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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

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Background: The efficacy of systemic chemotherapy against peritoneal dissemination from advanced gastric cancer (AGC) remains unclear, because the peritoneal dissemination was not defined as a measurable lesion in conventional phase II studies. In this study, we evaluated the efficacy and toxicity of sequential MTX and 5FU therapy (MF) in chemotherapy-naive patients with AGC accompanied by malignant ascites in a phase II setting.
Methods: The treatment schedule comprised weekly administration of MTX (100 mg/m^2, i.v. bolus) followed by 5FU (600 mg/m^2, i.v. bolus) with a 3 h interval. Leucovorin rescue (10 mg/m^2 every 6 h, for a total of six times) was commenced 24 h after MTX administration.
Results: Thirty-seven chemotherapy-naive patients with AGC presenting with malignant ascites were enrolled in this trial. The median age was 60 years (range, 25–74 years) and most patients (86%) had a performance status of 0–1. In total, 355 administrations of the sequential MTX/5FU therapy were performed. Major toxicity consisted of myelosuppression and gastrointestinal toxicity. Grade 4 neutropenia occurred in 10.8% of the patients. The overall objective response rate was 5.7% (two partial responses in 35 patients; 95% confidence interval: 0.7–19.2%). However, the response rate of ascites was 35.1% (complete disappearance in three patients and apparent decrease in 10 patients; 95% confidence interval: 20.2–52.5%).
Conclusions: Sequential MTX/5FU therapy is effective against AGC with malignant ascites with acceptable toxicity and warrants further investigations in a phase III setting.

Key words: sequential MTX/5FU chemotherapy – gastric cancer, peritoneal dissemination – ascites – clinical trial

INTRODUCTION

Despite a declining incidence in many industrial countries, gastric cancer remains one of the most common malignancies globally. Although this tumor is potentially curable with surgery when diagnosed at an early stage, the prognosis for patients with unresectable or metastatic disease is very poor, with a median survival of 3–4 months when they receive the best supportive care without palliative surgery or chemotherapy (1–3). Gastric cancer can progress to systemic disease through various routes such as direct invasion or lymphatic or vascular spread. Peritoneal dissemination, i.e. peritoneal carcinomatosis, which occurs mainly as a result of direct invasion and/or lymphatic spread, is very common in advanced gastric cancer and is considered an incurable disease state (4). Peritoneal dissemination may cause serious complications, such as

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intestinal obstruction, massive ascites and hydronephrosis associated with the clinical presentation of abdominal pain and fullness, vomiting, constipation, malnutrition and renal dysfunction. From the clinical point of view, palliative management of those complications warrants special considerations and represents a therapeutic challenge in oncology (5,6). Although the major treatment option for unresectable or metastatic gastric cancer is systemic chemotherapy, this strategy has been generally believed to have little effect on peritoneal dissemination, because the drugs could not be delivered sufficiently through the peritoneum–plasma barrier to the disseminated tumor cells (7). However, the efficacy of systemic chemotherapy against peritoneal dissemination from gastric cancer remains unclear, because peritoneal dissemination was not defined as a measurable lesion in conventional phase II studies and therefore few reports are available about the efficacy of systemic chemotherapy against peritoneal dissemination. 5-Fluorouracil (5FU) remains the mainstay for chemotherapy against gastric cancer and a variety of drugs have been tested as modulators to increase its chemotherapeutic efficacy. The modulators that have been most widely used in clinical practice against gastrointestinal tract cancers are folinic acid (leucovorin) and methotrexate (MTX) (8,9). MTX enhances 5FU cytotoxicity via DNA and/or RNA synthesis inhibition when the two drugs are administered in sequence, with 5FU administered a few hours after MTX (10,11). A meta-analysis of randomized trials of sequential MTX/5FU therapy revealed a higher response rate than for single agent bolus 5FU in colorectal cancer (12). The toxicity of these sequential MTX/5FU regimens was comparable to that of 5FU alone (i.e. vomiting, stomatitis, diarrhea and leukopenia). The sequential MTX/5FU therapy was found in phase II trials for advanced gastric cancer to have antitumor activity against advanced gastric cancer (13,14). A Japanese phase II trial of sequential MTX/5FU therapy against advanced gastric cancer demonstrated that low- and intermediate-dose MTX regimens achieved response rates of 23% (13 PRs/56 patients) and 41% (15 PRs/37 patients), respectively (15). Sequential MTX/5FU therapy is widely used as one of the standard treatment regimens for patients with unresectable or metastatic gastric cancer at present in Japan. Konishi et al. reported that sequential MTX/5FU therapy was effective in patients with peritoneal dissemination with a response rate of 23% (6/26) and that ascites disappeared in eight of 16 patients (50%) treated with this therapy (16). Those findings suggest that sequential MTX/5FU might be effective in advanced gastric cancer with peritoneal dissemination.

The objective of this study was to evaluate the efficacy and toxicity of sequential MTX/5FU chemotherapy in advanced gastric cancer with malignant ascites in order to determine whether this regimen is worthy of further investigation in a phase III trial for the treatment of patients with peritoneal dissemination from advanced gastric cancer. The primary endpoints planned for this study were tumor response rate and response rate in ascites. Secondary endpoints were overall survival and toxicity. To our knowledge, there has been no prior study that evaluated the efficacy and toxicity of systemic chemotherapy in a phase II setting in patients with advanced gastric cancer who have peritoneal dissemination with malignant ascites.

SUBJECTS AND METHODS

ELIGIBILITY

Patients enrolled in this study were required to fulfill the following eligibility criteria: (1) histologically confirmed gastric cancer; (2) unresectable or recurrent disease; (3) peritoneal dissemination with cytologically confirmed malignant ascites evaluable by CT scan or ultrasonography; (4) measurable or evaluable disease; (5) age 20–75 years; (6) performance status (PS) ≤2 on Eastern Cooperative Oncology Group (ECOG) scale; (7) no prior chemotherapy with the exception of one adjuvant chemotherapy; (8) adequate bone marrow function (WBC ≥4000/mm^3 and platelets ≥100 000/mm^3) (9) adequate liver function (serum bilirubin level ≤2.0 mg/dl and serum transaminase level ≤2.5-fold the upper limit of normal; (10) adequate renal function (serum creatinine and blood urea nitrogen within the upper limit of normal; (11) serum albumin ≥2.6 g/dl; (12) normal ECG; (13) currently hospitalized; (14) life expectancy at least 8 weeks; (15) written informed consent. Patients with active bleeding from the gastrointestinal tract, other active synchronous carcinoma, central nerve metastasis or concurrent uncontrolled medical illness and pregnant or lactating women were excluded. Patients with massive ascites that required drainage for the relief of symptoms were also excluded. The study protocol was approved by the JCOG Clinical Trial Review Committee and by the institutional review board of each participating center.

TREATMENT PLAN

The treatment schedule comprised weekly administration of MTX (100 mg/m^2, i.v. bolus) followed by 5FU (600 mg/m^2, i.v. bolus) with a 3 h interval. Leucovorin rescue (10 mg/m^2 orally or i.v. every 6 h, six times) was commenced 24 h after MTX administration. To prevent toxicity from MTX, acetazolamide (250 mg) was given intravenously immediately after the infusion of MTX and sodium bicarbonate (33.3 mequiv.) added to 500 ml of electrolyte solution was administered by drip infusion for urine alkalinization during the 3 h interval between the administration of MTX and 5FU. The plasma level of MTX was monitored 24 h after MTX administration and leucovorin rescue at 10 mg/m^2 was administered every 6 h until the plasma level of MTX was $<1 \times 10^{-6}$ mol/l. At the time of each administration, patients were required to fulfill the following criteria: leukocyte count ≥3000/ mm^3; platelet count ≥75 000/ mm^3; adequate liver and renal function as eligibility criteria; PS 0–2; and absence of toxicity grade 2 or greater. The treatment was repeated unless disease progression or severe toxicity was observed. The treatment was terminated when the ascites did

Table 1. Patients' characteristics

Characteristic	Total (n = 37)
Gender	
Male	21
Female	16
Age (years)	
Median	60
Range	25–74
ECOG performance status score	
0	8
1	24
2	5
Histological type	
Intestinal type	
Well-differentiated tubular adenocarcinoma	4
Moderately differentiated tubular adenocarcinoma	7
Papillary adenocarcinoma	1
Diffuse type	
Poorly differentiated adenocarcinoma	6
Mucinous adenocarcinoma	2
Signet-ring cell carcinoma	17
Macroscopic type of primary tumor	
Scirrhous type	21
Non-scirrhous type	16
Metastatic sites	
Lymph nodes	25
Liver	7
Krukenberg's tumor	2
Douglas's metastasis	1
Lung	2
Bone	1
Pleural effusion	4

not improve within 8 weeks or when toxicity did not disappear within 6 weeks.

