chemotherapeutic agents, no favorable candidate for second-line chemotherapy is available. The answer to the question of whether the combined use of chemotherapeutic agents is more effective than the sequential use of a single agent is being addressed in a randomized phase II study being performed in Japan (unpublished results of the Trial for Advanced Stomach Cancer (TASC) trial performed by the Epidemiological and Clinical Research Information Network (ECRIN) investigators), and the results will be reported.

Recent debates regarding differences in survival outcomes between East and West have largely focused on issues regarding surgical technique, especially in the field of adjuvant therapy. In addition, with the advent of universal coverage health insurance in Japan, Japanese patients are more likely to be diagnosed with a node-negative state that can be cured by surgical resection. The question of variability in diagnostic criteria for malignancy has also been raised, noting that gastric cancer is diagnosed early according to nuclear and structural criteria in Japan even when invasion is absent according to the Western viewpoint [6]. If all these assumptions (i.e., stage migration, difference in pathological diagnosis between East and West etc.) are confirmed, it is probable that stage-stratified gastric cancer survival would be decreased in Western countries compared with that in Asian countries. However, objections to the hypotheses of geographic differences have been raised in studies performed in Asian-American gastric cancer patients in the United States [7] and in Canada [8]. Results from the Los Angeles County Cancer Surveillance Program demonstrated that, in California, Asian-Americans showed a significantly better probability of survival compared with non-Asians (hazard ratio 0.76, 95% confidence interval 0.72–0.82, $p < 0.001$), regardless of the presence of a similar extent of surgery, and a similar treating facility [7]. Of note, nearly one-fourth of Asians in California are uninsured and they are thus less likely to receive preventive or timely healthcare. This disparity in healthcare access could indicate that Asians in California would be more likely to have delays in diagnosis and to present with more advanced disease. Similar studies have been performed by the British Columbia Cancer Agency in Canada, where universal coverage health insurance provides comprehensive medical access to all residents, and treatment policies are fairly standardized in accordance with practical guidelines. A proportional hazards model adjusted for age, grade, location, extent, chemotherapy, and surgery, did not show statistically significant differences in outcome between Asians and non-Asians. However, Asian Canadian patients who underwent curative gastric cancer resection showed a significantly lower risk of mortality compared with non-Asians (hazard ratio 0.60, 95% confidence interval 0.41–0.87, $p < 0.001$) [8]. As far as these results are concerned, we have to be a bit sceptical about the widely accepted opinion that Asian treatments for gastric cancer are superior to Western therapies.

Regarding the difference in subtypes of gastric cancer, gastric cancer in the cardia and esophagogastric junction adenocarcinoma were reported to be more common in Caucasians than in Asians. However, a study looking at the chronological changes in esophagogastric junction adenocarcinoma in the Japanese population has demonstrated that the incidence has increased from 2.3 to 10% (a factor of more than 4 times) during the past 40 years [9]; i.e., it has become comparable to the incidence in Caucasians [10]. One possible explanation could be the rapid expansion of programs for the eradication of *Helicobacter pylori* infection among Japanese. Since the 1990s, when *H. pylori* was determined as the major causative organism of atrophic gastritis, and the precancerous nature of atrophic gastritis for intestinal gastric cancer was shown, the eradication of *H. pylori,* using amoxicillin and levofloxacin hydrate, has been extensively performed in the older Japanese population at risk. As a result, the incidence of intestinal-type cancer has been decreased. However, in contrast, the preservation of gastric acid secretion due to the removal of *H. pylori* could have led to the development of esophagogastric junction diffuse-type adenocarcinoma in Japanese [11]. The prevalences of the different types and locations of gastric cancer may have been changing rapidly in certain Asian populations, especially among Japanese.

The most critical limitation of the study by Hsu et al. [11] is that the data obtained are mostly from the tabulated meta-analysis data of randomized clinical trials. It is now taken for granted that the meta-analysis of individual patient data will be able to give more detailed answers to most of the above-mentioned questions. Meta-analysis or meta-regression analysis may be biased to a certain extent by the lack of individual data for the patients enrolled in each clinical trial.

All in all, we should wait for the results of the Global Advanced/Adjuvant Stomach Tumor Research International Collaboration (GASTRIC) investigators' individual patient data meta-analysis [12] before further proclaiming the importance of geographic differences in the safety and efficacy of chemotherapies for gastric or esophagogastric junction adenocarcinoma.

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References

1. Hsu C, Shen YC, Cheng CC, Cheng AL, Hu FC, Yeh KH. Geographic difference in safety and efficacy of systemic chemotherapy for advanced gastric or gastroesophageal carcinoma: a meta-analysis and meta-regression. Gastric Cancer. 2011 (in press).

2. Haller DG, Cassidy J, Clarke SJ, Cunningham D, Van Cutsem E, Hoff PM, et al. Potential regional differences for the tolerability profiles of fluoropyrimidines. J Clin Oncol. 2008;26(13): 2118–23.
3. Ajani JA, Faust J, Ikeda K, Yao JC, Anbe H, Carr KL, et al. Phase I pharmacokinetic study of S-1 plus cisplatin in patients with advanced gastric carcinoma. J Clin Oncol. 2005;23(28):6957–65.
4. Liu JY, Qu K, Sferruzza AD, Bender RA. Distribution of the UGT1A1*28 polymorphism in Caucasian and Asian populations in the US: a genomic analysis of 138 healthy individuals. Anti-cancer Drugs. 2007;18(6):693–6.
5. Takano M, Kato M, Yoshikawa T, Goto T, Furuya K, Kikuchi Y. Indispensability of UGT1A1*6 genotyping in Japanese cancer patients treated with irinotecan. Int J Clin Oncol. 2010;15(2): 224–5.
6. Schlemper RJ, Itabashi M, Kato Y, Lewin KJ, Riddell RH, Shimoda T, et al. Differences in diagnostic criteria for gastric carcinoma between Japanese and western pathologists. Lancet. 1997;349(9067):1725–9.
7. Kim J, Sun CL, Mailey B, Prendergast C, Artinyan A, Bhatia S, et al. Race and ethnicity correlate with survival in patients with gastric adenocarcinoma. Ann Oncol. 2010;21(1):152–60.
8. Gill S, Shah A, Le N, Cook EF, Yoshida EM. Asian ethnicity-related differences in gastric cancer presentation and outcome among patients treated at a Canadian cancer center. J Clin Oncol. 2003;21(11):2070–6.
9. Kusano C, Gotoda T, Khor CJ, Katai H, Kato H, Taniguchi H, et al. Changing trends in the population of adenocarcinoma of the esophagogastric junction in a large tertiary referral center in Japan. J Gastroenterol Hepatol. 2008;23(11):1662–5.
10. Kubo A, Corley DA. Marked multi-ethnic variation of esophageal and gastric cardia carcinomas within the United States. Am J Gastroenterol. 2004;99(4):582–8.
11. Koike T, Ohara S, Inomata Y, Abe Y, Iijima K, Shimosegawa T. The prevalence of *Helicobacter pylori* infection and the status of gastric acid secretion in patients with gastroesophageal junction adenocarcinoma in Japan. Inflammopharmacology. 2007;15(2): 61–4.
12. GASTRIC Group, Paoletti X, Oba K, Burzykowski T, Michiels S, Ohashi Y, Pignon JP, et al. Benefit of adjuvant chemotherapy for resectable gastric cancer: a meta-analysis. JAMA. 2010;303(17): 1729–37.

