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ORIGINAL ARTICLE

Phase II study on hepatic arterial infusion chemotherapy using percutaneous catheter placement techniques for liver metastases from colorectal cancer (JFMC28 study)

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Abstract

Aim: This prospective multicenter study aimed to evaluate the efficacy and adverse events of hepatic arterial infusion chemotherapy (HAIC) using percutaneous catheter placement techniques for liver metastases from colorectal cancer (CRC).

Methods: We administered 5-fluorouracil at 1000 mg/m^2 over 5 h via hepatic arterial infusion on a weekly schedule. The primary endpoint was the overall response rate (RR). The secondary endpoints were the overall survival (OS), progression-free survival (PFS) and toxicities.

Results: Between February 2000 and March 2002, seventy-seven eligible patients were enrolled in this study. After a median of 26 treatment cycles, 4 patients achieved a complete response, 29 achieved a partial response, 28 had stable disease, 15 had progressive disease and the status of one patient was unknown. The overall RR was 42.9% and the disease control rate (DCR) was 79.2%. The median PFS and OS times were 203 and 560 days, respectively. The most common grade 3 or 4 hematological and non-hematological toxicities were total bilirubin level elevation (10.4%) and gamma-glutamyl transferase level elevation (10.4%). With regard to the relationship between the background factors and treatment outcomes, the DCR, RR, PFS and OS were different between patients with and without extrahepatic lesions (DCR: 86.5% *vs* 64%, RR: 46.2% *vs* 36.0%, PFS: 233 days *vs* 99 days, OS: 587 days *vs* 558 days).

Conclusion: The primary endpoint of this study was not met. HAIC using percutaneous catheter placement techniques did not improve the RR for liver metastasis from CRC.

Key words: colorectal cancer, hepatic arterial infusion, liver metastases.

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Conflict of interest: None declared

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INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignant disease worldwide.[1,2] Despite screening and early surgery, many patients eventually develop metastatic disease. The liver is the most frequent metastatic site of CRC, and thus managing liver metastasis is critical in the treatment of metastatic CRC.

Hepatic resection is considered the first-line treatment for liver metastasis from CRC.[3] The outcomes of hepatic resection have improved, and previous studies have reported a 5-year overall survival (OS) rate ranging from 43 to 58%. Additionally, the disease-free survival rate has been reported to be approximately 28%.[4,5] However, hepatic resection is only indicated for a limited number of patients, and most patients with liver metastases are treated with chemotherapy.

The efficacy of chemotherapy for CRC was dramatically improved in the 1990s owing to the development of new agents, such as irinotecan (CPT-11), oxaliplatin (L-OHP) and molecular-targeted agents.[6–12] Prior to the existence of these agents, chemotherapy for CRC mainly involved fluoropyrimidines, such as fluorouracil (5-FU), but its efficacy was limited.[13–17] Therefore, hepatic arterial infusion chemotherapy (HAIC), which delivers high drug concentrations to the tumor but results in less systemic toxicity, has been widely employed for the treatment of liver metastases from CRC. However, most randomized controlled trials comparing HAIC and systemic chemotherapy failed to demonstrate a survival benefit with HAIC, and some even reported a lower feasibility with hepatic arterial infusion due to catheter- or pump-related issues.[18,19] In these studies, HAIC was performed using surgical catheter placement techniques via laparotomy under general anesthesia. Accordingly, HAIC has not become a standard treatment for liver metastases from CRC.[19,20]

Percutaneous catheter placement techniques for HAIC were developed in the 1980s, mainly in Japan, and were fully established around 2000.[21–23] The procedure is less invasive than conventional surgical catheter placement because the catheter and port are placed percutaneously using interventional radiology techniques under local anesthesia. Additionally, the drug delivery can be evaluated by digital subtraction angiography (DSA) and computed tomographic angiography (CTA) through the implanted catheter and port system.[24–26] Using this technique for intermittent hepatic arterial infusion of high-dose 5-FU on a weekly schedule, Arai *et al.* reported a response rate (RR) of 78% in 1997.[27] Of note, novel standard systemic chemotherapy regimens such as FOLFIRI and FOLFOX had not been established in Japan at the time of Arai's study.

On the basis of these findings, we conducted a multi-institutional phase II trial to evaluate the efficacy and feasibility of HAIC using percutaneous catheter placement techniques for liver metastases from CRC.

METHODS

Patients

All patients were histologically diagnosed with colorectal adenocarcinoma with liver metastases. The patients' eligibility for surgical resection was determined by imaging studies according to the size and location of the hepatic tumors. Those with extrahepatic metastases were included at the investigators' discretion, provided that the liver was the dominant site of metastasis. The primary colorectal carcinoma had been previously resected in all cases. All patients had bidimensional measurable or assessable disease documented by imaging studies. Adequate hematological (white blood cell [WBC] count > 3000, platelet count > 750 000 and hemoglobin > 8.0 g/dL), liver (serum bilirubin 2 mg/dL) and coagulation (normal prothrombin time and partial thromboplastin time) profiles were required. No patients had received any prior treatment for liver metastasis from CRC before being enrolled in this study. All patients had an Eastern Cooperative Oncology Group performance status of 0–2.

Ethical considerations

The study data and informed consent were obtained in accordance with the Declaration of Helsinki and were approved by the Ethics Review Board of each participating institution. All patients received a written explanation of the study and provided written informed consent before participating.

Procedures and treatment

An indwelling catheter was inserted into the hepatic artery via the subclavian artery using standard interventional radiology techniques. We used the heparinized hydrophilic polymer catheters (Anthron, TORAY, Tokyo, Japan) for 75% of the patients in the present study. The others used another type of catheters. The proximal end of the catheter was connected to an implanted port. The optimal perfusion into the liver was confirmed by CTA, which was performed every 3

months. The details of the treatment procedure have been reported previously.[27] Briefly, patients received HAIC with a 5-h infusion of 1000 mg/m^2 5-FU once a week on an outpatient basis. Such treatment was repeated for as long as possible. Great care was taken to prevent or quickly detect any abnormalities resulting from technical issues such as catheter dislocation, vascular occlusion or inadequate drug distribution, and if necessary, appropriate countermeasures were taken.

Patient follow-up

The patient history was taken and physical and blood examinations were performed before each HAIC cycle. The puncture site was monitored for signs of bleeding, hematoma and infection throughout the procedure and after removal of the indwelling catheter. The National Cancer Institute common toxicity criteria were used to determine whether there was a need for dose modification or treatment discontinuation. In cases with a grade 2 WBC or platelet count decrease, or any grade 3 or 4 toxicity, the treatment was discontinued until full recovery, and the dose was reduced by 25% in the following cycle. If toxicity persisted, an additional 25% dose reduction was made when therapy resumed.

Treatment evaluation

The tumor response was assessed and evaluated according to the World Health Organization criteria.[28] The RR was defined as the combined proportion of complete response (CR) and partial response (PR), whereas the disease control rate (DCR) was the combined proportion of CR, PR and stable disease (SD) among all evaluable patients. Among the responders, relapse was defined as the appearance of new lesions or progression from the response at the time of maximum regression. The duration of the response was defined as the period from the first observation of the response to the time of documented relapse.

Statistical analyses and sample size

The primary endpoint of this study was the RR, whereas the secondary endpoints were the OS, progression-free survival (PFS) and safety. Although the RR for HAIC varied in previous reports, we aimed to achieve a 70% RR after reviewing the previously published data.[27] The required sample size to detect a difference between a threshold overall RR of 50% and a target overall RR of 70% using a one-sided binomial test with an alpha error of 2.5% and a statistical power of 90% was 65 patients.

Table 1 Patient characteristics

	Number ($N = 77$)	%
Age (years)		
Median	62	
Range	24–81	
Sex		
Male	49	63.6
Female	28	36.4
Primary site		
Colon	53	68.8
Rectum	24	31.2
Liver metastases		
Synchronous	53	68.8
Metachronous	24	31.2
Extrahepatic metastases		
Yes	25	32.5
No	52	67.5
ECOG performance status		
0	57	74.0
1	18	23.4
2	2	2.6
Prior chemotherapy		
Yes	10	13.0
No	67	87.0

ECOG, Eastern Cooperative Oncology Group.

