (Osaka, Japan) and maintained by implantation into the right axilla of nude mice at 3-week intervals. For enzyme assays, [6-^{3}H]-5-FU (525 GBq/mmol), [6-^{3}H]-thymidine (dThd; 2.41 TBq/mmol), [6-^{3}H]-FdUMP (625 GBq/mmol), and [^{14}C(U)]-cytidine-5'-diphosphate (CDP; 2.04 GBq/ mmol) were obtained from Moravek Biochemicals, Inc. (Brea, CA, USA). For immunoblot analysis of proteins, anti-TS antibody was provided by Okabe,[20] and all other antibodies used were purchased from Santa Cruz Biochemicals Inc. (San Diego, CA, USA).

Xenografts and Treatments

For the antitumor experiments, 4-1-ST, AZ-521, and SC-2 tumors were prepared by subcutaneous implantation of approximately 3 mm^3 fragments into the right axilla in groups of 5 mice each. After 7 days, animals received oral S-1 at 8.3 mg/kg for 14 consecutive days, irinotecan 40 mg/kg IV on days 1 and 8, or the combination. For studies of the effects of irinotecan on 5-FU–metabolizing enzymes, groups of 5 nude mice were prepared by the same methods and received saline or irinotecan alone 75 mg/kg once weekly for 2 weeks. In studies assessing dose response of TS activity, groups of 3 nude mice with AZ-521 tumors received no irinotecan or irinotecan 20, 40, or 60 mg/kg weekly for 2 weeks.

In the antitumor studies, tumor volume [½ × (the major axis) × (the minor axis)^2] was measured in all groups twice a week throughout the experiments, and relative tumor volume (RTV) was calculated as follows: RTV = (mean tumor volume during therapy)/(mean tumor volume at the start of therapy). The antitumor effects of S-1, irinotecan, and both drugs combined were estimated by the following equation: mean tumor growth inhibition (TGI, %) = [1-(mean RTV of drug-treated group/mean RTV of control group) × 100].

Enzyme Assay

The effects of irinotecan treatment on 5-FU–metabolizing enzymes in AZ-521, 4-1-ST, and SC-2 tumors and the effect of different doses of irinotecan on TS activity in AZ-521 tumors were analyzed. AZ-521, 4-1-ST, and SC-2 tumors were homogenized with 3 volumes of 50 mM Tris-HCl (pH 7.6) containing 10 mM 2-mercaptoethanol, 25 mM KCl, and 5 mM $MgCl_2$, centrifuged at 105,000g for 60 minutes, and the resulting supernatant was used to measure enzyme activity. The enzymes measured were TS, DPD, ribonucleotide reductase (RNR), orotate phosphoribosyltransferase (OPRT), thymidine kinase (TK), and thymidine phosphorylase (TP). TS was measured by [6-^{3}H]-FdUMP binding assay based on the method of Spears et al.[21] DPD and OPRT activity was determined according to the method of Shirasaka et al[22] using [6-^{3}H]-5-FU as the substrate. TK activity was measured by the method of Ikenaka et al[23] except that the reaction product, [6-^{3}H]-thymidine-5'-monophosphate, was separated from [6-^{3}H]-thymidine by Silica gel 60F$_{254}$ (2 × 10 cm) thin layer chromatography with a mixture of chloroform, methanol, and acetic acid (17:3:1, v/v/v) as the mobile phase. TP was measured according to the modified method described by Maehara et al.[24] Ribonucleotide reductase activity was determined using [^{14}C(U)]-CDP as the substrate.[25]

Western Blot Analysis

For analysis of expression of TS proteins, Western blot analysis was performed using the ECL Western blotting detection system and protocol (Amersham Corp., Arlington Heights, IL). Briefly, cells were scraped from the plates, washed with PBS, and lysed in cell lysis buffer. Forty micrograms of protein were electrophoretically separated on SDS-polyacrylamide gels and transferred to polyvinylide difluoride membrane (Millipore, Bedford, MA, USA). Anti-

Table 2. Effect of irinotecan administration on the activities of 5-FU–metabolizing enzymes in 4-1-ST, AZ-521, and SC-2 human gastric cancer xenografts in mice.