Response and Toxicity Evaluation

Tumor response was assessed by CT scan or ultrasonography of the target lesions every 4 weeks after the first administration of MTX. Complete response (CR), partial response (PR), no change (NC) and progressive disease (PD) were defined according to the response assessment criteria proposed by the Japanese Research Society for Gastric Cancer (17). The response in ascites was evaluated by abdominal CT scan or ultrasonography based on the following specific criteria used in this study: (1) disappearance of ascites – disappearance of ascites visualized by CT scan or ultrasonography for at least 4 weeks; (2) decrease of ascites – apparent decrease of ascites visualized by CT scan or ultrasonography for at least 4 weeks; (3) no response of ascites – no change of ascites volume visualized by CT scan or ultrasonography. The data for tumor response in all responders was confirmed by an extramural review. The toxicity was evaluated according to the JCOG common toxicity criteria (18).

Statistical Analysis

The sample size was determined based on the precision of the estimates. The efficacy for malignant ascites was expected to be 30%. Fifty subjects and an observed efficacy of 30% would provide a 95% confidence interval of 17.9–44.6% or width of 26.7%. The expected accrual period was 1.5 years. Interim analysis was planned to test for inefficacy of the treatment by examining whether a 90% upper confidence bound of efficacy would exceed 25% for first 20–25 patients. The overall survival was calculated for the period from the date of registration to the date of death. Overall survival was calculated by the Kaplan–Meier method and confidence intervals were calculated based on Greenwood's formula.

RESULTS

Patient Population and Study Treatment

Between February 1997 and October 1999, 37 patients were enrolled in this trial from nine out of 13 participating institutions. Although this study was originally planned as a phase II study in which 50 patients would be enrolled within 1.5 years of the start of the study, the patient enrollment was delayed and was finally terminated before the projected number of patients had been achieved based on the decision of the JCOG monitoring committee that the evaluation of efficacy and toxicity was possible even with only 37 enrolled patients. Table 1 lists the demographic data, baseline disease and pretreatment characteristics of all patients. Twenty-one males and 16 females were registered as receiving first-line chemotherapy. The median age of the patients was 60 years (range, 25–74 years) and the majority of the patients (86%) had a good performance status of 0–1. Twenty-one patients (57%) had macroscopically scirrhous-type advanced gastric cancer. Twenty-five patients had histologically diffuse types (six poorly differentiated adenocarcinoma, two mucinous carcinoma and 17 signet-ring cell carcinoma). Two patients had undergone surgery prior to enrollment in this trial (one palliative total gastrectomy and the other exploratory laparotomy resulting in no resection). One patient suffered from hemilateral hydronephrosis due to peritoneal dissemination with normal range of renal function tests.

In total, 355 administrations of the sequential MTX/5FU therapy were performed in 37 patients. The median number of administrations was eight (range, 1–42). Twenty-nine of 37 enrolled patients (78%) received at least four administrations of the sequential MTX/5FU therapy. All patients were assessable for toxicity and response of ascites to chemotherapy. Thirty-five patients were assessable for objective tumor

Table 2. Toxicity profiles

Toxicity	JCOG grade					Grade 4 (%)
	0	1	2	3	4	
Hematological toxicity						
Leukopenia	11	13	7	4	2	5.4
Neutropenia	17	5	5	6	4	10.8
Anemia	7	6	15	9	–	–
Thrombocytopenia	32	3	1	1	0	0
Non-hematological toxicity						
Nausea/vomiting	13	14	10	0	–	–
Diarrhea	25	4	6	2	0	0
Stomatitis	30	7	0	0	0	0
Alopecia	35	2	0	–	–	–
Allergic reaction	36	1	0	0	0	0
Fever	18	10	9	0	0	0
Peripheral neuropathy	36	1	0	0	–	–
Total bilirubin	20	–	8	8	1	2.7
AST	16	16	5	0	0	0
ALT	16	16	5	0	0	0
Alkaline phosphatase	16	17	2	2	0	0
Creatinine	33	2	0	2	0	0
Hyponatremia	12	17	7	0	1	2.7
Hypokalemia	21	12	4	0	0	0

response to chemotherapy. The most frequent reason for treatment termination was disease progression (27 patients, 73%). Other reasons for treatment termination were no response after 8 weeks from initiation of treatment in two, patient refusal in two, severe toxicity in two, death in three (one due to disease progression and two treatment-related) and medical judgment by the investigators in one.

TOXICITY

The toxicity observed in the study period is summarized in Table 2. The major toxicity was myelosuppression and gastrointestinal toxicity. Grades 3 and 4 neutropenia occurred in 16 and 11% of the patients, respectively. Severe thrombocytopenia was infrequent. The incidence of grade 3 diarrhea was 5%. Mild nausea and vomiting (grades 1 and 2) were frequently experienced (65%). An increase in total bilirubin of grade 4 was observed in one patient (2.7%) and was diagnosed as obstructive jaundice caused by the development of lymphadenopathy from the primary disease. An increase in total bilirubin grade 3 was observed in eight patients, three cases of which were judged to be treatment-related. An increase in serum creatinine grade 3 was observed in two patients (5.4%). One patient experienced grade 4 hyponatremia due to loss of oral intake associated with primary disease. Early death, which was defined as death within 30 days from the last administration of anti-cancer drugs, occurred in five patients. The causal relationship between the death and the study treatment was 'unlikely' in three of those five patients. However, the remaining two deaths were assessed to be treatment-related. One patient died of severe neutropenia and rapidly progressive disseminated intravascular coagulation (DIC), which was complicated with respiratory dysfunction, and the other patient died of progressive neutropenic sepsis.

EFFICACY

The efficacy-related data are summarized in Table 3. Only two of 35 response-assessable patients achieved objective partial response (response rate 5.7%; 95% confidence interval: 0.7–19.2%). However, in terms of the response of ascites, three disappearances and 10 decreases of ascites were obtained (response rate 35.1%; 95% confidence interval: 20.2–52.5%). The median duration of response of ascites was 103 days with a range of 52–337 days. The median survival time of all patients was 155 days (95% confidence interval: 131–225 days) and the 1 year survival rate was 16.2% (95% confidence interval: 4.3–28.1%).

DISCUSSION

Although unresectable or metastatic gastric cancer is potentially incurable, there is significant evidence that adding systemic chemotherapy to the best supportive care could provide benefits in survival and quality of life as compared with best supportive care alone (1–3). However, it has been difficult to assess which of many available regimens is the most effective, although several regimens have been tested in randomized controlled trials. Some randomized trials failed to demonstrate the superiority of 5FU-based combination regimens as compared with 5FU-monotherapy (19–21). A recent randomized controlled trial showed that three commonly used combination regimens, 5FU/adriamycin/MTX (FAMTX), 5FU/cisplatin (FP) and etoposide/leucovorin/5FU (ELF), have only modest activity and that there were no significant differences in overall survival among these regimens (22). More recently, infusional 5FU in combination with cisplatin and epirubicin (ECF) showed significant superiority over FAMTX in terms of response rate, quality of life and survival, suggesting that the ECF could be a new standard treatment for future clinical trials (23). However, regarding the median survival time in those large-scale trials, there was little substantial difference among the various regimens. Therefore, in general, 5FU-based or cisplatin-based combinations are widely accepted as a possible standard therapy (24). In clinical practice, oncologists need to select a regimen considered to be the most appropriate for each individual patient based on the medical condition of each patient, including such factors as age, performance status, organ function and extent of disease. The cisplatin-based regimens are usually inappropriate to be used for patients having peritoneal dissemination and retention of ascites, because such patients have potential renal impairment or poor performance

Table 3. Responses to treatment (total of 355 administrations of the sequential MTX/5FU therapy)

	No. of evaluable patients	CR	PR	NC	PD	NE	Response rate (%)	95% CI (%)
Objective response	35	–	2	21	6	6	5.7	0.7–19.2
Response in ascites	37	3	10	15	6	3	35.1	20.2–52.5

CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluable; CI, confidence interval.

status, which makes it difficult to tolerate the large volume hydration for the prevention of cisplatin-induced renal injury. Among several 5FU-based regimens, sequential MTX/5FU therapy is widely used because this regimen has definite anti-tumor activity against advanced gastric cancer with acceptable toxicity even in high-risk patients. The purpose of the present phase II study was to evaluate the efficacy and toxicity of the sequential MTX/5FU regimen in patients with unresectable gastric cancer with peritoneal dissemination accompanied by malignant ascites and to assess whether further investigation in a phase III setting is warranted.