To account for potential dropouts, the number of patients to be accrued was set at 80. The OS and PFS were calculated using the Kaplan–Meier method. A two-sided value of $P < 0.05$ was considered to be statistically significant. The OS was calculated from the date of enrollment to that of death or final follow-up. The PFS was calculated from the date of enrollment to that of disease progression, death or the final follow-up. All analyses were performed using the SAS 9.3. Software program (SAS Institute Japan Ltd., Tokyo, Japan).

RESULTS

Patients

A total of 77 patients were enrolled on the protocol between February 2000 and March 2002. All patients were evaluable regarding the treatment efficacy and safety. The clinical characteristics of all eligible patients are summarized in Table 1. The median age was 62 years (range, 24–81 years), with a male-to-female ratio of 49:28. The primary cancer site was the colon in 53 patients and the rectum in 24. Twenty-four patients presented with metachronous liver metastases, and eight

Table 2 Response to treatment ($n = 77$)

Type of response	No. of patients	Percentage (%, 95% CI)
Complete	4	5.2
Partial	29	37.7
Stable	28	36.4
Progression of disease	15	19.5
Unknown	1	1.3
Response rate	33	42.9 (31.8–53.9)

of these patients had received prior chemotherapy. The remaining 53 patients had synchronous liver disease at the time of their colon cancer diagnosis.

Extrahepatic metastases were present in 25 (32.5%) of the 77 patients, but the liver was the predominant site of metastatic disease in this group. The Eastern Cooperative Oncology Group performance status was 0 in 57 patients (74.0%), 1 in 18 patients (23.4%), and 2 in 2 patients (2.6%).

Treatment response

The overall RR was 42.9% (95% confidence interval [CI], 31.8–53.9%) after a median of 26 (range, 2–84) cycles of treatment. The null hypothesis could not be rejected on the basis of the one-sided binomial test ($P = 0.915$). Four patients achieved a CR, 29 achieved a PR, 28 had SD, 15 had progressive disease, and the status of one patient was unknown (Table 2). The DCR was 79.2%.

With regard to the relationship between the background factors and tumor response, the DCR was significantly different ($P = 0.023$) between the patients without extrahepatic lesions (45 of 52 patients; 86.5%) and those with extrahepatic lesions (16 of 25 patients; 64.0%). Furthermore, although there was no statistically significant difference, the RR was higher in patients without extrahepatic lesions than in those with extrahepatic lesions (46.2% *vs* 36.0%, $P = 0.400$). On the other hand, the RR for the liver metastasis alone was 64.0% (16 of 25) in the patients who had extrahepatic metastasis and 63.5% (33 of 52) in those who did not have extrahepatic metastasis. These values were not significantly different between the two groups ($P = 0.786$).

PFS and OS

The median PFS was 204 days (95% CI, 163–238 days). The median OS was 561 days (95% CI, 493–646 days). The median PFS was significantly different between the patients with and without extrahepatic lesions (99 days *vs* 233 days; log-rank $P = 0.004$, Fig. 1). The median OS was 587 days in patients without extrahepatic lesions and 558 days in those with extrahepatic lesions (log-rank $P = 0.163$, Fig. 2).

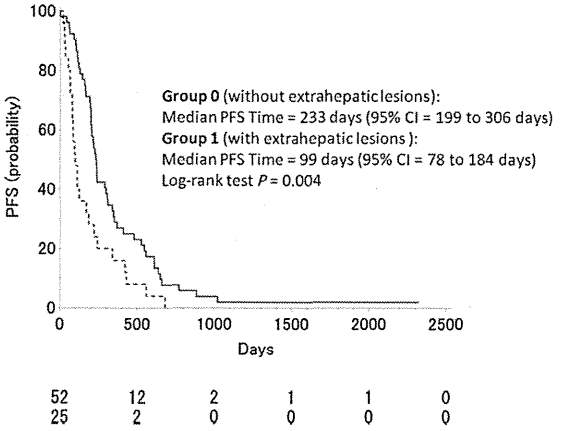

Figure 1 The progression-free survival of patients with and without extrahepatic lesions.

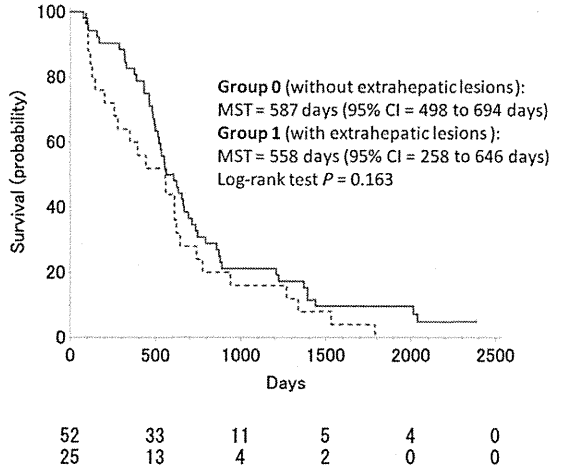

Figure 2 The overall survival of patients with and without extrahepatic lesions.

Treatment exposure and safety

The median number of cycles for hepatic arterial infusion treatment was 26 (range, 2–84). The median 5-FU dose was 37.5 g (range, 3–143 g). All 77 patients were evaluable for the safety of the treatment. To decrease toxicity, the 5-FU dose was reduced at least once in 27 patients. The toxicities observed are summarized in

Table 3 Hematological and biochemical toxicities observed during treatment

	All grades				Grade 3/4 (%)
	1	2	3	4	
Hematological toxicity					
Leucopenia	1	2	2	0	2.6
Neutropenia	1	1	1	0	1.3
Hemoglobin	12	3	0	1	1.3
Platelet	11	5	5	0	6.5
Bilirubin elevation	13	1	7	1	10.4
AST elevation	12	2	2	0	2.6
ALT elevation	6	1	3	0	3.9
γGTP elevation	7	3	8	0	10.4
Non-hematological toxicity					
Anorexia	4	7	1	0	1.3
Nausea	8	7	0	0	0
Vomiting	3	3	0	0	0
Fatigue	3	4	0	0	0
Constipation	0	1	1	0	1.3
Fever	3	5	0	0	0
Gastric ulcer	0	2	1	0	1.3
Duodenal ulcer	0	2	1	0	1.3

ALT, alanine aminotransferase; AST: aspartate aminotransferase; γGTP, γ-glutamyl transpeptidase.

Table 3. The most common grade 3 or 4 hematological toxicities were total bilirubin level elevation (10.4%) and gamma-glutamyl transferase level elevation (10.4%). However, non-hematological toxicities of grade 3 or higher were rare and included anorexia (1.3%) and gastric ulcers (1.3%). No treatment-related death was observed. The overall catheter-related complication rate was 19%. Early complications were more likely to involve inadequate drug distribution, as observed on CTA, and were frequently corrected by additional angiographic interventions. Complications that occurred more than 3 months after catheter and port placement were more likely to be catheter occlusions or arterial thrombosis. The late complications were less likely to be salvaged (30%) compared with those occurring early (70%). We used the heparinized hydrophilic polymer catheters (Anthron, TORAY) for 75% of the patients in the present study. We used another type of catheter in the others. However, there were no significant differences between the two groups in the rate of infusion- and catheter-related complications.

The most common causes of treatment discontinuation were disease progression (39/77 [50.7%]), catheter/procedure-related complications (12/77 [15.6%]) and patient refusal to continue treatment (4/77 [5.2%]).

Clinical course after HAIC treatment

After HAIC treatment failure, six patients underwent liver resection (7.8%). We evaluated their prognosis compared with patients who did not undergo liver resection. Five of these six patients and 69 of the 70 patients died. The median OS was 1418 days in patients who underwent liver resection and 555 days in those who did not (log-rank $P = 0.0023$). However, there were no standard criteria for resectability, and the choice to perform resection was left to the physician's discretion.