Tumor	4-1-ST		AZ-521		SC-2	
Enzyme	Control	Irinotecan (75 mg/kg/wk x 2)	Control	Irinotecan (75 mg/kg/wk x 2)	Control	Irinotecan (75 mg/kg/wk x 2)
TS	2.163±0.281	1.375±0.184*	0.622±0.095	0.082±0.019*	0.089±0.043	0.086±0.033
DPD	2.22 ± 0.27	1.84 ± 0.34	78.10±12.59	53.87±15.40†	8.580±3.45	8.120±1.46
OPRT	8.119±0.663	7.599±0.806	6.649±0.772	4.726±0.600†	21.01±2.68	22.10±4.00
TP	1.011±0.018	1.168±0.157	0.115±0.030	0.103±0.025	0.647±0.154	0.676±0.105
RNR	2.030±0.975	3.076±1.183	0.483±0.282	0.660±0.099	4.330±1.840	4.150±0.730
TK	15.28±1.90	15.29± 3.41	31.10± 3.75	35.97± 6.86	32.01± 4.20	68.70±13.61

For each tumor, mice received saline (n=5) or irinotecan 75 mg/kg (n=5) administered IV weekly for 2 weeks. At 24 hours after last treatment, tumors were removed and activities of 5-FU-metabolizing enzymes were measured. TS = thymidylate synthase; DPD = dihydropyrimidine dehydrogenase; OPRT = orotate phosphoribosyltransferase; TP = thymidine phosphorylase; RNR = ribonucleotide reductase; TK = thymidine kinase. *P< .01, †P< .05 compared with control group by Dunnett's test.

Table 3. Effect of irinotecan 20 to 60 mg/kg on TS activity in AZ-521 human gastric cancer xenografts in mice.

Dose (mg / kg)	N	TS activity* (pmol/mg ± SD)	% of Control
Control	3	0.994 ± 0.188	100.0
20	3	0.526 ± 0.118	52.7
40	3	0.292 ± 0.088	29.3
60	3	0.214 ± 0.054	21.4
Control	5	0.622 ± 0.095	100.0
75	5	0.082 ± 0.019	13.2*

Mice received no irinotecan or irinotecan 20, 40, or 60 mg/kg on days 1 and 8; 24 hours after final treatment, tumors were dissected and TS activity was measured. *Data on 75 mg/kg treatment and control are from Table 2.

Rh TS polyclonal antibody was applied and the membranes were enhanced by chemiluminescence using ECLTM Western blot detection reagents (Amersham Corp.) according to the manufacturer's instructions.

Statistical Analysis

The significance of differences between groups was assessed using Dunnett's test and the IUT test.

RESULTS

Clinical Study

The relationship between tumor response and DPD mRNA and TS mRNA expression was assessed in 29 primary gastric tumor samples from patients with advanced gastric cancer treated with S-1 alone (n=18) or S-1/irinotecan (n=11). The cutoff values for high vs. low mRNA expression, determined by ROC analysis, were 6.76 (range, 0.19–19.77) for DPD mRNA and 4.53 (range, 0.75–17.27) for TS mRNA. Tumor response occurred in 6 of 18 patients receiving S-1 alone and in 8 of 11 receiving S-1/irinotecan.

There was no relationship between high or low DPD mRNA expression and tumor response with either S-1 alone or with S-1/irinotecan (Figure 1). Response in patients receiving S-1 alone was significantly associated with TS mRNA expression, with none of the 6 responses occurring in tumors with high TS mRNA levels ($P = .009$) (Figure 2). There was no relationship between TS mRNA expression and tumor response in the S-1/irinotecan group, with responses being observed in some patients with high TS levels (Figure 2).