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ORIGINAL ARTICLE

Is there diversity among *UGT1A1* polymorphism in Japan?

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Abstract

AIM: To investigate into the diversity of *UGT1A1* polymorphism across three different districts in Japan and highlight genetic differences among the population in Japan.

METHODS: We enrolled 50 healthy volunteers from each of the Yamaguchi (western part of Japan), Kochi (southern part of Japan) and Akita (northern part of Japan) prefectures. Blood samples (7 mL) were collected from each participant and stored in EDTA for subsequent genotyping by fragment size analysis, direct sequencing and TaqMan assay of *UGT1A1**28, *UGT1A7**3/*UGT1A9**22 and *UGT1A1**93/*UGT1A1**6/*UGT1A1**27/*UGT1A1**60/*UGT1A7* (-57), respectively.

RESULTS: The only statistically significant differences in allele polymorphisms among the group examined were for *UGT1A1*6*. The Akita population showed more *UGT1A1*6* heterozygosity (P = 0.0496).

CONCLUSION: Our study revealed no regional diversity among *UGT1A1*, *UGT1A7* or *UGT1A9* polymorphisms in Japan.

Key words: *UGT1A1* gene; Polymorphism; Diversity

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INTRODUCTION

Irinotecan with fluoropyrimidine is approved worldwide as a first-line chemotherapeutic agent for metastatic colorectal cancer[1-5]. Although prolonged survival has been reported with the use of this drug, severe diarrhea and neutropenia have also been reported as dose-limiting

toxicities in 20%-35% of patients treated by the agent. Recent studies revealed that the risk of such severe toxicities might be associated with genetic variation in irinotecan metabolism, indicating a possible predictive factor.

Irinotecan is activated by hydrolysis to SN-38, a potent topoisomerase I inhibitor[6] that is primarily inactivated through biotransformation into SN-38 glucuronide (SN-38G) by the enzyme uridine diphosphate glucuronosyltransferase isoform 1A1 (*UGT1A1*)[7]. In addition, the toxicity of irinotecan has been correlated with polymorphisms in the number of TA repeats in one of the promoter regions of the *UGT1A1* gene (*UGT1A1 *28*), which affects transcriptional efficiency[8]. Because of the clinical importance of the glucuronidation pathway in irinotecan treatment, *UGT1A1 *28* was proposed as a potent predictor for severe toxicity[9-11]. Recently, a novel prospective dose-finding study of irinotecan alone based on *UGT1A1*6* and **28* genotyping was reported[4,12]. These results showed that the *UGT1A1 *6* or **28* genotype status could be used to determine RD (recommended doses) of irinotecan. We conducted a prospective phase II study of FOLFIRI for metastatic colorectal cancer in Japan, analyzed the *UGT1A1*28* and **6* polymorphisms and demonstrated that the combination of the *UGT1A1*28* and **6* polymorphism is important to predict the adverse event of the CPT-11[5].

The role of *UGT1A1*28* alleles in the toxicity and pharmacokinetics of irinotecan is considerably different between Asians and Caucasians. Only homozygotes of **28* have been associated with neutropenia in Caucasians[11,13-15], whereas both homozygote and heterozygote **28* patients have shown severe toxicity with irinotecan in Japan[4,9]. Other results revealed that SN-38 glucuronidation was highly impaired in heterozygotes, as previously reported[9,16]. Such ethnic differences may be associated with other genetic variants of UGT1A family polymorphisms, such as *UGT1A1*60*, **6*, *UGT1A7*3* and *UGT1A9*22*, which were demonstrated in linkage disequilibrium experiments with *UGT1A1*28*[17-22]. Such genotype variation could affect SN-38 glucuronidation and also the severe irinotecan-related toxicity. This study aimed to clarify the regional differences in *UGT* enzyme polymorphisms among three different districts in Japan that are widely different, both geographically and culturally.

MATERIALS AND METHODS

The 50 volunteers from Akita, Kochi and Yamaguchi prefectures comprised of 8 males and 42 females, 6 males and 44 females, and 11 males and 39 females, respectively, with an average age of 37.5, 43.8 and 38.4 years, respectively. The examinee demographics are shown in Table 1.

Blood samples (7 mL) were collected from each participant and stored in EDTA for subsequent analysis. Examinees were limited to those whose parents and grandparents came from the same region.

Written informed consent was obtained from all participants.

Table 1 Examinee characteristics

	Akita	Kochi	Yamaguchi
Sex			
Male	8	6	11
Female	42	44	39
Age (yr)	37.4 (23-55)	43.8 (24-66)	38.4 (18-67)

Table 2 Primers, probes used for genotyping

Gene	Variant	Primers and probes[1]	
*UGT1A1*28*	-53 TA6/TA7	F-FAM	5'-gtgacacagtcaaacattaacttgt-3'
		R	5'-gcctttgctcctgccagaggtt-3'
*UGT1A7*3*	N129K	F	5'-tacactctggaggatcagga-3'
	W208R	R	5'-tattgggcatcacgggtttg-3'
*UGT1A9*22*	-118 T10/T9	F	5'-acttaacattgcagcacagg-3'
		R	5'-atgggcaaaagccttgaact-3'
*UGT1A1*93*	-3156 G/A	F	5'-cagaagggctagagaggaggaa-3'
		R	5'-cttgctctcaaaactctgggataga-3'
		FAM	5'-cctgtccaagctca-3'
		VIC	5'-cacctgtctaagctca-3'
*UGT1A1*6*	211 G/A	C 559715 20	
*UGT1A1*27*	686 C/A	C 2307598 20	
*UGT1A1*60*	-3279 T/G	C 1432134 10	
UGT1A7 (-57)	-57 T/G	C 287265 10	

[1]Primers for fragment size assay: F-FAM: Forward primer labeled FAM; R: Reverse primer. Primers for Sequence assay: F: Forward primer; R: Reverse primer. TaqMan assay: F: Forward primer; R: Reverse primer; FAM: Reporter 1 probe; VIC: Reporter 2 probe. Number: TaqMan SNP genotyping assays number.