Forty-three patients received systemic chemotherapy. Among them, 10 patients received systemic chemotherapy and interventional radiology, 5 patients received systemic chemotherapy and radiation therapy, and 2 patients received systemic chemotherapy, interventional radiology and radiation therapy. Twenty-two patients received interventional radiology, radiation therapy, and combination interventional radiology and radiation therapy. Twelve patients did not receive any treatment after HAIC. When comparing the OS between the patients who received salvage systemic therapy and those who did not, there was a statistically significant difference ($P = 0.424$).

DISCUSSION

To the best of our knowledge, this trial is the first multi-institutional phase II study to evaluate the safety and efficacy of HAIC using percutaneous catheter and port placement techniques for treating liver metastases from CRC. Our primary objective was to confirm a RR of over 70%, which was previously reported in a single-institutional study.[27] However, the RR of HAIC in our study was lower than expected at 42.9%, and our statistical hypothesis in this phase II study was not met.

In this trial, we hypothesized that: (i) catheter-associated complications could be decreased by using percutaneous catheter and port placement techniques; (ii) patients could start HAIC without enduring a performance status decline due to surgical procedures; (iii) adequate HAIC could be repeated, with drug distribution evaluated by DSA and CTA; and (iv) better clinical outcomes of HAIC could therefore be achieved. The first possible explanation for this trial not meeting its primary endpoint is that HAIC might not substantially increase the RR compared with intravenous therapy. Theoretically, HAIC has several advantages over intravenous chemotherapy. For example, chemotherapeutic agents can be delivered more specifically to malignant cells. Normal hepatocytes that mostly rely on the portal venous system are thus exposed to fewer chemothera-

peutic agents. However, many chemotherapy agents used in HAIC have high first-pass hepatic clearance effect, such as 5-FU and floxuridine (FUDR), a prodrug of 5-FU. Over 90% of FUDR and 19–50% of 5-FU are cleared by the liver when they are administered by HAIC.[29] The second possible explanation is the heterogeneity in the level of expertise when performing percutaneous catheter and port placement among the participating institutions. To realize the theoretical benefits of HAIC, optimal drug distribution is critical, which means that the administered drug should be distributed to all intra-hepatic tumors, but not to any extrahepatic organs. Such drug distribution requires various and precise interventional radiology techniques. Furthermore, the procedural skill levels might have differed between this study and the above-mentioned single-institution study that reported better results.[27] In this study, we attempted to evaluate the patency of the hepatic artery and the position of the indwelling catheter every 3 months; however, catheter-related complications were observed in 19% of all patients, and 15.6% of the patients could not continue their treatment due to such complications. Similar rates of catheter-related complications were reported in HAIC performed using surgical procedures.[30–32] Scaife *et al.* reported an overall catheter- and pump-related complication rate of 16% in patients receiving HAIC between 1996 and 2001,[33] whereas Allen *et al.* reported that this rate was 22% in patients treated with a pump between 1986 and 2001.[34] Therefore, the percutaneous catheter and port placement techniques might not have succeeded in reducing catheter-related complications. However, the effects of the operators' skill level on the high incidence of catheter-related complications observed in our study cannot be ruled out. Of note, Campbell *et al.* found that a lack of surgical experience was associated with pump-related complications. The complication rate was 7% for surgeons who had placed more than 10 pumps, whereas it was 37% for surgeons who had placed fewer than 10 pumps.[30] Allen *et al.* also found that the complication rate was lower (19%) for surgeons who had placed more than 25 pumps, whereas this rate was higher (31%) for surgeons who had placed fewer than 25 pumps.[34]

In our study, patients were enrolled from nine different institutions. Arai *et al.* reported a RR of 72% for HAIC combined with systemic CPT-11 by percutaneous catheter placement in a multi-institutional study[35]; however, the above-mentioned study was conducted by well-experienced interventional radiologists at a limited number of institutions. Therefore, various technical factors, such as the operators' skills and/or experience levels, likely contributed to the catheter-associated complications. Moreover, we do not know the learning curve for the generalizability of these technical factors. This is one of the limitations of our study, and further studies should focus on this issue.

Another possible reason for our study's failing to meet its primary endpoint was the patients' background in terms of the presence or absence of extrahepatic lesions on the initial diagnostic images. Similar results were reported in previous studies. Arai *et al.* conducted phase I and II studies to examine the usefulness of HAIC in patients with liver metastasis from CRC, and found that the OS was 25.9 months in patients without extrahepatic lesions compared with 17.3 months in those with extrahepatic lesions.[27] However, the background characteristics between the two patient groups were statistically different in terms of their prior treatment with chemotherapy (20% [10/50] *vs* 0% [0/27], $P = 0.010$), their carcinoembryonic antigen levels (56.5 *vs* 143.6 ng/mL, $P = 0.020$) and their cancer antigen 19-9 levels (93.9 *vs* 409 ng/mL, $P = 0.056$). These background differences might have indicated that the condition of patients with extrahepatic lesions was more severe than that of patients without extrahepatic lesions. In addition, the RR for the liver metastasis only was similar between the patients who had extrahepatic metastasis and those who did not. On the other hand, the relationship between background factors and the tumor response, the DCR, was significantly different between the patients without extrahepatic lesions and those with extrahepatic lesions. Furthermore, although there was no statistically significant difference, the RR was higher in patients without extrahepatic lesions than in those with extrahepatic lesions. Therefore, the inclusion of patients with extrahepatic metastasis might have diluted the overall benefit of HAIC. Thus, it might be possible that HAIC would have been more effective in patients without extrahepatic lesions on diagnostic imaging studies at the time of treatment initiation. However, such a possibility remains unclear based on the present evidence.

Nonetheless, this trial demonstrated that HAIC with a percutaneous approach did not substantially increase the safety of HAIC. The safety profile of percutaneous HAIC in this study was consistent with that of previous reports.[27] No other complications were observed. Therefore, HAIC using percutaneous catheter and port system placement techniques is safe and feasible for liver metastases from CRC, but is not superior to treatment using a catheter placed using conventional surgical techniques.

In conclusion, HAIC using percutaneous catheter placement techniques did not improve the RR for liver metastasis from CRC, probably because these techniques could not reduce catheter-related complications in a multi-institutional setting. However, a difference in treatment outcomes was observed between patients with and without extrahepatic lesions on diagnostic images at the time of treatment initiation. In this regard, HAIC might provide a much better local disease control for patients without initial extrahepatic lesions. Therefore, percutaneous catheter placement techniques were feasible, but future studies on HAIC should focus on liver metastases from CRC in patients without extrahepatic lesions.

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RESEARCH ARTICLE

Prevalence of Esophageal Cancer in the Northern Part of Afghanistan

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Abstract

Background: Esophagogastroduodenoscopy (EGD) is the standard technique for diagnosis of patients presenting with upper gastrointestinal symptoms. Some reports have shown high prevalence of esophageal cancer in the northern part of Afghanistan. The aim of this study was to investigate epidemiological profile of esophageal cancer among patients in this region. Materials and Methods: We identified 364 consecutive patients that received EGD examinations to examine upper gastrointestinal tract at the endoscopy unit of Balkh regional Hospital from March 2012 to March 2013. The case subjects included both in-patients and out-patients aged 16 years or more. We evaluated the results retrospectively. Results: The cases consisted of 184 (51%) males and 180 (49%) females. The mean age was 47.3±17.8 and the age range 17-88 years. Ninety two cases had esophageal cancer, out of which 58 (63.0%) were male. The mean age at time of diagnosis was 57.8±13.2 years. Uzbek-Turkmen peoples were more common among patients with esophageal cancer (52.2%). Dysphagia was the most frequent symptom among patients with esophageal cancer at the time of presentation, seen in 77 (84.8%) of cases. Conclusions: Our results showed high incidence of esophageal cancer in the northern part of Afghanistan, especially in the Uzbek-Turkmen ethnic group.

Keywords: Esophagus cancer - endoscopy - northern part of Afghanistan - ethnicity

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Introduction

Esophagogastroduodenoscopy (EGD) is a standard and established upper gastrointestinal endoscopy technique that visually examines lining of gastrointestinal tract from esophagus through stomach, duodenal bulb and descending small intestine (Nguyen et al., 2010). EGD has become more and more popular in the past 40 years with increasing frequency for examination of the upper gastrointestinal symptoms (McColl et al., 2002; Chan et al., 2004). The aim of EGD is to help both physician and patient to evaluate and to understand clinical symptoms besides acting as a tool to prove the presence of peptic ulcer or tumors (Maaroos et al., 2004).