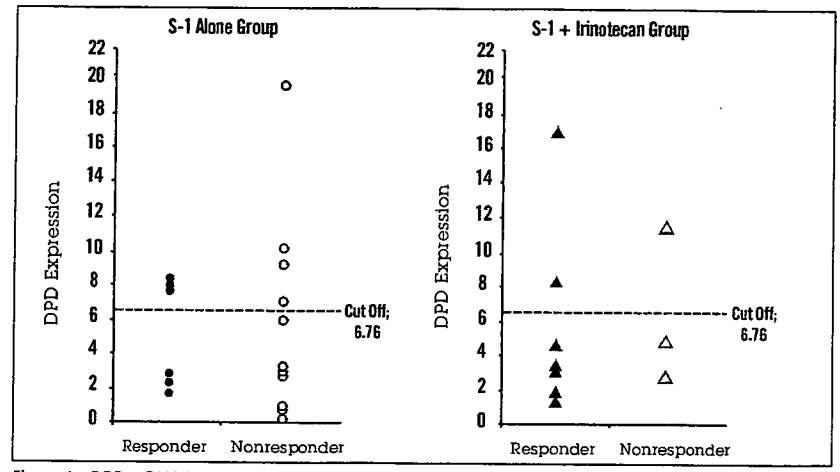


Figure 1. DPD mRNA in responding (partial or complete response) and nonresponding (no change or disease progression) primary gastric tumors in patients receiving S-1 (n=18) or S-1 plus irinotecan. Cutoff value demarcates high vs. low values. **[Author: Difficult to tell, but looks like 17 data points for S-1 and 10 for S-1/CPT-11 on graph? Also, units of measure for DPD expression?]**

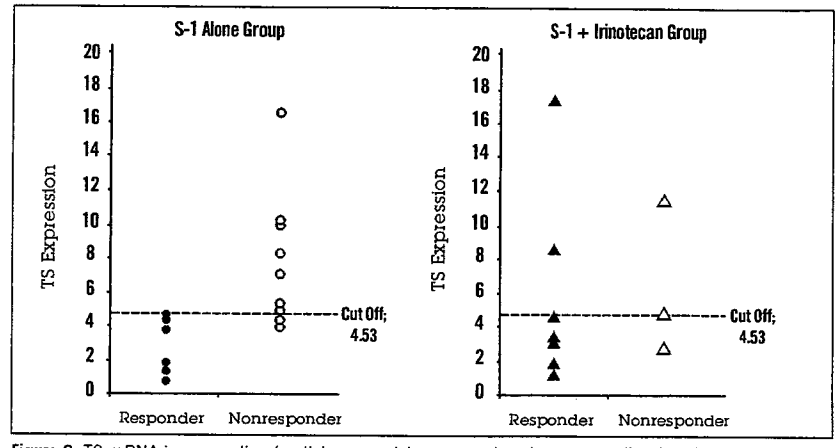


Figure 2. TS mRNA in responding (partial or complete response) and nonresponding (no change or disease progression) primary gastric tumors in patients receiving S-1 (n=18) or S-1 plus irinotecan. Cutoff value demarcates high vs. low values. $P = .009$ for **[Author: Please specify comparison that P value applies to.]**

Experimental Study

Antitumor Effects of S-1 and Irinotecan

The antitumor activities of S-1 and irinotecan were evaluated in nude mice with human gastric cancer xenografts with high TS activity (4-1-ST and AZ-521) or low TS activity (SC-2). As shown in Figure 3, for 4-1-ST and AZ-521 tumors, the tumor growth inhibition rate (IR) with the combination of S-1/irinotecan was significantly augmented ($P < .01$) compared with either agent alone, reaching approximately 60% for both tumor types. For SC-2 tumors, S-1 alone had IR of approximately 70%, with no further augmentation observed with the addition of irinotecan.

Effects of Irinotecan on the Activity of 5-FU–Metabolizing Enzymes

To investigate the mechanism for augmented antitumor activity of the combination of S-1 and irinotecan on 4-1-ST and AZ-521 tumors, activities of the 5-FU–metabolizing enzymes TS, DPD, OPRT, TP, RNR, and TK were assessed in 4-1-ST, AZ-521, and SC-2 tumor xenografts in mice treated with irinotecan or saline. As shown in Table 2, irinotecan 75 mg/kg weekly for 2 weeks significantly reduced TS activity in 4-1-ST and AZ-521 xenografts compared with controls, and produced no change in TS activity in SC-2 tumors. Other 5-FU–metabolizing enzymes were not affected by treatment with irinotecan. Moreover, TS activity in AZ-521 tumor xenografts decreased dose-dependently following administration of 20, 40, and 60 mg/kg of irinotecan (Table 3). Overall, the doses of irinotecan examined in these studies were associated with a dose-dependent antitumor effect, with IR ranging from approximately 30% to 60% (data not shown). Doses of 20 to 40 mg/kg correspond to clinically applicable doses of irinotecan.

Immunochemical Detection of TS Proteins and Rate-Limiting Proteins in G1/S Phase of Cell Cycle

To confirm the decrease in TS activity in 4-1-ST and AZ-521 tumors treated with irinotecan, we assessed expression of TS proteins.