Genotyping

Genomic DNA was extracted from peripheral blood anti-coagulated with EDTA-2Na, using a conventional NaI method[23]. *UGT1A1**28, *UGT1A7**3/*UGT1A9**22 and *UGT1A1**93/*UGT1A1**6/*UGT1A1**27/*UGT1A1**60/*UGT1A7* (-57) were genotyped by fragment size analysis, direct sequencing and TaqMan assay, respectively. Primers and probes used in this study are shown in Table 2.

For fragment size analysis, PCR reactions were performed in a total volume of 10 μL containing template DNA (80 ng/μL) according to the manufacturer's instructions (Ex Taq; Takara, Tokyo, Japan). The amplification was carried out with a Gene Amp PCR System PC808 (ASTEC, Tokyo, Japan), with an initial denaturation at 95 ℃ for 2 min followed by 27 cycles of denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 20 s, and extension at 72 ℃ for 30 s. The PCR products of TA6 and TA7, whose sizes were 94 *bp* and 96 *bp*, respectively, were mixed with Hi-Di formamide, including the internal size standard (GeneScan 500, Applied Biosystems, CA, USA) at a 1:10 (*vol/vol*) ratio. Then, samples were run in the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Fragment sizes were determined by comparison with the internal size standard (GeneScan LIZ-500) using the local Southern algorithm and the data were analyzed by GeneMapper™ software version 3.5 (Applied Biosystems).

For direct sequencing, PCR amplifications were performed using the Gene Amp PCR System PC808

Table 3 Polymorphisms of *UGT1A1* *n* (%)

	*UGT1A1**28 (*P* = 0.663)			*UGT1A1**6 (*P* = 0.0496)			*UGT1A1**27 (*P* = 1.000)			*UGT1A1**60 (*P* = 0.766)			*UGT1A1*-3156		
	6/6	6/7	7/7	A/A	G/A	G/G	A/A	C/A	C/C	G/G	T/G	T/T	A/A	G/A	G/G
A	41 (82)	8 (16)	1 (2)	1 (2)	20 (40)	29 (58)	0 (0)	0 (0)	50 (100)	2 (4)	19 (38)	29 (58)	1 (2)	8 (16)	41 (82)
K	37 (74)	13 (26)	0 (0)	0 (0)	14 (28)	36 (72)	0 (0)	1 (2)	49 (98)	1 (2)	25 (50)	24 (48)	0 (0)	13 (26)	37 (74)
Y	37 (74)	12 (24)	1 (2)	3 (6)	9 (18)	38 (76)	0 (0)	0 (0)	50 (100)	2 (4)	22 (44)	26 (52)	1 (2)	12 (24)	37 (74)

A: Akita prefecture; K: Kochi prefecture; Y: Yamaguchi prefecture.

Table 4 Polymorphisms of *UGT1A7* and *UGT1A9* *n* (%)

	UGT1A7 N129K (*P* = 0.853)			*UGT1A7* W208R (*P* = 0.409)			*UGT1A7* -57 (*P* = 0.409)			*UGT1A9**22 (*P* = 0.993)		
	G/G	T/G	T/T	C/C	T/C	T/T	G/G	T/G	T/T	9/9	9/10	10/10
A	7 (14)	24 (48)	19 (38)	2 (4)	23 (46)	25 (50)	2 (4)	23 (46)	25 (50)	5 (10)	24 (48)	21 (42)
K	8 (16)	20 (40)	22 (44)	4 (8)	17 (34)	29 (58)	4 (8)	17 (34)	29 (58)	6 (12)	22 (44)	22 (44)
Y	5 (10)	23 (46)	22 (44)	4 (8)	14 (28)	32 (64)	4 (8)	14 (28)	32 (64)	5 (10)	23 (46)	22 (44)

A: Akita prefecture; K: Kochi prefecture; Y: Yamaguchi prefecture.

(ASTEC, Tokyo, Japan) with Ex Taq polymerase. Amplification conditions were 30 cycles of 95 ℃ for 30 s, each annealing temperature for 20 s, and 72 ℃ for 30 s. PCR products were purified using ExoSAP-IT (Amersham Bioscience, Tokyo, Japan) for 20 min at 37 ℃ and then for 20 min at 80 ℃. Sequencing reactions were carried out using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan). After purification with ethanol, the reaction products were analyzed using an ABI 3100-Avant Genetic Analyzer (Applied Biosystems).

TaqMan assays of PCR products were performed according to the manufacturer's protocol. Specific forward/reverse PCR primers and TaqMan probes for *UGT1A1**93 were custom-synthesized by Applied Biosystems. Primers and probes for *UGT1A1**6, *UGT1A1**27, *UGT1A1**60, *UGT1A7* (-57) were purchased from Applied Biosystems (TaqMan SNP Genotyping Assays). Reaction mixtures were loaded into 384 well plates and placed in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). PCR amplifications were performed as follows: initial denaturation at 95 ℃ for 10 min, followed by 40 cycles of PCR with a denaturation at 95 ℃ for 15 s, and one step annealing/extension for 1 min at 60 ℃.

Statistical analysis and power calculation

Proportions of wild-type, hetero-type and homo-type were calculated with 95% Agresti-Coull confidence intervals (95% CI)[24]. Fisher's exact test with a two-sided significance level of 0.05 was used for comparing the areas. For a two-sided 95% CI for a binomial proportion whose true value is varied from 0.5 to 0.1, a sample size of 50 yields a half-width of, at most, 14% in any situations of the true value.

RESULTS

Tables 3 and 4 list the polymorphisms of *UGT1A1* allele *28, *6, *60, *27 and *93 (-3156), *UGT1A7 *3* (N129K, W208R, -57) and *UGT1A9*22*. The incidence of wild-type *UGT1A1*28* in the Akita, Kochi and Yamaguchi cohorts was 82% (95% CI: 69 to 90), 74% (95% CI: 60 to 84) and 74% (95% CI: 60 to 84), respectively (*P*-value = 0.663). The incidence of homozygous *UGT1A1*28* across the three districts was only 1.3% (95% CI: 0.0 to 5.0).