There are a lot of differences in distribution of gastrointestinal diseases among different population. Esophageal cancer shows a specific geographical distribution that distinguishes it from other malignancies. Very high rates of esophageal cancer have been reported in several other areas of Central and East Asia including the northern part of Afghanistan (Mir et al., 2009). Several statistics have shown that esophageal cancer is one of the 10 most common cancers, and sixth most common cause of cancer deaths worldwide (Ghavamzadah et al., 2001; Rasouli et al., 2011). It is the fourth most common gastrointestinal cancer after gastric, colorectal and hepatocellular cancers (Mashhadi et al., 2011). Squamous cell carcinoma of esophagus is a common type of esophageal tumor (Amini et al., 2014); of which more than 80% of cases and deaths occur in developing countries (Igissinov et al., 2012; Kiadaliri, 2014). This is a unique epidemiological feature that differentiated it from other cancer types. The contrast variation of 3/100,000 per year in western countries and 140/100,000 per year in Central Asia (Alidina et al., 2004) is a remarkable need for more researches to identify factors of the high incidence rate. Esophageal cancer stretches eastward through Asian Esophagus Cancer Belt in the region of Central Asia, from Iran through Turkmenistan, Northern Afghanistan, Uzbekistan, and Kazakhstan to Northern China (Mir et al.,

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2009; Igissinov et al., 2013). Afghanistan, especially the northern region falls under the Asian Esophagus Cancer Belt and is well known with high incidence rate of the disease (Sobin, 1969; Ghazzawi et al., 2004).

Some studies showed high prevalence of esophageal cancer in the northern part of Afghanistan. However, there has been still limited data; the purpose of this study was to perform an additional investigation to determine the epidemiology profile of esophageal cancer in the region.

Materials and Methods

Three hundred sixty four consecutive patients were included in this study who received EGD examination at Balkh regional hospital from March 2012 to March 2013. The case subjects included both inpatient and outpatient of age 16 years or more.

The examinations were performed with pharyngeal spray lignocaine as local anesthesia. The procedures were done with a fibro-optic endoscope without sedation in dedicated endoscopy unit. The endoscopic facility served both inpatient and outpatients. The study was endoscopy-based diagnostic methods and is considered the gold standard in diagnosing esophageal cancer. Demographic characteristics, clinical presentations, and endoscopic findings were examined.

Data are presented as mean±standard deviation. Categorical variable are expressed as count and percentages. Continuous data were compared by using unpaired t test. Categorical data were compared by means of X^2 test. p value<0.05 was considered statistically significant. All analyses were performed using the SPSS 18.0 software package (SPSS, Chicago, IL). The study was approved by the scientific review committee of Balkh regional hospital.

Results

Baseline clinical characteristics in all patients are shown in Table 1. Of these patients, 184 were male (51%) and 180 were female (49%). The mean age of the patients was 47.3±17.8 and the age range 17-88 years. Two hundred forty patients (66%) were out-patient and 124 patients were in-patient (34%).

The main clinical presentations were dysphagia in 77 patients (21%), epigastric pain in 69 patients (19%), and chest pain in 52 patients (14%).

Table 1. Demographic Characteristics of All Patients

Mean age±SD	47.3±17.8		
Age range	17-88		
Gender Male, n (%)	184	51%	
Female, n (%)	180	49%	
Ethnicities			
Uzbeks-Turkmen, n (%)	111		30%
Tajiks, n (%)	154		42%
Pashtoons, n (%)	46		13%
Hazaras, n (%)	53		15%
Outpatient *vs* Inpatient			
Outpatient, n (%)	240		66%
Inpatient, n (%)	124		34%
Main Clinical Presentations			
Dysphagia	77		21%
Epigastric pain	69		19%
Chest pain	52		14%
Melana	45		12%
Anemia	31		9%
Nausea	32		9%
Vomiting	23		6%
Heart burn	16		4%
Weight loss	10		3%
Hematemesis	9		2%

*n=364

Table 2. Main Endoscopic Findings

Endoscopic findings	n=364	%
1-Normal	51	14.00%
2-Esophageal Cancer	92	25.30%
3-Gastric Cancer	13	3.60%
4-Duodenal Cancer	2	0.50%
5-Other benign findings	206	56.70%

The EGD findings are shown in Table 2. A total of 92 cases had esophageal cancer (25%), gastri and duodenal cancerws also being found.

Table 3 shows the comparison between the patients with esophageal cancer and without esophageal cancer. The patients with esophageal cancer were older than those without esophageal cancer (57.8±13.2 *vs* 43.7±17.7, p<0.01), the rate of males was higher (63% *vs* 43%, p=0.006). The Uzbek-Turkmen was more common among the patients with esophageal cancer (48/92:52.2%). Dysphagia was the most frequent symptom among patients with esophageal cancer at time of presentation, seen in 77 (84.8%) of cases.

Table 3. Comparison between the Patients with and without Esophageal Cancer

		Esophageal Ca (+) n=92	Esophageal Ca (-) n=272	p-value
Age, y	57.8±13.2	43.7±17.7	<0.001	
Male, n (%)	58 (63.0%)	126 (46.3%)	0.006	
Ethnicity, n (%)	Uzbeks-Turkmen	48 (52.2%)	63 (23.2%)	<0.001
	Tajiks	25 (27.2%)	129 (47.4%)	
	Pashtoons	7 (7.6%)	39 (14.3%)	
	Hazaras	12 -13.00%	41 (15.1%)	
Main clinical presentation, n (%)	Dysphagia	77 (83.7%)	0 (0%)	<0.001
	Weight loss	8 (8.7%)	2 (0.7%)	<0.001
	Outpatient, n (%)	53 (57.6%)	187(68.8%)	0.051

Discussion

The high incidence rates of esophageal cancer were observed among patient in this region especially in the Uzbek-Turkmen and predominantly with symptom of dysphagia. Previous study of esophageal cancer in Jordanian field hospital in the northern part of Afghanistan has reported similar finding (Ghazzawi et al., 2004). However, the incidence of esophageal cancer is low in western countries compared to Asian countries (Bloomfled et al., 2006). The high rates of esophageal cancer in the northern part of Afghanistan reflected that this area lies in Central Asian Esophageal Cancer Belt (Mir et al., 2009; Mansour-Ghanaei et al., 2012). This shows the variable geographic distribution of esophageal cancer which might due to many known and unknown environmental and ethnic reasons.

The esophageal cancer male to female ratio found in this study was 1.8:1, which is similar to the ratio reported in several studies done in north and northeast of Iran (Ghavamzadeh et al., 2001), Kazakhstan, Turkmenistan, and Uzbekistan (Melhado et al., 2010). However, a study found conversely in western countries where the rate is several times higher in male than female (Islami et al., 2004). Despite the common presumption that smoking and alcohol intake are the main risk factors of esophageal cancer in some areas, this disease displayed a unique epidemiological feature whereby female has the similar risk as male. This pattern was also observed in Linxian, China (Islami et al., 2009).

In our study, Uzbek-Turkmen ethnic group has the highest incidence of esophagus cancer. Insight to the custom of Uzbek-Turkmen people in northern part of Afghanistan, they like to drink tea at rather high temperature and eat solid food together with boiling fluids (Sobin,1969). Islami et al. (2009) suggested that the common hot beverages or fluids consumption in both sexes may have a role in carcinogenesis of esophageal cancer. Correlation between drinking hot beverages and risk of esophageal cancer has been demonstrated in many studies from different part of the world (De Jong et al., 1974; Launoy et al., 1997; Castellsague et al., 2000; Mir et al., 2009; Rasouli et al., 2011). It is believed that this long lasting and spontaneous thermal irritation is likely to damage the esophagus and may facilitate carcinogen through the esophagus lining (Sadjadi et al., 2010). Moreover, phenol content in the tea was reported to have cancer-promoting effects (Kaiser, 1967).