Progression to peritoneal dissemination is very common in advanced gastric cancer and is frequently a component of the first episode of failure after surgery for primary gastric cancer (25). Therefore, intraperitoneal chemotherapy has previously been investigated for peritoneal dissemination for the purposes of palliation and the prevention of peritoneal metastasis after surgery in high-risk patients. The pharmacokinetic rationale for intraperitoneal therapy is that drug concentrations within the peritoneal cavity are several-fold to 1–2 logs higher than concentrations that can be achieved after oral or intravenous treatment (26,27). In ovarian cancer, a large randomized trial demonstrated a small but statistically and clinically significant survival advantage in patients receiving intraperitoneal therapy (28). However, generally the efficacy of intraperitoneal chemotherapy is considered to be modest because the penetration of intraperitoneally injected drug into submesothelial tissue is too limited to achieve anti-tumor activity. Moreover, intraperitoneal chemotherapy sometimes induces systemic adverse events similar to systemic chemotherapy in addition to local complications such as chemical peritonitis. No definite data are currently available to specify which treatment option, intraperitoneal or systemic chemotherapy, is more suitable for patients with peritoneal dissemination in terms of benefit regarding survival and quality of life.

When we perform systemic chemotherapy in patients who have fluid retention such as ascites or pleural effusion, we have to consider the pharmacokinetic alterations of the anti-tumor agents administered. Intravenously administered MTX penetrates the ascites or pleural effusion and the clearance rate of MTX from ascites and plasma is ~5 and ~120 ml/min, respectively (29). Therefore, the retention of body fluid prolongs the terminal plasma half-life of intravenously administered drug owing to the slow re-entry of the sequestered drug into the bloodstream. Such phenomena should be associated with both favorable anti-tumor activity against peritoneal or pleural dissemination and with the potential risk of systemic toxicity. In another phase II study of sequential MTX/5FU therapy against unresectable or metastatic gastric cancer previously conducted by the JCOG, in which the same dosage and schedule as in the present study were utilized but the patients having ascites were ineligible for entry (JCOG 9207 study), none of 56 enrolled patients experienced grade 3 or 4 neutropenia (data not shown). In the present study, grades 3 and 4 neutropenia were observed in six (16 %) and two patients (11 %), respectively. The incidence of leukopenia, anemia, increase in total bilirubin and increase in serum creatinine of grade 3 or 4 tended to be more frequent in the present study than in the JCOG 9207 study (data not shown). Therefore, the toxicity of the sequential MTX/5FU therapy might be more severe in patients with malignant ascites than in those without. Two treatment-related deaths were observed in the present study. These two patients developed progressive neutropenic sepsis, which is a major cause of death. Although these two patients had met the eligibility criteria required in the study, both patients were retrospectively shown to be at high-risk for neutropenic infection, because pretreatment serum CRP values were highly elevated in both patients and leukocytosis was also observed at the baseline in one patient. Therefore, we consider that patients with apparent inflammatory signs such as elevation of CRP or leukocytosis should be excluded from future studies to prevent neutropenic sepsis. It is known that the different methods of administration of 5FU, either as a bolus or by infusion, represent different efficacy and toxicity profiles, thus infusional 5FU has more clinical benefit in efficacy (response rate) and safety in metastatic colorectal cancer. At present, however, we do not have sufficient data to establish whether these clinical observations hold true in patients with peritoneal dissemination with malignant ascites and it seems to be important to investigate the infusional 5FU-based regimens in this clinical setting, which may contribute to reducing the toxicity.

It is difficult to evaluate the efficacy of chemotherapy against peritoneal dissemination in clinical trials as well as in clinical practice, because most disseminated tumor cells do not form a measurable mass but rather constitute a diffuse lesion. Clinicians have to assess the efficacy of treatment and disease status in each patient based on the integration of clinical information such as clinical imaging, tumor markers and clinical symptoms. In the present study, the therapeutic efficacy was assessed according to the specific criteria for the study based on the change in the volume of ascites visualized by abdominal CT scan or ultrasonography as a surrogate marker. Using these criteria, we found that the ascites disappeared or was decreased by the MTX/5FU therapy in 35% of the patients. Konishi et al.

also reported that ascites disappeared in 50% (8/16) of patients with peritoneal-disseminated gastric cancer after MTX/5FU therapy (16). These results show that sequential MTX/5FU therapy is effective in controlling malignant ascites and also suggest that this regimen is effective against peritoneal dissemination from advanced gastric cancer.

Although the present study was originally planned as a phase II study involving 50 patients, patient enrollment had been delayed and finally terminated before the projected number of patients was achieved. The delay in patient enrollment was probably caused by the eligibility criteria for this study. Although peritoneal dissemination of advanced gastric cancer is very common in clinical practice, most patients with peritoneal dissemination accompanied by malignant ascites tend to have relatively poor performance status and impaired organ function, which was considered to be a critical issue delaying patient enrollment. The JCOG monitoring committee accepted the investigators' decision that the objectives of this study, which were to calculate the response rate in ascites and to evaluate the safety of sequential MTX/5FU therapy for decision-making to pursue further investigation in a phase III study, were achieved even with the actual sample size of 37 patients and that the response rate in ascites of 35% (95% confidence interval: 20.2–52.5%) observed in this study was positive.

It is well known that peritoneal dissemination of gastric adenocarcinoma occurs more commonly as the histologically diffuse type than the intestinal type. Konishi et al. reported that sequential MTX/5FU therapy was more effective against undifferentiated gastric cancer (i.e. histologically diffuse type) than differentiated gastric cancer (i.e. histologically intestinal type), with a response rate of 32% (9 PRs/28 patients) vs 0% (0 PRs/10 patients) (16). A similar tendency was observed in the present study, namely that the response rate of ascites was higher for the histologically diffuse type than for the intestinal type (44%, 11 responders among 25 patients, versus 17%, two responders among 12 patients). The difference in the efficacy of the sequential MTX/5FU therapy depending on the histological type might be explained by the difference in the activities of two enzymes, thymidylate synthetase and thymidine kinase, in the various histological types of gastric cancer (30). However, other reports have suggested that there were no significant differences according to the histological type. (15)

In conclusion, the findings of the present study suggest that sequential MTX/5FU therapy is effective in controlling malignant ascites from gastric cancer with overall acceptable toxicity and that further investigations are warranted. However, the present study also suggests that severe toxicity may occur more frequently in patients with malignant ascites than in those without malignant ascites. Whether there is true clinical benefit in this regimen for patients with peritoneal dissemination from advanced gastric cancer should be evaluated in future randomized clinical trials. Since the peritoneal dissemination from gastric cancer is considered to be an incurable disease, the patient's survival and quality of life will be important endpoints to be assessed in the future clinical trials. Recently, various new drugs with different mechanisms of action have been developed. However, since the patients whose main diseases are peritoneal dissemination are usually excluded from the phase II trials of new drugs or new combination regimens because of the lack of measurable lesions in those patients, the available data as to the efficacy against peritoneal dissemination are very limited unless we conduct trials specifically designed for this purpose as the present study. We think it is important to assess the roles of new drugs from the viewpoint of how we can maximize the potential value of each drug or regimen in disease-specific clinical situations. In this study we have focused on peritoneal dissemination with malignant ascites from advanced gastric cancer, which is very common and a major clinical problem. At present, any 5FU-based combination chemotherapies cannot prolong overall survival compared with 5FU alone. However, the present study brought us to the hypothesis that if we choose an appropriate regimen and administer it to the appropriate patient population (for example, to choose MTX/5FU therapy for the patients with peritoneal dissemination), survival may be prolonged compared with 5FU alone. We think that MTX/5FU therapy is the most reasonable regimen to be tested as a first-line chemotherapy in patients with peritoneal dissemination from advanced gastric cancer. From this clinical standpoint, a phase III randomized controlled trial comparing sequential MTX/5FU therapy with infusional 5FU-monotherapy (800 mg/m^2 of 5FU continuous infusion over 5 days every 4 weeks) in patients with advanced gastric cancer who have peritoneal dissemination with or without ascites is currently being carried out by the Japan Clinical Oncology Group (JCOG 0106-MF study). As a final note, we suggest that in future trials we should investigate the therapeutic strategy not only with newer cytotoxic drugs including irinotecan, taxanes and oxaliplatine, but also with new molecular targeting drugs such as antibody, VEGF drugs and EGF drugs, to bring about a breakthrough in this dire clinical condition.