The only statistical difference in allele polymorphisms examined among the three groups was in *UGT1A1*6*. The incidence of wild-type *UGT1A1*6* across the Akita, Kochi and Yamaguchi populations was 58% (95% CI: 44 to 71), 72% (95% CI: 58 to 83) and 76% (95% CI: 62 to 86), respectively, while the incidence of heterozygous-type *UGT1A1*6* was 40%, 28% and 18%, respectively. Volunteers from Akita showed the most heterozygosity in *UGT1A1*6*, although the *P*-value was 0.0496.

DISCUSSION

The participants in this study were mostly nurses and other medical staff from hospitals in the three Japanese prefectures. Around 95% of the nurses in Japan are women; thus the predominance of female subjects in this study.

There are several reports about the distribution of *UGT1A1* polymorphisms worldwide. However, these studies were limited to the promoter region, *UGT1A1*28*[8,25-27], and demonstrated that *UGT1A1*28* homozygosity is frequent in Europe (5.0%-14.8%), Africa (5.9%-17.9%) and the Indian subcontinent (19.2%-24.0%), compared to East Asia, which comprises mainly of the Chinese (1.2%-5.0%)[25,26]. Hall *et al*[25] showed that sub-Saharan Africa, especially Cameroon, was 33% homozygous for

Figure 1 The location of the three prefectures. Akita represents the northern part of Japan, while the Kochi prefecture on Shikoku Island was obstructed from communication with other prefectures by the Shikoku mountain (dotted line) range in ancient times. Yamaguchi is one of the nearest prefectures to the Korean Peninsula in Japan.

*UGT1A1*28*, which is a fairly high frequency even compared to Caucasians and Indians.

The incidence of homozygous *UGT1A1*28* across the three districts of our data in Japan was only 1.3%, which is comparable to the 1.0% reported by Hall *et al*[25]. Premawardhena *et al*[26] also reported a wider diversity of repeat numbers among individuals from North and Central America with varying degrees of African ancestry. Our data demonstrated that the repeat number of (TA) was 6/6, 6/7 and 7/7, which is the same as those reported for Europeans and other Asians. Hitherto, no studies have investigated the regional diversity in *UGT1A1*-family polymorphism within one country, although our study now indicates that there is no diversity of *UGT1A1*28* polymorphism in Japan.

In this study, we selected the Akita, Kochi and Yamaguchi prefectures (Figure 1). Akita represents the northern part of Japan, while the Kochi prefecture on Shikoku Island was obstructed from communication with other prefectures by the Shikoku mountain range in ancient times. Thus, both prefectures have developed a unique dialect and less communication with each other historically. On the other hand, Yamaguchi is one of the nearest prefectures to the Korean Peninsula in Japan. All the prefectures chosen have also developed a unique culture.

Our study revealed no regional diversity of *UGT1A1*, *UGT1A7* and *UGT1A9* polymorphisms in Japan. Only *UGT1A1*6* showed a statistically significant difference among these three regions in Japan, with more G/A type in the Akita prefecture compared to the other two regions. However, the *p*-value for the *UGT1A1*6* polymorphism was marginal (*P*-value = 0.0496) and the statistical significance is easily changeable due to the selection of the sampling population. The number of *UGT1A1*6* homozygotes was not different among the three districts, with allele frequencies for Akita, Kochi and Yamaguchi of 2.2%, 1.4% and 1.5%, respectively.

Our study is an exploratory research about the diversity of *UGT1A1* in Japan. Before the study, we speculated that Akita may have the same tendency of *UGT1A1* polymorphism as Caucasians, i.e. Akita may have more polymorphism in *UGT1A1*28* and less polymorphism in *UGT1A1*6*. However, our study revealed that *UGT1A1*28* showed no diversity and *UGT1A1*6* did not show less polymorphism, although this was not random sampling and generalizability of our population could not be guaranteed.

As described, heterozygotes of *UGT1A1*28* are extremely rare in the Japanese population compared to Caucasians and the incidence of heterozygotes and homozygotes of *UGT1A1*28* across the three districts combined was 22.0% and 0.013%, respectively.

Our study also demonstrated that the *UGT1A1*6* polymorphisms, G/A and A/A, occurred at a rate of 28.7% and 2.7%, respectively, in Japan. Kaniwa *et al*[28] examined the variants of *UGT1A1*6* in Caucasian and African-American populations. Caucasians showed only two heterozygotes among 150 blood samples, while none were found among the African-Americans. Our study confirmed the Japanese standard data for *UGT1A1* polymorphism frequencies, which shows more variants for *UGT1A1*6* compared to Caucasian and African-American samples.

Jinno *et al*[29] examined the glucuronidation of SN-38, a potent inhibitor of topoisomerase 1, by human UGT1A1 variants in Cos-1 cells. The variant 211G<A (G71R) (*UGT1A1*6*) reduced the glucuronidation activity more than 686C>A (P229Q) (*UGT1A1*27*). Moreover, hyperbilirubinemia observed in Japanese and Taiwanese patients with the P229Q variant is mainly attributable to the TA7 variation. Thus, *UGT1A1*6* plays an important role during chemotherapy with irinotecan in East Asian populations[28,30].

Finally, the variant sequences in exon 1, *UGT1A1*6* and *UGT1A1*27*, have been identified only in the Japanese. Thus, Japanese studies could focus more on these two genotypes, which might be more closely associated with drug sensitivity in Japanese patients than in Caucasians[31-33].

Our ongoing studies will compare *UGT1A* gene polymorphism worldwide, starting in Asian populations and gradually spreading to Europeans. Such investigations may also clarify the movement of people throughout history.

COMMENTS

Background

Irinotecan with fluoropyrimidine is approved worldwide as a first-line chemotherapeutic agent for metastatic colorectal cancer. Although prolonged survival has been reported with the use of this drug, severe diarrhea and neutropenia have also been reported as dose-limiting toxicities in 20%-35% of patients treated by the agent. Recent studies revealed that the risk of such severe toxicities might be associated with genetic variation in irinotecan metabolism, indicating a possible predictive factor.

Research frontiers

This study aimed to clarify the regional differences in *UGT* enzyme polymorphisms among three different districts in Japan that are widely distant, both geographically and culturally.

Innovations and breakthroughs

The authors enrolled 50 healthy volunteers from each of the Yamaguchi (west-

ern part of Japan), Kochi (southern part of Japan), and Akita (northern part of Japan) prefectures. Blood samples were collected from each participant and stored in EDTA for subsequent genotyping by fragment size analysis, direct sequencing, and TaqMan assay of *UGT1A1*28*, *UGT1A7*3*/*UGT1A9*22, and *UGT1A1*93*/*UGT1A1*6*/*UGT1A1*27*/*UGT1A1*60*/*UGT1A7* (-57), respectively.