A study done in the northern part of Afghanistan showed high esophageal cancer incidence rate in the Uzbek-Turkmen ethnicity, outlying other ethnicities (Sobin, 1969). This ethnic group and geographical distribution agrees with our observations from this study and other studies of neighboring Esophagus Cancer Belt countries including Turkmenistan, Uzbekistan, Karakalpakstan and Kazakhstan (Saenko et al., 1975; Zaridze et al., 1978; Kairakbaev et al., 1992). Nevertheless, Turkmen ethnicity who resides adjacent to Afghanistan and Turkmenistan was found to have the highest esophageal cancer risk in northeastern part of Iran (Kamangar et al., 2007).

Besides smoking and alcohol drinking, environmental and genetic factors are also presumed to play a role in the carcinogenesis of esophageal cancer, particularly for squamous cell carcinoma of the esophagus (Kamanger et al., 2009). In some studies, it is advocated that genetic factors have higher influence in changing the vulnerability to endogenous and exogenous carcinogenic factors, as compared to widely variable lifestyle and habits (Wang et al., 2007; Ganesh et al., 2009). Whereas, the environmental carcinogens link to genetic factors through irreversible changes and trigger mutations which lead to cancer development (Bartsch et al., 2000; Hecht et al., 2001). Nonetheless, interactions between environmental and genetic factors have been suggested as subjective to individual, whereby genetically susceptible individual would be at higher risk than less-susceptible individual when exposed to moderate intensities of environmental risk factors (Marjani et al., 2010). Studies in the region from China through Central Asian republics toward northeastern part of Iran found positive relationship between genetic factors and the prevalence of esophageal cancer and especially high incidence rates were reported among population with the so-called "Mongolian phenotype" genes type (Kamangar et al., 2007). Although no study on association of high-penetrance germline mutations with SCCE risk at time, somatic mutation in TP53 and other tumor suppressor genes have been extensively studied in SCCE tumors. The study of 98 ESCC tumor samples obtained from hospitals in Tehran that found TP53 mutations among half of the tumors. (Sephehr et al., 2001).

According to the result of our study, the median age at diagnosis of esophageal cancer was 60 years (range 17-88). The median age at presentation of esophageal cancer is 72 years in Scotland (Alidina et al., 2004). Therefore, this cancer is a disease of the younger age group in the northern part of Afghanistan.

In our study, dysphagia was the most frequent symptom observed among patients with esophageal cancer at the time of examination .This could be justified by locally advanced esophageal cancer where the esophagus obstructed by the tumor and causes progressive solid food dysphagia which often accompanied by weight loss.

Limitations identified in this study are the retrospective review protocol of the medical records. At time, there were no stratified ethnic groups and residential regions cancer registration available to investigate this issue. Furthermore, the diagnosis of esophageal cancer was completely based on endoscopy finding because the histopathological examination was inaccessible in this region. Moreover, there were the lack of data on laboratory examination, and several established risk factors including chewing a mixture of tobacco and lime is common habit throughout the country in both sexes and one associated with high level of esophageal cancer in some other countries. Also the sample size was a relatively small. However, there are limited data about the esophageal cancer among the Afghan population living in the northern part, and our results would be of benefit to accumulate data regarding this issue.

We concluded that our results confirmed the high incidence of esophageal cancers in the northern part

of Afghanistan, especially in Uzbek-Turkmen ethnic group. Therefore, more frequent EGD screening might be imperative for early detection of esophageal cancer especially in those high risk population.

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Original Article

Efficacy of Molecular Response at 1 or 3 Months after the Initiation of Dasatinib Treatment Can Predict an Improved Response to Dasatinib in Imatinib-Resistant or Imatinib-Intolerant Japanese Patients with Chronic Myelogenous Leukemia during the Chronic Phase

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Dasatinib is a BCR-ABL kinase inhibitor with improved potency compared with imatinib, for which efficacy and safety in imatinib-resistant and imatinib-intolerant patients with chronic myelogenous leukemia (CML) have been established. Here, an open-label phase II study evaluated the efficacy and safety of dasatinib in 50 Japanese patients with imatinib-resistant or imatinib-intolerant CML during the chronic phase (CML-CP). Dasatinib was effective in imatinib-resistant and imatinib-intolerant patients. After 12 months of dasatinib therapy, 35 patients (70%) had achieved a major molecular response (MMR) and 16 patients (32%) had achieved a complete molecular response (CMR). Among the imatinib-resistant CML-CP cohort, 21 and 8 patients had achieved an MMR and a CMR after 12 months of dasatinib therapy, respectively. Among the imatinib-intolerant CML-CP cohort, 14 and 8 patients had achieved an MMR and a CMR after 12 months of dasatinib therapy, respectively. After 18 months of dasatinib therapy, 38 out of 50 patients (76.0%) had achieved an MMR and 19 patients (38.0%) had achieved a CMR. A lower level of *BCR-ABL* transcript at 1 or 3 months after the initiation of dasatinib treatment was more strongly correlated with the *BCR-ABL* transcript level at 12 and 18 months ($p < 0.001$) than a higher level of *BCR-ABL*. The *T315I* mutation was identified in two patients receiving dasatinib therapy. Dasatinib was generally well tolerated, with only 3 patients (5%) having treatment discontinuation as a result of adverse hematologic events (thrombocytopenia, anemia, neutropenia) and/or non-hematologic events at a 12-month follow-up evaluation. Dasatinib was a safe and effective treatment for Japanese patients with imatinib-resistant or imatinib-intolerant CML. In addition, the molecular response at 1 or 3 months predicted a response to dasatinib at 12 or 18 months. [*J Clin Exp Hematop 54(3) : 197-204, 2014*]

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INTRODUCTION

Chronic myeloid leukemia (CML) is caused by abnormalities in hematopoietic stem cells resulting in the uncontrolled proliferation of cells originating from the bone marrow. The BCR-ABL fusion protein produced by the Philadelphia chromosome (Ph) is a major molecular cause of CML.[1]

Imatinib is a selective BCR-ABL inhibitor that is effective against CML.[2-4] However, resistance to imatinib gradually develops in many patients with CML who are treated with imatinib, and 31% of these patients discontinue imatinib treatment within 5 years because of insufficient responses or unacceptable toxicity.[5] As major factors responsible for the development of resistance to imatinib, numerous point mutations in *BCR-ABL* have been reported.[6-8] Additional factors including BCR-ABL gene amplification,[6,9] excretion of the drug through a P-glycoprotein efflux pump,[10,11] and the activation of a signal transduction pathway for SRC family kinase and other signals[12,13] have also been implicated in the development of resistance. In this regard, the development of new treatments is needed for patients with insufficient responses to imatinib and in whom imatinib cannot be continued at an effective dose because of toxicity.

Dasatinib (BMS-354825) is an oral tyrosine kinase inhibitor that exerts inhibitory activity against BCR-ABL and SRC family kinase. It binds in vitro to both active and inactive BCR-ABL and is 325 times more potent than imatinib and 16 times more potent than nilotinib against wild-type BCR-ABL-expressing cells.[14] Five phase 2 studies, collectively known as START (SRC/ABL Tyrosine kinase inhibition Activity Research Trials of dasatinib), have demonstrated that dasatinib is safe and can elicit a hematologic and cytogenetic response in patients with imatinib-resistant or imatinib-intolerant CML or Ph-positive acute lymphocytic leukemia.[15-18] Dasatinib was shown to be highly effective, with 91% of patients in the chronic phase of CML (CML-CP) exhibiting a complete hematologic response and 62% exhibiting a major cytogenetic response. The efficacy of dasatinib for CML-CP was durable, and the rate of a major cytogenetic response was 88%; the progression-free survival rate was 80% and the overall survival rate was 94% at a 2-year follow-up.[19] However, clinical data for second-line dasatinib therapy are lacking for imatinib-resistant and imatinib-intolerant CML-CP in Japan.

In the present study, we conducted an open-label phase 2 study of dasatinib in Japanese patients with imatinib-resistant or imatinib-intolerant CML-CP. To determine the molecular responses to dasatinib, *BCR-ABL* transcripts in the peripheral blood were evaluated using quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR) at baseline and every month during the study; the results were then compared with the levels of total *ABL* transcripts.