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Genetic Variations of the AHR Gene Encoding Aryl Hydrocarbon Receptor in a Japanese Population

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SNP Communication

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Summary: Aryl hydrocarbon receptor (AhR), encoded by the *AHR* gene, is a transcriptional factor that induces various drug metabolizing enzymes in response to diverse endogenous and exogenous ligands. In order to identify genetic variations of the *AHR* gene, genomic DNA from 242 Japanese individuals was sequenced. We identified 32 single nucleotide variations, including 25 novel ones [7 were in the coding exons, 7 in the introns, 1 in the 5′-untranslated region (UTR), 5 in the 3′-UTR, 2 in the 5′-flanking region, and 3 in the 3′-flanking region] and a GGGGC repeat polymorphism (a novel microsatellite marker) in the promoter region. The novel nonsynonymous variations were 50A>C (Lys17Thr), 1202A>G (Lys401Arg), 1459A>G (Asn487Asp), and 1541T>C (Ile514Thr). The allele frequencies were 0.010 for 1459A>G (Asn487Asp) and 0.002 for the other 3 variations. Also detected in this analysis was the known nonsynonymous single nucleotide polymorphism 1661G>A (Arg554Lys) at a 0.444 frequency.

Key words: ***AHR*; genetic variation; nonsynonymous alteration; microsatellite marker**

Introduction

Aryl hydrocarbon receptor (AhR), encoded by the *AHR* gene, is a member of the basic-loop-helix/Per-Arnt-Sim family of transcriptional factors.[1] *AHR* mRNA is dominantly expressed in the placenta, lung, heart, pancreas, and liver.[2] Under resting conditions, AhR exists as a cytosolic complex with Hsp90, the co-chaperone p23, and the immunophilin-like protein

On May 20, 2004, these variations were not found in the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB (http://www.pharmgkb.org/do/) databases.

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XAP2. Upon binding a ligand, AhR translocates into the nucleus, followed by the replacement of its associated molecule with Arnt, and binds to the xenobiotic responsive elements (XRE) found in the regulatory elements of diverse genes. For example, AhR ligands activate the transcription of drug metabolizing enzymes *CYP1A1*, *CYP1A2*, *CYP1B1*, *UGT1A1*, and *UGT1A6* through XREs in the enhancer regions of these genes.[3-8]

AhR has been reported to be activated by various exogenous aromatic hydrocarbons: e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene.[9] Recently, several endogenous ligands were also identified, such as tryptophan derivatives (e.g., indirubin) and arachidonic acid metabolites (e.g., lipoxin A4).[9,10]

The human *AHR* gene is located on chromosome 7p15 and consists of 12 exons, including a non-coding exon (exon 12).[11] Over 30-fold interindividual differences in CYP1A1 inducibility by aromatic hydrocarbons have been reported in mitogen activated lymphocytes.[12,13] Furthermore, it was suggested that the 7p15 region was involved in such interindividual differences.[11] From these findings, it is possible that differences in AhR transcriptional activity caused by genetic polymorphisms in the *AHR* gene might affect the inducibility of target genes.

Several genetic polymorphisms have been reported in the *AHR* gene.[14] The most common single nucleotide polymorphism (SNP) is 1661G>A (Arg554Lys), which was first found by polymerase chain reaction (PCR)-single strand conformational polymorphism analysis, followed by direct sequencing of these products from 25 Japanese subjects.[13] The functional effect of this variation was marginal in *in vitro* TCDD-induced *CYP1A1* mRNA expression.[15] However, another study suggested that 3-methylcholanthrene-induced CYP1A1 activity in lymphocytes was significantly higher in 554Lys-positive Caucasian subjects than in 554Lys-negative ones.[16] Furthermore, the less frequent variation found in African populations, Val570Ile, is linked with Arg554Lys, and this haplotype shows abrogated TCDD-induced *CYP1A1* mRNA expression.[17] Thus, it is suggested that the genetic polymorphisms in *AHR* at least partly contribute to the interindividual differences in AhR transcriptional activity. However, there has been no comprehensive sequence analysis of *AHR* for the Japanese population.

In this study, all the exons and surrounding introns of *AHR* were sequenced for 242 Japanese subjects. Sequence analysis revealed the identification of 33 genetic variations, including 26 novel ones.

Materials and Methods

Human DNA samples: DNA was extracted from the blood leukocytes of 118 Japanese cancer patients (with lung, stomach and colon cancers) administered irinotecan, and 76 Japanese arrhythmic patients administered mexiletine. Written informed consent was obtained from all participating patients. Forty-eight DNA samples from Epstein-Barr virus-transformed lymphoblastoid cells were also used. They were prepared from healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP Consortium (Tokyo, Japan). Informed consent was also obtained from all healthy subjects. The ethical review boards of the National Cancer Center, the National Cardiovascular Center, the National Institute of Health Sciences, the Pharma SNP Consortium, and the Tokyo Women's Medical University approved this study.

Polymerase chain reaction (PCR) conditions and DNA sequencing: First, the entire *AHR* gene was amplified by three mixed primer sets (Mix 1, Mix 2, and Mix 3 in the "1st PCR" section) shown in **Table 1**. Amplification was performed from 100 ng of genomic DNA using 1.25 units of Ex-Taq (Takara Bio. Inc., Shiga, Japan) with 0.2 μM of the primer sets. Since the exon 1 region is highly GC-rich, this exon was amplified by using the GC-buffer Kit (for LA-Taq, Takara Bio. Inc.). The first PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. Then, each exon was amplified by Ex-Taq (0.625 units) with a set of primers (0.2 μM) listed in the "2nd PCR" section of **Table 1** (primers were designed in the intronic regions). The second-round PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. Thereafter, the PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the primers listed in the "Sequencing" section of **Table 1**. The excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). For exons 2, 3, and exons 5 through 9, the primer sets for the 2nd PCR were also utilized for sequencing. As for exons 1 (with the 5′-flanking region), 10 and 11, more than 2 primer sets were utilized for sequencing since these regions were long. The 3′ end (about 180 bases) of exon 11 and the 5′ end (about 200 bases) of exon 12 [both are in the 3′-untranslated region (UTR)] were excluded from the current analysis because of the presence of successive thymine or adenine nucleotides in these regions, respectively. All the detected variations were confirmed by repeating the PCR from the genomic DNA and sequenc-