Applications

The authors found that the only statistically significant differences in allele polymorphisms among the group examined were for *UGT1A1*6*. The Akita population showed more *UGT1A1*6* heterozygosity. This study revealed no regional diversity among *UGT1A1*, *UGT1A7* or *UGT1A9* polymorphisms in Japan.

Peer review

Kobayashi *et al* aimed to clarify the regional differences in UGT enzyme polymorphisms among three different districts in Japan that are widely distant, both geographically and culturally. The study seems interesting, but the sample size is somewhat small.

REFERENCES

1 **Douillard JY**, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L, Rougier P. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000; **355**: 1041-1047

2 **Saltz LB**, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL, Miller LL. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000; **343**: 905-914

3 **Tournigand C**, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**: 229-237

4 **Hazama S**, Nagashima A, Kondo H, Yoshida S, Shimizu R, Araki A, Yoshino S, Okayama N, Hinoda Y, Oka M. Phase I study of irinotecan and doxifluridine for metastatic colorectal cancer focusing on the UGT1A1*28 polymorphism. *Cancer Sci* 2010; **101**: 722-727

5 **Okuyama Y**, Hazama S, Nozawa H, Kobayashi M, Takahashi K, Fujikawa K, Kato T, Nagata N, Kimura H, Oba K, Sakamoto J, Mishima H. Prospective phase II study of FOLFIRI for mCRC in Japan, including the analysis of UGT1A1 28/6 polymorphisms. *Jpn J Clin Oncol* 2011; **41**: 477-482

6 **Kawato Y**, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991; **51**: 4187-4191

7 **Iyer L**, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, Coffman BL, Ratain MJ. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 1998; **101**: 847-854

8 **Beutler E**, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci USA* 1998; **95**: 8170-8174

9 **Ando Y**, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000; **60**: 6921-6926

10 **Massacesi C**, Terrazzino S, Marcucci F, Rocchi MB, Lippe P, Bisonni R, Lombardo M, Pilone A, Mattioli R, Leon A. Uridine diphosphate glucuronosyl transferase 1A1 promoter polymorphism predicts the risk of gastrointestinal toxicity and fatigue induced by irinotecan-based chemotherapy. *Cancer* 2006; **106**: 1007-1016

11 **Innocenti F**, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramírez J, Rudin CM, Vokes EE, Ratain MJ. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004; **22**: 1382-1388

12 **Ura T**, Satoh T, Tsujinaka T, Sasaki Y, Yamazaki K, Munakata M, Okamura S, Yamada Y, Hyodo I, Sakata Y. A genotype-directed dose-finding study of irinotecan based on UGT1A1 *28 and *6 polymorphisms in Japanese patients with gastrointestinal cancer (UGT0601). *Ann Oncol* 2008; **19** Suppl 8: abstr 406P

13 **Toffoli G**, Cecchin E, Corona G, Russo A, Buonadonna A, D'Andrea M, Pasetto LM, Pessa S, Errante D, De Pangher V, Giusto M, Medici M, Gaion F, Sandri P, Galligioni E, Bonura S, Boccalon M, Biason P, Frustaci S. The role of UGT1A1*28 polymorphism in the pharmacodynamics and pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006; **24**: 3061-3068

14 **Mathijssen RH**, Marsh S, Karlsson MO, Xie R, Baker SD, Verweij J, Sparreboom A, McLeod HL. Irinotecan pathway genotype analysis to predict pharmacokinetics. *Clin Cancer Res* 2003; **9**: 3246-3253

15 **Stewart CF**, Panetta JC, O'Shaughnessy MA, Throm SL, Fraga CH, Owens T, Liu T, Billups C, Rodriguez-Galindo C, Gajjar A, Furman WL, McGregor LM. UGT1A1 promoter genotype correlates with SN-38 pharmacokinetics, but not severe toxicity in patients receiving low-dose irinotecan. *J Clin Oncol* 2007; **25**: 2594-2600

16 **Araki K**, Fujita K, Ando Y, Nagashima F, Yamamoto W, Endo H, Miya T, Kodama K, Narabayashi M, Sasaki Y. Pharmacogenetic impact of polymorphisms in the coding region of the UGT1A1 gene on SN-38 glucuronidation in Japanese patients with cancer. *Cancer Sci* 2006; **97**: 1255-1259

17 **Han JY**, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Jang IJ, Lee DH, Lee JS. Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J Clin Oncol* 2006; **24**: 2237-2244

18 **Minami H**, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, Shirao K, Yamada Y, Ohmatsu H, Kubota K, Yoshida T, Ohtsu A, Saijo N. Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. *Pharmacogenet Genomics* 2007; **17**: 497-504

19 **Saito Y**, Sai K, Maekawa K, Kaniwa N, Shirao K, Hamaguchi T, Yamamoto N, Kunitoh H, Ohe Y, Yamada Y, Tamura T, Yoshida T, Minami H, Ohtsu A, Matsumura Y, Saijo N, Sawada J. Close association of UGT1A9 IVS1+399C& gt; T with UGT1A1*28, *6, or *60 haplotype and its apparent influence on 7-ethyl-10-hydroxycamptothecin (SN-38) glucuronidation in Japanese. *Drug Metab Dispos* 2009; **37**: 272-276

20 **Yamamoto N**, Takahashi T, Kunikane H, Masuda N, Eguchi K, Shibuya M, Takeda Y, Isobe H, Ogura T, Yokoyama A, Watanabe K. Phase I/II pharmacokinetic and pharmacogenomic study of UGT1A1 polymorphism in elderly patients with advanced non-small cell lung cancer treated with irinotecan. *Clin Pharmacol Ther* 2009; **85**: 149-154

21 **Lankisch TO**, Schulz C, Zwingers T, Erichsen TJ, Manns MP, Heinemann V, Strassburg CP. Gilbert's Syndrome and irinotecan toxicity: combination with UDP-glucuronosyltransferase 1A7 variants increases risk. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 695-701

22 **Fujita K**, Ando Y, Nagashima F, Yamamoto W, Eodo H, Araki K, Kodama K, Miya T, Narabayashi M, Sasaki Y. Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. *Cancer Chemother Pharmacol*