PATIENTS AND METHODS

Patients and treatment

This study was a phase II analysis of a clinical trial conducted by the Kanto CML Study Group (registered at http://clinicaltrials.gov as NCT00866736). The study was conducted in accordance with the Declaration of Helsinki. The protocol was reviewed and approved by a recognized ethics review committee in each institution that participated in this study. Informed consent was obtained from all patients before entry into this study.

Japanese patients with CML-CP who were resistant or intolerant to first-line imatinib therapy participated in a prospective phase II study in order to evaluate the efficacy and safety of second-line dasatinib therapy. Patients who were at least 15 years of age were eligible for entry if they had imatinib-resistant or imatinib-intolerant CML-CP. The chronic phase of CML was defined as less than 15% blasts in the peripheral blood and bone marrow, less than 20% basophils in the peripheral blood, less than 30% blasts plus promyelocytes in the peripheral blood and bone marrow, a platelet count of at least 100×10^9/L unless thrombocytopenia was caused by recent therapy, and no extramedullary involvement other than in the liver or spleen. Patients with prior accelerated-phase or blast crisis CML were not eligible for inclusion in the trial.

Imatinib resistance was defined as the lack of a partial cytogenetic response after 3 months of imatinib treatment, the lack of a complete cytogenetic response (CCyR, 0.1-1.0% of *BCR-ABL/ABL*) after 6 months of treatment, or the lack of a major molecular response (MMR, less than 0.1% of *BCR-ABL/ABL*) after 12 months of treatment. Imatinib intolerance was defined as a non-hematologic toxicity of at least grade 2 or a hematologic toxicity of grade 3 or 4 persisting for more than 7 days in response to treatment with imatinib at a dose of 400 mg or more.

Patients with *BCR-ABL* mutations, which are associated with dasatinib resistance,[14] were excluded from the analysis. None of the patients exhibited neutropenia (< 1,000/μL) at the start of dasatinib treatment.

The real-time quantitative reverse transcription-polymerase chain reaction (RQ-PCR) analysis was performed by Bio Medical Laboratories (BML, Inc., Tokyo, Japan). Thus, the level of *BCR-ABL* transcripts is shown using conversion factor (CF).[20] Specifically, the level of measured *BCR-ABL* transcripts was multiplied by CF and the level of international-scale *BCR-ABL* transcripts is shown in this manuscript.[20,21] A complete molecular response (CMR) was

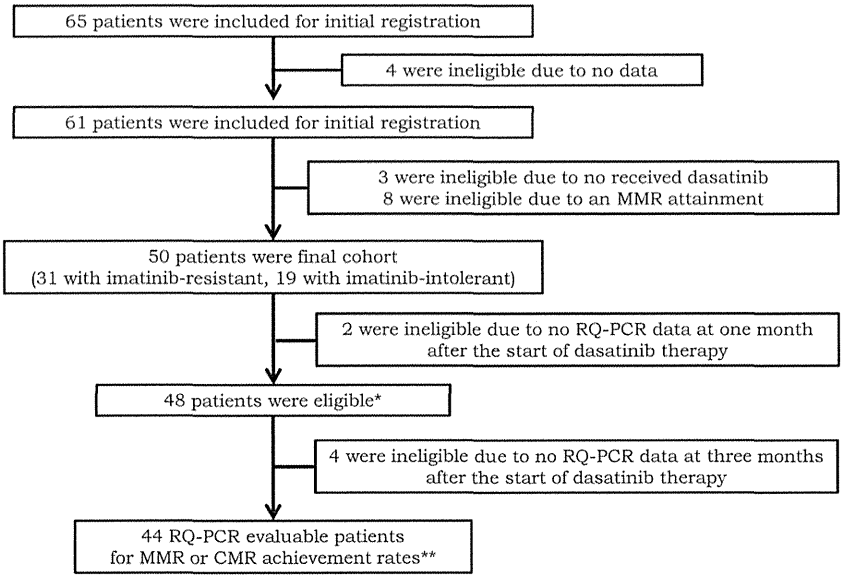

Fig. 1. Patient flow diagram. There was more than one reason for exclusion of patients. *, number of analyzed patients in Table 3; **, number of analyzed patients in Table 4; MMR, major molecular response; RQ-PCR, real-time quantitative reverse transcription-polymerase chain reaction; CMR, complete molecular response

defined as a peripheral *BCR-ABL/ABL* transcript ratio below the detection limit of the RQ-PCR analysis widely used throughout Japan, that is, a peripheral *BCR-ABL/ABL* ratio < $10^{-4.16}$ (0.0069%) on the international scale (IS).[20] MMR was defined as 0.1% of *BCR-ABL/ABL*.

Treatment with dasatinib

Patients received an oral dose of 100 mg of dasatinib once daily. Therapy could be interrupted or reduced to 70 mg daily or 50 mg daily in response to a hematologic toxicity of at least grade 3 or a non-hematologic toxicity of at least grade 2. When therapy was interrupted, the treatment was then reinitiated at a reduced dose level or discontinued altogether, depending on the severity of the adverse event and on the number of times the same event had occurred. In addition, the investigator and the sponsor made all dose reduction or discontinuation decisions for patients with any signs of bleeding or hemorrhage of any grade. Follow-up for 18 months has been performed in all cases.

Patient evaluations

The cytogenetic and hematologic responses to dasatinib were monitored using peripheral blood samples. Complete blood counts were analyzed once weekly for the first 12 weeks and every 3 months thereafter. The assessment of drug toxicities was continuous and included a physical examination to monitor adverse events that was conducted weekly for the first month and every 4 weeks thereafter. mRNA was collected from the peripheral blood samples and was analyzed for *BCR-ABL* gene point mutations using denaturing high-performance liquid chromatography and sequencing; the level of expression was examined using RQ-PCR. To determine the molecular responses to dasatinib, the *BCR-ABL* transcripts in peripheral blood were evaluated using Q-RT-PCR at baseline, one month, and every three months during the study, and the results were compared with the levels of total *ABL* transcripts.[20,21] Cytogenetic responses were based on the prevalence of Ph-positive interphase among at least 100 leukocytes in each peripheral blood sample. We used fluorescence *in situ* hybridization with BCR and ABL double-color probes to detect Ph-positive leukocytes.[22,23] The criteria for cytogenetic responses according to the percentage of Ph-positive cells in interphase among the peripheral leukocytes were as follows: CCyR, 0%; a partial cytogenetic response, 1% to 35%; minor cytogenetic response, 36% to 65%; minimal cytogenetic response, 66% to 95%; and no cytogenetic response, 96% to 100%.

Statistical analysis

The primary endpoint was MMR rate in CML patients at 12 months. Secondary endpoints were safety after treatment with dasatinib, CMR rate, and efficacy for patients with *BCR-ABL* point mutations. On the basis of previous evidence,[24] we

Table 1. Patient characteristics

Clinical feature		Total	Imatinib-resistant	Imatinib-intolerant
Registration number		61 (50)*	36 (31)*	25 (19)*
Median age, year (range)		57 (16-91)	57 (16-74)	57 (24-91)
Sex	Male	41	24	17
	Female	20	12	8

*, Data is for the final analyzed cohort.

set our goal at an MMR rate of 35%. In this situation, the required sample size was 55 patients to detect a difference between a threshold MMR rate of 15% and a target MMR rate using exact test for single proportion with one-sided alpha error of 2.5% and statistical power of 90%. To account for dropouts, the number of patients to be accrued was set at 60 in total.

The observed MMR rate at 12 months was compared with 15% using binomial test in the primary analysis. We also calculated the exact 95% confidence intervals (CI) for each proportion. The MMR rate and the CMR rate were estimated at each follow-up time. The associations between molecular response at 1 month or 3 months and the response at 12 months were evaluated using a chi-squared test. All analyses were performed using SAS version 9.3.