Table 1. Primer sequences utilized for the analysis of human *AHR*

		Amplified and sequenced region	Forward primer (5′ to 3′)	Position of the forward primer*	Reverse primer (5′ to 3′)	Position of the reverse primer*	Amplified length (bp)
1st PCR	Mix 1	Exon 1	GTCTCTCAAACAGGTGAAGT	16633629	AGGAGATTTCAAGACAGGTT	16634962	1,334
	Mix 2	Exon 2	CACTGTGCTACAAATGCTTG	16644944	CTGTTGGGTGAATAAAACTG	16645590	647
		Exon 4 to 6	CAGGAGTGTATGTTTTGGCT	16662888	GGGAATAGTTCTCTGCTGAA	16666264	3,377
		Exon 10	GTGTCAGGTAGGGATGTAAC	16674009	CTGGAAAAAGTACAGGCTTG	16675682	1,674
	Mix 3	Exon 3	GCTGACAACTTGACTAAACC	16657542	TTACGGGACCACTGTCGCAT	16658119	578
		Exon 7 to 9	TGACCATTAGGAACAAGGAG	16668945	AATCACTGCTGGGTTTAGAG	16671399	2,455
		Exon 11 to 12	GTTTACAACTCTCAGGGGTA	16678086	GAATACTGGTAAGTCTTCAG	16681215	3,130
2nd PCR		Exon 1	GTCTCTCAAACAGGTGAAGT	16633629	AGGAGATTTCAAGACAGGTT	16634962	1,334
		Exon 2	CACTGTGCTACAAATGCTTG	16644944	CTGTTGGGTGAATAAAACTG	16645590	647
		Exon 3	GCTGACAACTTGACTAAACC	16657542	TTACGGGACCACTGTCGCAT	16658119	578
		Exon 4	CAGGAGTGTATGTTTTGGCT	16662888	TATCCTCTGCTATCCATAAG	16663445	558
		Exon 5	GCAAGCACCCACTAATCTAA	16665133	ACTCTGTTGCCTGAAACTCA	16665570	438
		Exon 6	GTATTCAGAACACAGACTCC	16665921	GGGAATAGTTCTCTGCTGAA	16666264	344
		Exon 7	TGACCATTAGGAACAAGGAG	16668945	AGGCTACACTGGAAGAATGT	16669531	587
		Exon 8	TCTGTTACCCATACATCTGC	16669881	GAACAAAGAGTGTAACCCTG	16670432	552
		Exon 9	CCAGAACTATGTCACAAGAG	16670829	AATCACTGCTGGGTTTAGAG	16671399	571
		Exon 10	GTGTCAGGTAGGGATGTAAC	16674009	CTGGAAAAAGTACAGGCTTG	16675682	1,674
		Exon 11	GTTTACAACTCTCAGGGGTA	16678086	GGACAGTAAAGTTGGTAGGG	16679865	1,780
		Exon 12	TGCTTACCTACTTTCTTCAG	16679041	GAATACTGGTAAGTCTTCAG	16681215	2,175
Sequencing		Exon 1	GTCTCTCAAACAGGTGAAGT	16633629	GCCGTCTATTTAGAATCCTG	16634194	
			GGTCGGGGGTGCTCCTGCTA	16633879	CGGTGTAGGCTGGGACCACT	16634481	
			CAGGATTCTAAATAGACGGC	16634175	GCTGTCAACAAATCAGGACC	16634906	
		Exon 4	TGTTTTGGCTGTGTTTGTGA	16662898	TATCCTCTGCTATCCATAAG	16663445	
		Exon 10	TGTCAGGTAGGGATGTAACC	16674010	GTATCATTTCCCATCGGTGC	16674621	
			TCTCTATCCTGCTTCAAGTA	16674505	TAGGGGAACTCTTGACTGAT	16675161	
			TGTGTCAGAAGATGAAGCAC	16675014	TGAATGCTGTAGATAACCGA	16675614	
		Exon 11	GTTTACAACTCTCAGGGGTA	16678086	GGAGGAAAGCACTGAGATTA	16678866	
			CTACAGTCAAGATAGAAAGG	16678538	TTCAACATAAGGCACATAGC	16679152	
			TGCTTACCTACTTTCTTCAG	16679041	ACACATAGTTTCCACTGTTC	16679506	
			GAACAGTGGAAACTATGTGT	16679487	GGACAGTAAAGTTGGTAGGG	16679865	
		Exon 12	ATAAAAATGGCTTCGGACAA	16680646	TAAATCCCACAATGTAGCAG	16681116	

* The position of the 5′ end of each primer on NT_007819.14.

Table 2. Summary of *AHR* variations detected in a Japanese population.

SNP ID			Reference	Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Number of subjects			
This Study	NCBI (dbSNP)	JSNP			NT_007819.14	From the translational initiation site or from the nearest exon			Wild-type	Hetero-zygote	Homo-zygote	Frequency
MPJ6_AHR001[b]	rs11330131		20)	5′-flanking	16633733_16633741	−808_−800[c]	ATGGCTACCGGCG/−GGGGGGGG CGTCCTTACGTC		0	2	240	0.996
MPJ6_AHR002[a,b]				5′-flanking	16633740	−801[c]	CCGGCGGGGGGGG/AGCGTCCTTACGT		241	1	0	0.002
MPJ6_AHR003[a]				5′-flanking	16633775	−766[c]	CACGTGCCGGGAT/CGAGGGTGGGGCC		241	1	0	0.002
MPJ6_AHR004	rs10249788		20)	5′-flanking	16633799	−742[c]	CCTCAAGGAAGAC/TGGAATGGAATCC		114	107	21	0.308
MPJ6_AHR005[a]				5′-flanking	16633861_16633870	−680_−671[c]	CGGGATCTGGGC(GGGGC)nCGGTGAGGGGT[e]					
MPJ6_AHR006[a,b]				Exon 1 (5′-UTR)	16633910	−631[c]	ATTCAGCCGGTGC/TGCGCGGCGGCGG		241	1	0	0.002
MPJ6_AHR007	rs7796976		20)	Exon 1 (5′-UTR)	16634082	−459[c]	CACCCTGGATTTG/AGGAAGTCCCGGG		101	111	30	0.353
MPJ6_AHR008[a,b]				Exon 1	16634590	50[c]	GCAAGCGGCGGAA/CGCCGGTGCAGAA	Lys17Thr	241	1	0	0.002
MPJ6_AHR009			13)	Exon 2	16645278	132[c]	AGACCGACTTAAT/CACAGAGTTGGAC	Ala44Ala	229	12	1	0.029
MPJ6_AHR010[a]				Intron 3	16657928	IVS3+45	AGGACAGTTGTAA/GATGGAAAATGAA		241	1	0	0.002
MPJ6_AHR011[a]				Intron 4	16663417	IVS4+293	AAATTATTTGCTA/CTCAAATAACTTA		241	1	0	0.002
MPJ6_AHR012[a]				Intron 5	16665372	IVS5+21	TGATGGTACAAAA/CAATAGTGTTGGT		241	1	0	0.002
MPJ6_AHR013[a]				Intron 6	16669025	IVS6−163	GATTTAATGGGCG/ATCCCATGGAGAG		239	3	0	0.006
MPJ6_AHR014	rs2074113	IMS-JST000840		Intron 7	16669423	IVS7+33	TTTATTTTATTGG/TATGTACATTATG		70	129	43	0.444
MPJ6_AHR015[a,b]				Intron 7	16669983	IVS7−180	AAATTTGCATCA/CTAAGCAAAGAAG		241	1	0	0.002
MPJ6_AHR016[a,b]				Intron 8	16670310	IVS8+38	GTCAGAAGAAAAC/TGGCATATACTGT		241	1	0	0.002
MPJ6_AHR017[a,b]				Intron 8	16670885	IVS8−36	TATATTGATTTGG/TGGGTTTGATAAT		241	1	0	0.002
MPJ6_AHR018[a,b]				Exon 10	16674303	1202[c]	AACGAAATACGAA/GGTTGCCTTTTAT	Lys401Arg	241	1	0	0.002
MPJ6_AHR019[a]				Exon 10	16674307	1206[c]	AAATACGAAGTTG/ACCTTTTATGTTT	Leu402Leu	241	1	0	0.002
MPJ6_AHR020[a]				Exon 10	16674560	1459[c]	AACAACTTTTTCA/GACGAATCTATGA	Asn487Asp	237	5	0	0.010
MPJ6_AHR021[a,b]				Exon 10	16674642	1541[c]	AACATGAGCAAAT/CTGACCAGCCTCA	Ile514Thr	241	1	0	0.002
MPJ6_AHR022	rs2066853		13)	Exon 10	16674762	1661[c]	TTGAAGACATCAG/AACACATGCAGAA	Arg554Lys	70	129	43	0.444
MPJ6_AHR023[a,b]				Exon 10	16674835	1734[c]	CATTGACTTAACG/AGATGAAATCCTG	Thr578Thr	241	1	0	0.002
MPJ6_AHR024[a,b]				Exon 10	16674850	1749[c]	TGAAATCCTGACG/ATATGTCCAAGAT	Thr583Thr	241	1	0	0.002
MPJ6_AHR025[a]				Exon 11 (3′-UTR)	16678583	2790[c]	GTGGTGAGGTACC/TGTCTACATTTCA		241	1	0	0.002
MPJ6_AHR026[a]				Exon 11 (3′-UTR)	16678728	2935[c]	TTTGAGCTACTGG/CATTCTTATTAGT		241	1	0	0.002
MPJ6_AHR027[a]				Exon 11 (3′-UTR)	16679080	3287[c]	GCAAATAATGATC/TGAAAAAATAATT		241	1	0	0.002
MPJ6_AHR028[a]				Exon 11 (3′-UTR)	16679087	3294[c]	ATGATCGAAAAAA/GTAATTATTTATT		236	6	0	0.012
MPJ6_AHR029[a]				Exon 11 (3′-UTR)	16679593	3800[c]	ATGGTGCATTGTA/TTAGATATAATGA		240	2	0	0.004
MPJ6_AHR030[a]				3′-flanking	16681128	+84[d]	ATTTCTAGATGAT/CGTGCACATCTAA		239	3	0	0.006
MPJ6_AHR031[a,b]				3′-flanking	16681156	+112[d]	TATGGATGTGTCT/CAATTTAGTCTTT		241	1	0	0.002
MPJ6_AHR032	rs11400459			3′-flanking	16681158_16681159	+114_115[d]	GGATGTGTCTAA−/TTTTAGTCTTTTC		98	112	32	0.364
MPJ6_AHR033[a]				3′-flanking	16681171	+127[d]	TTTAGTCTTTTCC/ATGTACCAGGTTT		241	1	0	0.002