2007; **60**: 515-522
23 **Wang L**, Hirayasu K, Ishizawa M, Kobayashi Y. Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS. *Nucleic Acids Res* 1994; **22**: 1774-1775
24 **Agresti A**, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. *Am Stat* 1998; **52**: 119-126
25 **Hall D**, Ybazeta G, Destro-Bisol G, Petzl-Erler ML, Di Rienzo A. Variability at the uridine diphosphate glucuronosyltransferase 1A1 promoter in human populations and primates. *Pharmacogenetics* 1999; **9**: 591-599
26 **Premawardhena A**, Fisher CA, Liu YT, Verma IC, de Silva S, Arambepola M, Clegg JB, Weatherall DJ. The global distribution of length polymorphisms of the promoters of the glucuronosyltransferase 1 gene (UGT1A1): hematologic and evolutionary implications. *Blood Cells Mol Dis* 2003; **31**: 98-101
27 **Mercke Odeberg J**, Andrade J, Holmberg K, Hoglund P, Malmqvist U, Odeberg J. UGT1A polymorphisms in a Swedish cohort and a human diversity panel, and the relation to bilirubin plasma levels in males and females. *Eur J Clin Pharmacol* 2006; **62**: 829-837
28 **Kaniwa N**, Kurose K, Jinno H, Tanaka-Kagawa T, Saito Y, Saeki M, Sawada J, Tohkin M, Hasegawa R. Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide polymorphism 686C> T (P229L) found in an African-American. *Drug Metab Dispos* 2005; **33**: 458-465
29 **Jinno H**, Tanaka-Kagawa T, Hanioka N, Saeki M, Ishida S, Nishimura T, Ando M, Saito Y, Ozawa S, Sawada J. Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38), an active metabolite of irinotecan (CPT-11), by human UGT1A1 variants, G71R, P229Q, and Y486D. *Drug Metab Dispos* 2003; **31**: 108-113
30 **Huang CS**, Luo GA, Huang ML, Yu SC, Yang SS. Variations of the bilirubin uridine-diphosphoglucuronosyl transferase 1A1 gene in healthy Taiwanese. *Pharmacogenetics* 2000; **10**: 539-544
31 **Bosma PJ**, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995; **333**: 1171-1175
32 **Akaba K**, Kimura T, Sasaki A, Tanabe S, Ikegami T, Hashimoto M, Umeda H, Yoshida H, Umetsu K, Chiba H, Yuasa I, Hayasaka K. Neonatal hyperbilirubinemia and mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene: a common missense mutation among Japanese, Koreans and Chinese. *Biochem Mol Biol Int* 1998; **46**: 21-26
33 **Maruo Y**, Nishizawa K, Sato H, Doida Y, Shimada M. Association of neonatal hyperbilirubinemia with bilirubin UDP-glucuronosyltransferase polymorphism. *Pediatrics* 1999; **103**: 1224-1227

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STUDY PROTOCOL Open Access

A randomized phase II trial to elucidate the efficacy of capecitabine plus cisplatin (XP) and S-1 plus cisplatin (SP) as a first-line treatment for advanced gastric cancer: XP ascertainment vs. SP randomized PII trial (XParTS II)

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Abstract

Background: On the basis of international clinical trials, capecitabine plus cisplatin (XP) as a first-line treatment of advanced gastric cancer is considered a global standard regimen. However, the usefulness of XP as compared with S-1 plus cisplatin (SP), which is considered standard therapy in Japan, has not yet been assessed.

Methods/design: This is a multicenter randomized phase II trial to elucidate the efficacy of XP as compared with SP for first-line treatment of advanced gastric cancer. Patients with unresectable metastatic or recurrent gastric cancer, 20–74 years of age and human epidermal growth factor 2 (HER2)-negative status, will be assigned in a 1:1 ratio to receive either S-1 40 mg/m^2 bid for 21 days plus cisplatin 60 mg/m^2 (day 8) every 5-week cycle or capecitabine 1000 mg/m^2 bid for 14 days plus cisplatin 80 mg/m^2 (day 1) every 3-week cycle. Patients will be also asked to the analysis of tumor tissues for translational investigations. The Primary endpoint is progression-free survival and secondary endpoints are overall survival, time to treatment failure, tumor response rate and safety. These comparisons will also be evaluated in terms of biomarkers. Planned sample size is 100 (50 in each arm), which is appropriate for this trial.

Discussion: Fluoropyrimidine plus cisplatin combination is the standard regimen of the first line treatment for advanced gastric cancer. Both S-1 and capecitabine are the prodrug of 5-FU but differ from their process of metabolism. Result of this trial and translational research will provide the important clues to prepare the individualized therapy for advanced gastric cancer in the near future.

Trial registration: ClinicalTrials.gov Identifier NCT01406249

Keywords: Biomarker, Capecitabine, Cisplatin, Clinical trial, Gastric cancer, S-1

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Background

Gastric cancer is the fourth most common malignancy in the world (988 602 cases in 2008, 7.8% of total) and the second leading cause of cancer death (737 419 deaths, 9.7% of total) [1]. For the treatment of advanced or recurrent gastric cancer (AGC), the most commonly used regimens are combination chemotherapy consisting of a fluoropyrimidine (5-fluorouracil or oral fluoropyrimidine) plus a platinum agent with or without docetaxel or anthracyclines [2-6].

S-1 is an oral anticancer drug composed of the 5-fluorouracil (5-FU) prodrug tegafur and two 5-FU modulators; it has achieved high response rates in patients with gastric cancer in phase II studies [7,8]. In a phase III trial (SPIRITS trial) that compared S-1 alone to S-1 plus cisplatin (SP), SP showed a significantly longer overall survival (OS; 13 months vs. 11 months; HR = 0.77, 95% CI 0.61–0.98, p = 0.04) and longer progression-free survival (PFS; 6.0 months vs. 4.0 months; HR = 0.57, 95% CI 0.44–0.73, p < 0.0001) [4]. Therefore, SP is now considered to be one of the standard first-line regimens for AGC in Japan.

Capecitabine is also an oral fluoropyrimidine, which is metabolized primarily in the liver and converted in tumor tissues to 5-FU by the enzyme thymidine phosphorylase (TP), which is associated in higher concentrations in tumor cells than in normal cells [9]. Kang and colleagues evaluated the non-inferiority of capecitabine plus cisplatin (XP) compared with 5-FU plus cisplatin (FP). The median PFS showed significant non-inferiority (5.6 months vs. 5.0 months; HR = 0.81, 95% CI 0.63–1.04, P < 0.001) [5]. On the basis of these results, XP is now considered one of the standard treatments of AGC [10], and XP was adopted as the reference arm in two recent global studies of molecular targeting agents [11,12]. However, data is scarce with respect to XP treatment in Japanese patients, and also the usefulness of XP as compared with SP has not yet been assessed.