RESULTS

Sixty-five patients were enrolled between March 2009 and March 2010. Four of these 65 patients were determined to be ineligible for inclusion in the study, so they were excluded (Fig. 1). Three patients with imatinib-intolerant CML-CP ultimately did not receive dasatinib, so these patients were also excluded from the present analysis. Since eight of the registered patients had attained an MMR at the time of registration, these eight patients were additionally excluded.[20] The remaining 50 patients were analyzed statistically. Table 1 shows the characteristics of the patients. The results presented are for the final cohort of 50 patients: 31 with imatinib-resistant CML-CP and 19 with imatinib-intolerant CML-CP.

The patient demographics and baseline disease characteristics are representative of the patient population with imatinib-resistant or imatinib-intolerant CML-CP (Table 1).

Dosage of dasatinib

All the patients received an oral dose of 100 mg of dasatinib once daily at baseline. When a hematologic toxicity of at least grade 3 or a non-hematologic toxicity of at least grade 2 was observed, the dosage of dasatinib was reduced or transiently interrupted. Thus, dasatinib was continued at 100 mg/day for 12 months in 20 (40%) of the 50 patients. The dose intensity at 12 months was 82.0 mg/day. Six patients discontinued dasatinib treatment after 12 months. There were no patients who died in 12 months.

Molecular response

After 12 months of dasatinib therapy, 35 patients (70%; 95% CI = 55.4%-82.1%) had achieved an MMR and 16 patients (32%; 95% CI = 19.5%-46.7%) had achieved a CMR. Among the imatinib-resistant CML-CP cohort, 21 and 8 patients achieved an MMR and a CMR, respectively. The observed MMR rate showed a statistically significant difference with a threshold MMR rate of 15% ($p < 0.0001$). Among the imatinib-intolerant CML-CP cohort, 14 and 8 patients achieved an MMR and a CMR, respectively. All of the 50 patients were followed for 18 months. After 18 months of dasatinib therapy, 34 out of 50 patients (68.0%; 95% CI = 53.3 to 80.5%) had achieved an MMR and 22 patients (44.0%; 95% CI = 30.0 to 58.8%) had achieved a CMR.

Prediction of an improved response to dasatinib at 12 or 18 months based on the efficacy of the molecular response at 1 or 3 months after the initiation of dasatinib treatment (Fig. 2)

To investigate the relationships between response and outcome, the 50 patients were divided into four groups according to the therapeutic effect of dasatinib (Table 2): Group A exhibited more than 10% *BCR/ABL* mRNA, Group B exhibited 1%-10% *BCR/ABL* mRNA, Group C exhibited 0.1%-1% *BCR/ABL* mRNA, and Group D exhibited less than 0.1% *BCR/ABL* mRNA. At baseline, Group A was composed of 16 patients, Group B was composed of 13 patients, Group C was composed of 21 patients, and Group D did not contain any patients. After one month of dasatinib treatment, Group A was composed of 9 patients, Group B was composed of 10 patients, Group C was composed of 18 patients, and Group D was composed of 11 patients (Table 3). Three months after dasatinib treatment, Group A was composed of 4 patients, Group B was composed of 4 patients, Group C was composed of 15 patients, and Group D was composed of 21 patients (Table 4). Figure 2 shows the rates of MMR and CMR achievement at both 12 and 18 months. As shown in Fig. 2 and Table 3, the efficacy of the molecular response at 1 month

Table 2. Number of patients at baseline and responses of patients to dasatinib after 12 and 18 months of treatment

Group	Ratio of copy number of *BCR/ABL* mRNA to *ABL* mRNA at baseline	Percentage of patients who obtained an MMR, %*		Percentage of patients who obtained a CMR, %**	
		At 12 months	At 18 months	At 12 months	At 18 months
A	10% or more	43.8 (7/16)	50 (8/16)	25 (4/16)	25 (4/16)
B	1-10%	92.3 (12/13)	92.3 (12/13)	38.5 (5/13)	38.5 (5/13)
C	0.1-1%	76.2 (16/21)	85.7 (18/21)	33.3 (7/21)	47.6 (10/21)
D	0.1% or less (MMR)	NE	NE	NE	NE

*, number of patients who obtained an MMR/number of patients at baseline; **, number of patients who obtained an CMR/number of patients at baseline; CMR, complete molecular remission; MMR, major molecular remission; NE, not evaluable

Table 3. Major molecular remission and complete molecular remission achievement rates in four groups at one month after the start of dasatinib therapy

Group	Ratio of the copy number of *BCR/ABL* mRNA for *ABL* mRNA at 1 month after the start of dasatinib	Percentage of patients who obtained an MMR %*		Percentage of patients who obtained a CMR %**	
		At 12 months	At 18 months	At 12 months	At 18 months
A	10% or more	22.2 (2/9)	22.2 (2/9)	0 (0/9)	0 (0/9)
B	1-10%	80 (8/10)	80 (8/10)	50 (5/10)	50 (5/10)
C	0.1-1%	77.8 (14/18)	77.8 (14/18)	33.3 (6/18)	44.4 (8/18)
D	0.1% or less (MMR)	100 (11/11)	100 (11/11)	45.5 (5/11)	54.5 (6/11)

*, number of patients who obtained an MMR/number of patients at baseline; **, number of patients who obtained an CMR/number of patients at baseline; CMR, complete molecular remission; MMR, major molecular remission; NE, not evaluable

Table 4. Major molecular remission or complete molecular remission achievement rates in four groups at three months after the start of dasatinib therapy

Group	Ratio of copy number of *BCR/ABL* mRNA to *ABL* mRNA at 3 months after the start of dasatinib	Percentage of patients who obtained an MMR, %*		Percentage of patients who obtained a CMR, %**	
		At 12 months	At 18 months	At 12 months	At 18 months
A	10% or more	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
B	1-10%	25 (1/4)	50 (2/4)	0 (0/4)	0 (0/4)
C	0.1-1%	66.7 (10/15)	73.3 (11/15)	13.3 (2/15)	44.4 (8/18)
D	0.1% or less (MMR)	100 (21/21)	100 (21/21)	57.1 (12/21)	66.7 (14/21)

*, number of patients who obtained an MMR/number of patients number at baseline; **, number of patients who obtained an CMR/number of patients at baseline; CMR, complete molecular remission; MMR, major molecular remission; NE, not evaluable

after the initiation of dasatinib treatment was capable of predicting a significantly improved response consisting of the achievement of an MMR at 12 months ($p < 0.003$) or at 18 months ($p < 0.003$) and the achievement of a CMR at 18 months ($p < 0.027$). The efficacy of the molecular response at 3 months after the initiation of dasatinib treatment was also capable of predicting an improved response consisting of the achievement of an MMR at 12 months ($p < 0.001$) or an MMR at 18 months ($p < 0.001$) and the achievement of a CMR at 12 months ($p < 0.002$) or a CMR at 18 months ($p < 0.001$) (Table 4).

Response according to BCR-ABL mutation status

Eight types of *BCR-ABL* mutation were detected at baseline in 6 (12%) of the 50 CML-CP patients. Double mutations were detected in two patients (No. 11: M244V and Q252H, and No. 47: P359V and F359I). Four patients (No. 24: A397P, No. 36: E459K, No. 47: P359V and F359I, and No. 62: M351T) had achieved a CMR at 12 months. One of the double-positive patients (No. 11) was unable to achieve an MMR or a CCyR, whereas the other double-positive patient (No. 47) was able to achieve a CMR at 12 months, as described above. Patient No. 15, who had an H396R mutation, was unable to achieve an MMR or a CCyR. A T315I