[a] Novel variations detected in our study.
[b] Detected only from the cancer patients.
[c] A of the translation initiation codon ATG is numbered +1.
[d] The nucleotide number from the end of exon 12.
[e] Microsatellite; n=2>n=4, 5 and 6.

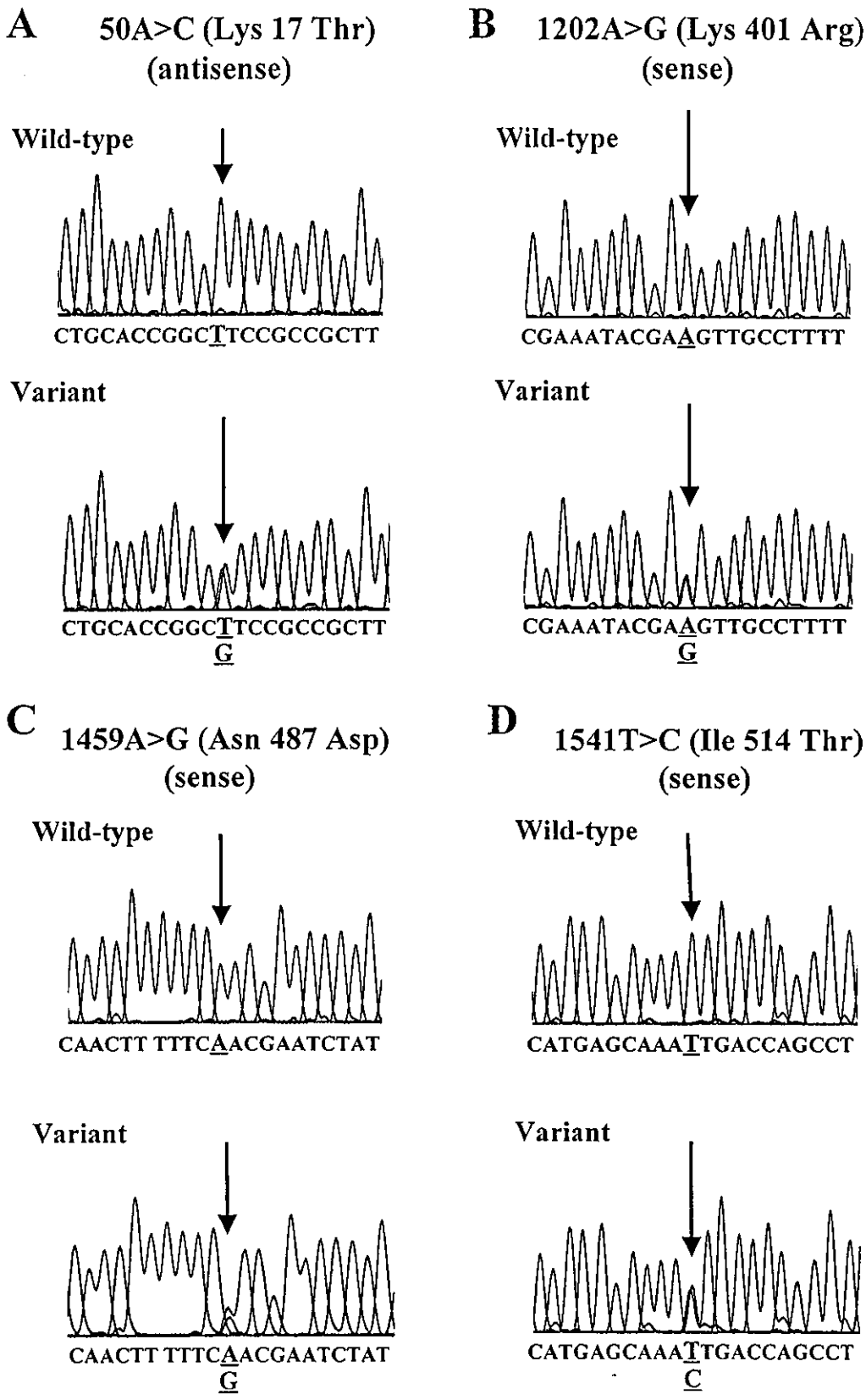

Fig. 1. Electropherograms for the novel nonsynonymous variations of *AHR*. (A) MPJ6_AHR008 (wild-type 50A/A; variant 50A/C). (B) MPJ6_AHR018 (wild-type 1202A/A; variant 1202A/G). (C) MPJ6_AHR020 (wild-type 1459A/A; variant 1459A/G). (D) MPJ6_AHR021 (wild-type 1541T/T; variant 1541T/C). Arrows indicate the variant nucleotide positions.

ing the newly generated PCR products. Furthermore, the rare variations found in only one subject were confirmed by sequencing the PCR fragments produced by amplification with a high fidelity DNA polymerase KOD-Plus- (TOYOBO, Tokyo, Japan). All variable sites in all samples were clearly visible in the electropherograms.

Results and Discussion

NT_007819.14 (Genbank Accession number) was utilized as the reference sequence of *AHR*. Thirty-three genetic variations were identified, including 25 novel single nucleotide variations and 1 novel microsatellite marker, from 242 Japanese individuals (see **Table 2**). Of the 32 single nucleotide variations, 9 were in the coding exons, 8 in the introns, 2 in the 5′-UTR, 5 in the 3′-UTR, 4 in the 5′-flanking region, and 4 in the 3′-flanking region. One novel microsatellite marker was found in the 5′-flanking region. All 32 single nucleotide variations were in Hardy-Weinberg equilibrium.

In the coding region, we detected the known nonsynonymous SNP 1661G>A (Arg554Lys) with a frequency of 0.444, which was similar to the previously reported frequency of 0.43.[13] This SNP was perfectly linked to IVS7+33G>T. SNP 1661G>A (Arg554Lys) was the most frequent in the coding exons, and the other variations were relatively rare (below 0.03). Of these, four novel nonsynonymous variations, 50A>C (Lys17Thr), 1202A>G (Lys401Arg), 1459A>G (Asn487Asp), and 1541T>C (Ile514Thr) were found. Variation 50A>C (Lys17Thr) was detected in a patient with colon cancer. Variation 1202A>G (Lys401Arg) or 1541T>C (Ile514Thr) was found in a patient with small cell lung cancer. Variation 1459A>G (Asn487Asp) was detected from 2 cancer patients (colon and non-small cell lung cancers) and 3 healthy volunteers. All these novel variations were detected as heterozygotes. The electropherograms of these variations are shown in **Fig. 1**. The allele frequencies were 0.010 for 1459A>G (Asn487Asp) and 0.002 for the other variations. Lys-17 is located within the nuclear localization signal sequence. Though a previous mutational study showed no apparent effect of the Lys17Ala mutation on nuclear localization,[18] the effect of the Lys-to-Thr substitution on receptor localization is unknown. Successive deletions of the C-terminal domain showed that the region containing Lys-401 and Asn-487 was important for both ligand binding and ligand-independent binding to DNA, and also that the region including Ile-514 was required for ligand-dependent binding to DNA.[19] Thus, these amino acid alterations might influence AhR function. Among 242 Japanese samples analyzed, we did not detect the low-activity variation 1708G>A (Val570Ile) found in Africans.[17]

In the 5′-flanking region, $-808\_-800G_9>G_8$ and $-801G>A$ were found in 9 successive G repeats within the GC-rich region, which has been recently characterized as the element important for *AHR* basal expression.[20] Notably, the 8 G repeat was dominant (allele frequency of 0.996) in the Japanese population, and the 10 G repeat, which is dominant in Caucasians (0.741), was not found, while the 9 G repeat is described in the reference sequence NT_007819.14. However, the 8 G and 9 G repeats have been reported not to affect *AHR* mRNA expression levels, compared to the 10 G repeat.[20]

AhR is one of the key regulators for many drug metabolizing enzymes. In addition, this receptor has also been suggested to be involved in the induction of various genes related to cell cycle control.[21] Our findings provide fundamental and useful information for genotyping *AHR* in the Japanese, and could be utilized for haplotype determination.