As another issue, these 2 types of oral fluoropyrimidine show some different characteristics in the mechanisms of their antitumor effect. A subset analysis of the FLAGS trial showed that S-1 seemed to be better than 5-FU in the subgroup with diffuse-type gastric cancer [6]. This result was consistent with the results of a subset analysis of the JCOG9912 trial, which showed that S-1 was better than 5-FU in patients with diffuse-type gastric cancer or with gastric cancer associated with high dihydropyrimidine dehydrogenase (DPD), with diffuse-type tumors associated more commonly than intestinal type with high DPD [13]. This result was expected, since S-1 consists of tegafur, otastat potassium, and gimestat which is a potent competitive inhibitor of DPD. Capecitabine is transformed to 5-FU in several steps, to be finally converted by TP as above [9]. A phase II trial in Japan showed that response rate (RR) was significantly higher (Fisher's exact test, $p = 0.028$) in patients with TP-positive and DPD-negative tumors (60%, 6/10) than in the remaining patients (13%, 2/15) [14]. In contrast, high expression of TP is reported to be negatively associated with efficacy of 5-FU or S-1 in gastric cancer [15,16].

On the basis of the above reports, histological type (diffuse or intestinal) and biomarkers (TP, DPD, and others) may be candidates to select whether S-1 or capecitabine be used for each patient, although validation with a randomized study is necessary. We planned the current clinical trial to elucidate the efficacy of XP and SP for the first-line treatment of AGC. This comparison will be also evaluated in terms of several biomarkers.

Method/design

Study objective

This randomized phase II trial is planned to elucidate the efficacy of SP and XP and also to explore predictive or prognostic biomarkers with additional research. This trial protocol has been approved by the Institutional Review Board (IRB) of each participating institution and the Kanagawa Cancer Center.

Study endpoints

Primary endpoint is PFS and secondary endpoints are OS, RR, time to treatment failure (TTF), and incidence of adverse events (safety).

Eligibility criteria

Inclusion criteria

(i) Histologically confirmed gastric adenocarcinoma with unresectable metastatic or recurrent disease
(ii) Lesions confirmed by imaging no more than 28 days before registration (not required for measurable lesions as defined in RECIST version 1.1)
(iii) No previous chemotherapy or radiotherapy. However, prior adjuvant chemotherapy is allowed if more than 6 months has passed since the end of adjuvant chemotherapy
(iv) Eastern Cooperative Oncology Group (ECOG) Performance Status of 0, 1, or 2
(v) Life expectancy of at least 3 months after registration
(vi) Written informed consent
(vii) Between the ages of 20 and 74 years at the time informed consent is obtained
(viii) Adequate major organ function including:
(a) Neutrophil count: ≥1500/mm^3
(b) Platelet count: ≥10.0 × 10^4/mm^3
(c) Hemoglobin: ≥9.0 g/dL
(d) AST, ALT: ≤2.5 × upper limit of normal (ULN) in each institution (≤5 times in cases of metastases to liver)
(e) ALP: ≤2.5 × ULN in each institution (≤5 times in cases of metastases to liver, and ≤10 times in cases of metastases to bone)

(f) Total bilirubin: ≤1.5 × ULN in each institution
(g) Creatinine clearance: ≥60 mL/min (as estimated by Cockcroft-Gault equation)

Exclusion criteria

(i) HER2- positive status
(ii) Previous history of fluoropyrimidine therapy within 6 months prior to registration
(iii) Previous treatment with platinum agents within 12 months prior to registration
(iv) Previous treatment with cisplatin more than total dose of 120 mg/m^2
(v) Previous history of serious hypersensitivity to fluoropyrimidines or platinum agents
(vi) Previous history of adverse reactions suggestive of dihydropyrimidine dehydrogenase (DPD) deficiency
(vii) More than 1 cancer at the same time or more than 1 cancer at different times separated by a 5-year disease-free interval. However, multiple active cancers do not include carcinoma *in situ* or skin cancer which is determined to have been cured as a result of treatment.
(viii) Obvious infection or inflammation (pyrexia ≥38.0°C)
(ix) Active hepatitis
(x) Heart disease that is serious or requires hospitalization, or history of such disease within the past year
(xi) Having a complication that is serious or requires hospitalization (intestinal paralysis, intestinal obstruction, interstitial pneumonia or pulmonary fibrosis, poorly controlled diabetes mellitus, renal failure, liver disorders, or hepatic cirrhosis)
(xii) Being treated or in need of treatment with flucytosine, phenytoin, or warfarin potassium
(xiii) Chronic diarrhea (watery stools or ≥4 times/day)
(xiv) Active gastrointestinal bleeding
(xv) Body cavity fluids requiring drainage or other treatment
(xvi) Clinical suspicion or previous history of metastasis to brain or meninges
(xvii) Women who are pregnant, breastfeeding, or potentially (hoping to become) pregnant
(xviii) Unwillingness to practice contraception
(xix) Poor oral intake
(xx) Psychiatric disorders which are being, or may need to be, treated with psychotropics
(xxi) Otherwise determined by investigators or site principal investigators to be unsuitable for participation in study

Registration

Physicians or coordinators will send a Case Registration Form to the data center (Epidemiological and Clinical Research Information Network, ECRIN) with all the required items filled out. Enrollment has started from July 2011.

Startification

Eligible patients will be randomized to either Arm-A (SP treatment) or Arm-B (XP treatment) by dynamic allocation via a centralized randomization method using 5 stratification factors as balancing variables:

(i) baseline ECOG Performance Status (0–1/2)
(ii) measurable lesion (yes/no)
(iii) prior adjuvant chemotherapy (yes/no)
(iv) histopathological classification (intestinal/diffuse)
(v) institution.

Statistical analysis

PFS has been set as the primary endpoint and is defined as the time from date of registration until the date that progression is determined or the date of death for any reason, whichever is sooner. "Progression" will be evaluated on the basis of Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 [17]. More information about the definition of PFS and Progression are pre-specified (Table 1).

The primary objective of this trial is to evaluate the PFS of SP and XP as the first-line treatment for advanced gastric cancer. The 24-week progression-free rate (PFR) will be estimated for each group, calculating point estimates and 2-sided 90% confidence intervals. The 2-sided 90% confidence interval of the difference between the 2 groups will be also estimated. Exploratory analysis will be done to test the null hypothesis that PFS is equal in both groups. Cumulative PFS curves will be constructed as time-to-event plots by the Kaplan-Meier method.