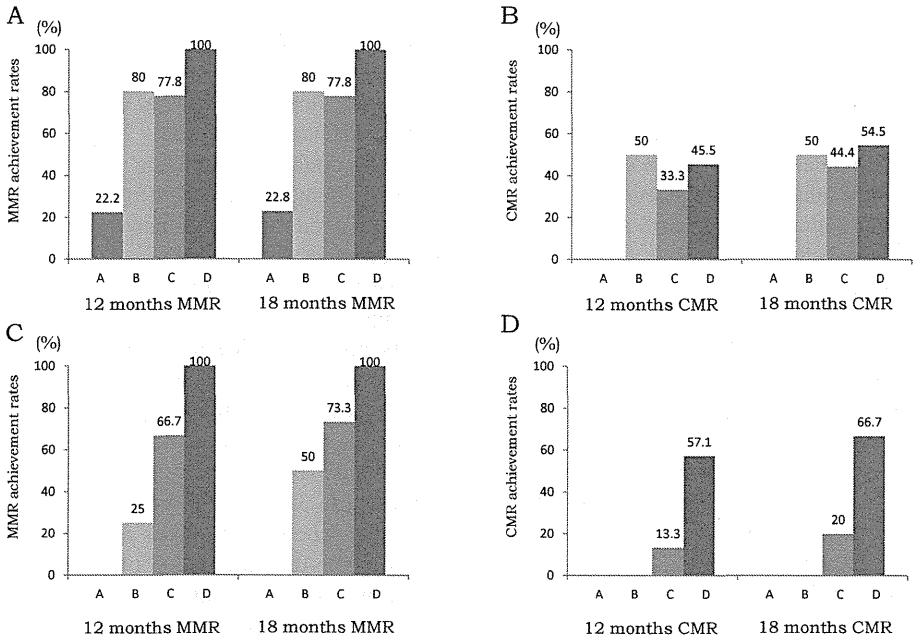

Fig. 2. Major molecular response (MMR) and complete molecular response (CMR) achievement rates in four groups at 1 and 3 months after the start of dasatinib therapy. (***2A***) MMR and (***2B***) CMR achievement rates in four groups at 1 month after the start of dasatinib therapy. (***2C***) MMR and (***2D***) CMR achievement rates in four groups at 3 months after the start of dasatinib therapy. The numbers on the bar graphs show the rates. *2A*, *2B*, *2C*, and *2D* are the four patient subgroups (Table 2) divided according to the therapeutic effect of dasatinib.

mutation was identified in two patients (No. 25 & 42) at three months. Both patients achieved a CCyR at 12 months, since hematopoietic stem cell transplantation was performed in both patients. A total of 14 different *BCR-ABL* mutations affecting 12 different amino acids were detected.

Safety

Dasatinib was generally well tolerated, with 3 patients (5%) having to discontinue treatment as a result of adverse hematological events (thrombocytopenia, anemia, neutropenia) at the 12-month follow-up.

Grade 1-2 cytopenia was a common hematologic adverse event, but was generally reversible and was effectively managed using dose adjustments (reductions or temporary interruptions). Six patients with grade 3-4 thrombocytopenia and three patients with grade 3-4 anemia were observed. However, no packed cells or platelet transfusions were administered in the present cohort.

Non-hematologic events that were considered by the investigator to be related to dasatinib therapy were generally mild to moderate in intensity (grade 1 or 2), with rashes, gastrointestinal disorders (diarrhea, nausea), and mild fever being occasionally observed. Dose reductions or interruptions were used effectively to treat the causes of elevated liver enzyme activities, headaches, bone pain, rashes, renal failure, cardiac abnormalities, and diarrhea. Twelve patients (24%) experienced grade 1 or 2 pleural effusion; in general, however, most cases of pleural effusion were uncomplicated and were resolved with temporary dose interruptions, the administration of diuretics, or, in some cases, the administration of steroids.

The majority of the serum chemistry changes observed with dasatinib were also mild to moderate in intensity (grade 1 or 2); grade 3 to 4 aspartate aminotransferase elevation was observed in 2 patients (4%), and 1 patient (2%) experienced grade 3 to 4 alanine transaminase elevation.

DISCUSSION

This clinical trial examined imatinib-resistant and imatinib-intolerant patients with CML-CP who were treated with dasatinib, a second-generation BCR-ABL inhibitor.

Imatinib, the first BCR-ABL TKI approved for the treatment of Ph-positive CML and Ph-positive acute lymphocytic leukemia, has been shown to be clinically effective. However, resistance develops in some patients, and treatment options for patients who are resistant or intolerant to imatinib have been very limited. Dasatinib is a more potent inhibitor of the BCR-ABL protein tyrosine kinase.[15,16]

In a recent single-institution study of 119 imatinib-resistant patients receiving dasatinib, nilotinib, or bosutinib as a second-line therapy for CML-CP, Milojkovic *et al.*[25] found that patients achieving a *BCR-ABL* ≤ 10% at 3 months had significantly improved rates of progression-free survival, overall survival, CCyR, MMR, and CMR. Shah *et al.*[26] supported the value of early molecular and cytogenetic responses in predicting the outcome of patients treated with second-line dasatinib therapy after imatinib failure.

Dasatinib (100 mg) administered once daily appears to offer a favorable long-term risk-benefit profile in patients with imatinib-resistant or imatinib-intolerant CML-CP. The present findings indicate that a consistent subgroup of CML-CP patients who were resistant or intolerant to imatinib were able to obtain a molecular benefit from dasatinib therapy. We previously showed that a *BCR-ABL* level had a significantly negative correlation with a relative increase in lymphocyte count at 1 and 3 months.[21] Here, we were able to show that molecular and cytogenetic responses at 1 and 3 months were highly predictive of the outcomes at 12 and 18 months, and that the achievement of a *BCR-ABL* ≤ 0.1% (MMR) at 3 months was a particularly strong predictor. A lower level of *BCR-ABL* transcript at 1 or 3 months was more strongly correlated with the *BCR-ABL* transcript level at 12 and 18 months ($p < 0.001$) than a higher level of *BCR-ABL*. In particular, those with faster and deeper responses to dasatinib (*BCR-ABL* ≤ 10% at 1 month and *BCR-ABL* ≤ 1% at 3 months) were more likely to have a significantly improved MMR, and those with a *BCR-ABL* level of ≤ 1% at 3 months were significantly more likely to have a CMR at 12 or 18 months ($p < 0.001$ or $p < 0.001$).

These results are consistent with the findings reported by Hanfstein *et al.*,[27] showing that a group with a 1% IS at 3 months had the best CCyR and MMR, compared with a group with a 1%–10% IS or a group with a 10% IS. Hanfstein *et al.* proposed that a cut-off of a 1% IS at 3 months seemed to be the best response-predictive surrogate, regardless of the treatment line (i.e., 1st line or 2nd/3rd line) and TKI drug (i.e., imatinib vs. dasatinib vs. nilotinib). Since the transcriptional level of *BCR-ABL* had a significantly negative correlation with the relative increase in lymphocyte count at 1 and 3 months,[21] we speculated that the increase in the number of lymphocytes due to dasatinib therapy would be associated with improved responses to dasatinib, and that the lymphocytes themselves could inhibit the proliferation of leukemic cells through an immune-mediated effect. We here surmised that dasatinib may have a synergistic effect of direct TKI and anti-leukemic immunity.

The results of the present study show a high level of clinical activity for dasatinib in Japanese patients with CML. The rates of MMR and CMR were higher than overseas data.[28] Overall, imatinib resistance or intolerance and the baseline *BCR-ABL* mutation status did not appear to have an impact on the response to dasatinib for CML-CP.[29]

The most common drug-related adverse events were hematological suppression. Although there were side effects, such as rash, headache, nausea, vomiting, and pyrexia, grade 3 or 4 events were uncommon. The reduction or discontinuation of dasatinib prevented the appearance of grade 3 or 4 peripheral edema or pleural effusion when grade 1 or 2 side effects appeared. Although neutropenia and thrombocytopenia occurred in 43% and 49% of the patients, respectively, these events were generally manageable with dose discontinuation and/or reduction. In the present study, none of the patients required occasional support with hematopoietic growth factors or transfusions. Grade 3 or 4 hemorrhage from the gastrointestinal tract was not reported. The majority of serum biochemistry abnormalities that were observed were mild to moderate in severity. Although imatinib intolerance was present in 38% (19/50) of the CML-CP patients, none of the patients experienced serious side effects or any side effects that led to the discontinuation of dasatinib administration.

In conclusion, although the number of Japanese patients that could be analyzed was relatively small, dasatinib exhibited a favorable long-term risk-benefit profile in patients with imatinib-resistant or imatinib-intolerant CML-CP. The present study is the first to show that a molecular response at 1 month was a strong predictor of outcome among patients with imatinib-resistant or imatinib-intolerant CML-CP.

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