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Non-surgical treatment for primary gastric lymphoma

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Non-surgical treatment for primary gastric lymphoma

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Recent reports including Japanese studies have demonstrated that conservative treatments could be alternative to surgery in the treatment of primary gastric lymphoma. In patients with high grade lymphoma, CHOP followed by involved field radiation with 40 Gy has shown favorable survival and incidence of critical toxicities such as perforation or hemorrhage is very rare. For low grade MALT lymphoma, most studies resulted in approximately 80% of complete remission by *H. pylori* eradication. Our Japanese studies support the optimal treatment series for MALT lymphoma: primarily eradication, second line radiation therapy to eradication-resistant tumors, and salvage surgery if these conservative treatments fail.

Introduction

Primary gastric lymphoma is a rare tumor, accounting for less than 5% of primary gastric neoplasms. However, it is the most common extranodal lymphoma, representing 4-20% of all extranodal lymphomas and at least 60% of gastrointestinal lymphomas arise in the stomach.[1] The majority of primary gastric lymphoma are B-cell non-Hodgkin's lymphoma, either low-grade mucosa-associated lymphoid tissue (MALT) lymphoma or a high grade, diffuse large cell lymphoma. MALT lymphoma was firstly described in 1983 and usually develop after chronic inflammation induced by *H. pylori* infection.[2, 3] The association between *H. pylori* chronic gastritis and MALT lymphoma has been confirmed in large population based studies where immunological evidences of *H. pylori* infection has been shown to be more in patients with gastric lymphomas than in matched controls.[4,5] This discovery has altered the therapeutic approach to patients with early stage MALT lymphoma: durable complete remissions might be achieved in patients with early stage MALT lymphoma following eradication of *H. pylori*.[6] The relationship between low grade MALT lymphoma and high grade primary gastric lymphoma is still controversial. A close relationship between the two entities is suggested by the frequent presence of foci of both grades of lymphoma in the same lesion and the high frequency of the same cryptogenic abnormality, suggesting this transformation from low to high grade.[7,8] However, their clinical behaviors are quite different: low grade MALT lymphoma presents at an early stage, grow slowly, and remain localized for years, in contrast, high grade lymphoma demonstrates rapid growth and less favorable behavior. Therefore, treatment strategy for these diseases should be distinctively separated.

Importance of staging

Staging is essential for planning the treatment, particularly to rule out secondary involvement of the stomach. A combination of clinical, radiological, and endoscopic procedures are required to define accurately the stage of each patient. CT scan of chest and abdomen colonoscopy, and bone marrow examination are essential to make accurate staging. In addition, examination for Waldeyer's ring involvements is also important, because of their possible association with gastric lymphoma. Endoscopic ultrasonography (EUS) can provide accurate estimation of depth of invasion and regional lymph nodes involvements.

Regarding the staging system of primary gastric lymphoma, the poor applicability of the Ann Arbor system

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Table 1. Modified Blackledge staging system for gastrointestinal lymphoma

Stage	Definition
I	Tumor confined to gastrointestinal tract without serosal penetration
II	Tumor extending into abdomen from primary site: Nodal involvement II_1 Local (gastric / mesenteric) II_2 Distant (paraaortic / paracaval)
II_E	Penetration of serosa to involve adjacent 'structures': Enumerate actual site of involvement, e. g. stage II_E (pancreas), stage II_E (large intestine) perforation / peritonitis
IV	Disseminated extranodal involvement or a gastrointestinal-tract lesion with supradiaphragmatic nodal involvement

into this disease is generally recognized, since the most critical prognostic discrimination pertains to stage II: prognosis between patients with regional (paragastric) lymph nodes and with distant (mesenteric or retroperitoneal) nodes are quite different.[9] Several investigators revised this staging system and modified Musshoff or Blacklede staging system have been the most widely accepted in general use (Table 1).[10, 11]

Treatment for primary gastric lymphoma

Overview of the Western studies

The optimal role of each anticancer modality for treatment of primary gastric lymphoma is still controversial. A review of the literature regarding treatment approaches is difficult to interpret for several reasons. Most of the reports were based on retrospective analysis of single institution experiences, which does not accumulate a sufficient number of cases to draw meaningful conclusions. Additionally, most of the earlier reports included diagnostic issues: concept of MALT tumors has not been considered in many reports, neither the staging nor the histologic classification is uniform across reports, and the widely variable therapeutic options were included. Therefore, these earlier reports analyzed as retrospective manner should be excluded when interpreting the treatment outcomes.

Historically, surgical resection has been the mainstay of treatment for gastric lymphoma. In addition to the favorable survival, surgery was considered to have other several advantages: it can collect definitive tissues for pathologic examination and prevent gastric hemorrhage or perforation which would be complicated in medical treatment for gastric lymphoma. However, it seems that the advances and expertise in endoscopic biopsy techniques and in immunohistopathology have allowed for acceptably more accurate histologic classification. The noninvasive radiologic investigations are providing fairly accurate clinical staging. The main concern with nonsurgical treatment is that chemotherapy and radiotherapy can lead to necrosis of the tumor with resultant gastric perforation or bleeding. In a review of the literature involving 188 patients, Gobbi et al[12] reported an incidence of 3.2% and 2.7% for perforation and bleeding, respectively. In another review of 17 articles, Mittal et al.[13] found three instances of gastric perforation in 75 patients after receiving radiotherapy and 25 instances in 626 patients who did not receive radiotherapy and had perforation on presentation. Based on the results showing similar risk in both groups, it seems that risk of perforation is due to development of the tumor and is not increased by medical treatment.

Recently, a prospective study with large sample size has been reported from Germany.[14] In this study, treatments were divided based on histology (low / high grade) and modality (surgical + conservative / conservative alone). Whether therapy included surgery or conservative management only was left to the discretion of each participating center. In surgical treatment group, patients received primarily surgical resection followed by extended field radiotherapy with 30 Gy for low grade lymphoma or CHOP x 4 + extended field radiotherapy with 40 Gy for stage IE or CHOP x 6 + involved field radiotherapy with 40Gy for stage IIE high grade lymphoma. In conservative treatment group, patients received extended radiotherapy with 40 Gy alone for stage IE or additional COP x 6 for stage IIE low grade lymphoma; CHOP x 4 + extended field radiotherapy with 40 Gy for stage IE or CHOP x 6 + involved field radiotherapy with 40Gy for stage IIE high grade lymphoma. A total of 277 patients were accrued but 185 (low grade MALT 82, high grade 70, high grade with low grade MALT component 31, and mantle cell 2) patients could be analyzed for treatment results. Conservative treatment only was performed in 106 patients, whereas 79 patients were treated with surgical resection in combination with conservative treatment. Although this study was not a randomized comparison, no significant differences in survival between surgical + conservative and conservative treatment alone were observed with 5-year cause specific survival of around 90% (Figure 1). Additionally, only one out of 106 patients

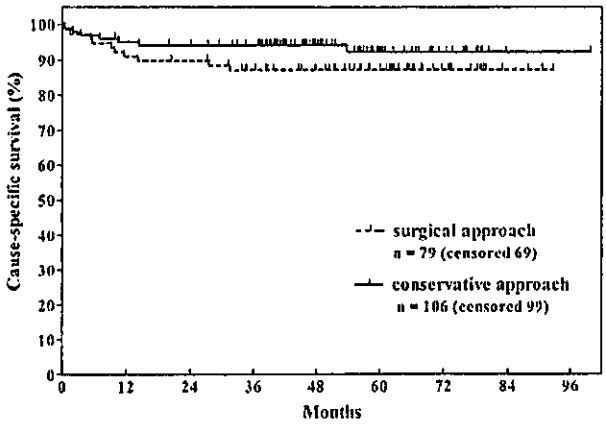

Figure 1. Cause-specific survival of surgical versus conservative treatments for gastric lymphoma in German study

treated with conservative modality alone died of perforation during the chemotherapy. The authors concluded that a stomach-conserving approach may be favored, although the study was not a randomized manner and its end points are uncertain.

Primary treatment for low grade MALT lymphoma has been altered to conservative approach. Numerous reports of the *H. pylori* eradication therapy have shown the efficacy for this disease and this therapy is considered the well accepted initial therapy.[15-18] Although the eradication is accepted to be the initial treatment, there still remain some issues to be elucidated. The treatment of low grade MALT lymphoma with diffuse large component is controversial. Regression of these cases with eradication has been documented in scattered reports but the long-term outcomes are still uncertain. It is also undetermined how to predict patients resistant to eradication, when and what kind of second-line treatment is indicated. Some reports revealed that tumors deeper than submucosa in wall invasion and involvement of perigastric lymph nodes were more resistant to the antibiotic therapy.[19, 20] Molecular markers may be able to predict tumor response: tumors with t(11, 18) rearrangement may represent less likely to result in a complete response.[21] Histological response sometimes does not correlate with macroscopic findings and it is still uncertain whether treatment failure should be based on histological or macroscopic findings. The second-line treatment in patients who do not respond to eradication therapy can be surgery, radiation, and chemotherapy. Gastrectomy is an established and safe treatment in Japan. However, total gastrectomy, which is usually recommended due to multi-focal lesions, would restrict patient's quality of life. Radiation therapy has also shown promising results: one study with radiation alone revealed 100% complete response of MALT lymphoma at a median follow-up time of 27 months.[22] Monochemotherapy with alkylating agents such as chlorambucil or cyclophosphamide has been successfully used in patients who have failed to respond eradication, though the results were immature. [23]

Recently, long-term results of *H. pylori* eradication for low grade MALT lymphoma was reported as a multicenter study in Germany and Austria.[24] In this study, a total of 95 patients were accrued and 90 patients were analyzed for efficacy. With a median follow-up period of 44.6 months, complete regression was achieved in 56 (62%) patients, minimal residual disease, which meant normalization of macroscopic findings but with persistent residual lymphoma infiltrates on histologic examination, in 17 (18%) patients, partial remission in 11 (12%) patients, progressive disease in two (2%) patients. Four patients with complete regression relapsed after 6, 8, 8, and 15 months. Based on the results, this study was concluded that the majority of patients with low grade MALT lymphoma treated by exclusive *H. pylori* eradication have a favorable long term outcomes, offering a real chance of cure. However, precise information was not described; how to evaluate, when and which second-line treatments were indicated. Optimal therapy for low grade MALT with diffuse large component and second-line treatment after failure of eradication for low grade MALT lymphoma still remain uncertain.

Results from Japanese prospective study with discussions

In Japan, two nation-wide multicenter prospective studies for primary gastric lymphomas were conducted: feasibility study for high grade lymphoma and confirmation study for low grade MALT lymphomas.[25, 26] The objective of the first study for high grade lymphoma was to evaluate feasibility, incidence of critical toxicity as a primary end point, of conservative treatment. Eligibility criteria required intermediate to high grade gastric lymphoma, stage I-II_1, no prior therapy, adequate organ function, and written informed consent. Treatment consisted of 3 cycles of CHOP every 3 weeks and followed by radiotherapy for a total dose of 40.5 Gy in 27 fractions over 5.5 weeks (Figure 2). Diagnosis was confirmed by central pathological review in all cases. Fifty-five patients

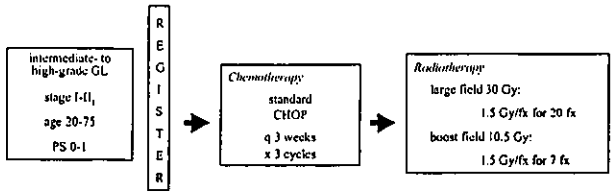

Figure 2. Study schema of the Japanese study for high grade gastric lymphoma

were enrolled during December 1999 and February 2003. Three patients were ineligible and 52 patients were included in this analysis. All patients completed 3 cycles of CHOP and all but one patient, who suffered disease progression, completed planned radiotherapy. There was no serious non-hematological toxicity such as treatment related death or perforation of the stomach. Complete response was observed in 48 of 52 patients (92%, 95% confidence interval: 82%-98%), partial response in 1, and progressive disease in 3. With a median follow-up period of 25 months for surviving patients, the 2-year progression-free survival and overall survival were 88% and 94%, respectively.

The second study was designed to confirm the efficacy and safety of *H. pylori* eradication and second-line radiation therapy in patients failed to respond to eradication for low grade MALT as well as those with diffuse large component. The primary endpoint was failure-free survival (FFS). Eligibility criteria required histologically proven low grade MALT with/without diffuse large component, stage I-II1, no prior therapy, adequate organ function, and written informed consent. Patients received eradication therapy consisting of a PPI-based triple regimen, and RT of 30 Gy in cases of residual or recurrent disease after eradication (Figure. 3). Between 2000 and

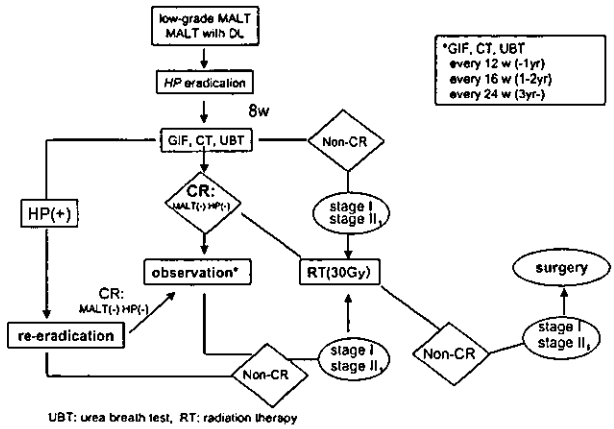

Figure 3. Study schema of the Japanese study for low grade MALT lymphoma of the stomach

2003, 150 patients were registered and 141 were included in the preliminary analysis. Of these, 16 (11%) were ineligible: 14 by central pathological review and two for other reasons. Thus 125 patients including 15 low grade MALT with diffuse large component in histology were evaluated for efficacy and safety. Eradication was administered completely to all patients. Twenty-five patients (20%) underwent second line radiation therapy due to persistent or recurrent disease after eradication and 22 (88%) of these achieved complete remission with radiation therapy. No serious adverse events, such as hemorrhage or perforation of the stomach, were observed. With a median follow-up of 21.5 months, five events were considered "treatment failures": death not related to the treatment, 1; salvage gastrectomy, 1; transformation to diffuse large B-cell lymphoma, 2; and development to stage IV, 1. Two-year failure-free and overall survivals were 97.0%, and 99.2%, respectively.

These Japanese studies as well as European studies demonstrated that these conservative treatments showed possibly comparable survival with those in Japanese surgery[27] and could be alternative to surgery in the treatment of primary gastric lymphoma. In patients with high grade lymphoma, three to four courses of CHOP followed by involved field radiation with a total of 40 Gy have shown favorable and similar survival in both studies. Incidence of critical toxicities such as perforation or massive hemorrhage is very rare: only one patient with perforation among more than 100 patients with high grade lymphoma and more than 200 patients with low grade MALT lymphoma occurred during all studies. For low grade MALT lymphoma, both studies as well as the retrospective analyses resulted in approximately 80% of complete remission by eradication and the remaining 20% would be the candidate of second line therapy. Whether treatment failure was based on histological or macroscopic findings is still uncertain: the Japanese study defined treatment failure when consecutive two times of follow-up biopsy reveled lymphoma cells, while the German study did not clarify the decision making to undergo second line treatment. In the German study, second line treatment was not determined and permitted various modalities such as surgery, radiation, chemotherapy. On the contrary, our Japanese study recommended radiation therapy if the residual or relapsed tumors are still localized. The results seemed to be promising with a complete remission rate of around 90%. Although the number is small, patients with