With respect to secondary endpoints, efficacy endpoints OS and TTF will be evaluated according to the method of analysis of the primary endpoint. Overall response rate (RR) is defined as the proportion of patients with complete response (CR) or partial response (PR) by RECIST out of the patients with measurable lesions, and the chi-square test will be used to compare the 2 groups. The 2-sided 95% confidence interval of the difference between the 2 groups will also be estimated. For the analysis of safety, Fisher's exact test will be used if necessary, and the exact confidence intervals for the binomial distribution will be estimated.

Sample-size calculation

Assuming a threshold 24-week PFR of 40% and an expected 24-week PFR of 55% (clinically promising), and a 1.5-year registration period and a 1.5-year follow-up period, 49 patients are required in each group to ensure a 1-sided alpha of 5% and statistical power of 90%. Assuming that the 24-week PFR of the biomarker-positive (any FU-related enzyme or expression of intestinal type) population

Table 1 Definition of PFS and progression

Definition of PFS and progression are predefined as below
1.) PFS will be determined as the time from the date of registration until the date that progression is determined or the date of death for any reason, whichever is sooner.
2.) "Progression (PD)" means both PD confirmed by routine diagnostic imaging in each course and PD confirmed by as-needed diagnostic imaging in the case that there is clinical suspicion of PD. In the latter case, it is preferable that there is at least objective evidence.
3.) When progression is determined based on diagnostic imaging, the date of progression will be the date on which imaging is assessed. When clinical progression is first determined independently of diagnostic imaging, and then later objectively determined on the basis of diagnostic imaging, the date of progression will be back-dated to the date of determination of clinical progression. If no objective evidence is obtained, it will be treated as a censoring event in the formal analysis, and sensitivity analysis will be also conducted as if this were PD.
4.). When considering tumor regrowth and determining PD according to RECIST, it is considered a PD as PFS event regardless of tumor diameter. But even if it is decided as PD according to RECIST, investigators can continue the protocol treatment if they consider continued treatment to be beneficial to the patient.
5.) If treatment discontinuation is needed due to symptomatic deterioration without any objective evidence at that time, it is reported as "symptomatic deterioration". Investigators should endeavor to obtain objective evidence of the progression even after discontinuation of treatment. In this case, the event shall be judged to be clinical PD and handled as mentioned in 2) above. When progression is determined on the basis of diagnostic imaging, the date of progression will be back-dated to the date of diagnosis of symptomatic deterioration.
6.) Survivors for whom progression has not been determined will be censored based on the last date on which the absence of progression was clinically confirmed (the last day that PFS was confirmed).
7.) Cases of discontinuation of protocol treatment because of toxicity or patient refusal, even if another therapy is added as a post-treatment, will be censored at the date of discontinuation or the date that post-treatment was started.
8.) In cases where progression is diagnosed on the basis of imaging, the event will be determined based not on evaluation dates where the result is "suspected" on imaging but on a subsequent evaluation date where progression is "confirmed" on imaging.
9.) Secondary cancer (multiple cancers in metachronous) will not be regarded as either an event or censored.

in the SP arm is 45%, and the risk reduction rate in the XP arm is 40%, 46 patients in total are needed to ensure a 2-sided alpha of 10% and statistical power of 70%. Under the hypothesis that the targeted biomarker-positive population is 50%, 92 patients in total are required. Considering the likelihood of some ineligible cases in the whole setting outlined above, the total sample size is set to 100. A following Phase III study will be designed for both randomized comparison and biomarker-oriented comparison of XP and SP (4 groups).

Treatment program

Patients who allocated SP will be treated with S-1 and cisplatin every 5-week cycle. S-1 will be administered orally at a dose of 40 mg/m^2 twice-daily (equivalent to a total daily dose of 80 mg/m^2) for 3 weeks (day 1 to 21). Cisplatin 60 mg/m^2 on day 8 of each cycle will be given by intravenous infusion over 2 hours. On the other hand, patients who allocated XP will be treated with capecitabine and cisplatin every 3-week cycle. Capecitabine will be administered orally at a dose of 1000 mg/m^2 twice-daily (equivalent to a total daily dose of 2000 mg/m^2) for 2 weeks (day 1 to 14). Cisplatin 80 mg/m^2 on day 1 of each cycle will be given by intravenous infusion over 2 hours.

Treatment continuation is intended until disease progression or unacceptable toxicity. If treatment continuation with cisplatin is determined to be unfeasible before any progression is confirmed, continuously monotherapy of S-1 or capecitabine will be continued until PD.

Follow-up

During treatment under this protocol, patients will have a physical check-up and a blood examination before every drug administration. PFS and RR will be monitored by using abdominal CT or MRI every 6 weeks and by measuring levels of tumor markers CEA and CA19-9.

Translational research project

Translational research will be conducted to elucidate the clinical utility of the following biomarkers. These biomarkers will be analyzed Immunohistochemistry (IHC) and mRNA expression by using tissue specimen. Tumor tissue samples from primary lesions and/or biopsy material will be collected and centralized assessment.

(i) Immunohistochemistry (IHC): Expression of TP, DPD, ERCC1, Ki67, LGALS4, and CDH17
(ii) mRNA: Expression of TP, DPD, thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT), and excision repair cross-complementation group1 (ERCC1)

Discussion

Recently, molecular target drugs has resulted in the opportunity to provide individualized treatment in the field of AGC. Especially in patients with HER2-positive AGC (defined as assessed by IHC 3+ on a scale of 0 to 3+, and/or fluorescence in-situ hybridization; FISH, *HER2*:CEP17 ratio ≥2.0), ToGA study showed that adding trastuzumab was significantly improved overall survival comparing with standard chemotherapy consists of cytotoxic drugs [11]. This study excludes HER2-positive gastric cancer since these patients should be recommended trastuzumab containing regimen. The individualized treatment for cytotoxic agents also needs to be developed to have more effect and less toxicity.

This is the first study to compare two standard regimens for AGC. Additionally, the translational research is

performed to explore the biomarker for chemo-sensitivity and make the individualized treatment possible. When the difference of treatment is found in efficacy or safety from this analysis, we will conduct a phase III trial to examine the possibility of individualized treatment. We believe the result of this study will play the important role to prepare the individualized therapy for advanced gastric cancer in the near future.

Competing interests
All authors declare that they have no competing interest.

Authors' contributions
AT drafted the manuscript and wrote the original protocol for the study. All authors participated in the design of the study. SM performed the statistical analysis. All authors read and approved the final manuscript.

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References
1. International Agency for Research on Cancer: *GLOBOCAN*.: 2008. http://www-dep.iarc.fr/CancerMondial.htm.
2. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML, Ajani JA, V325 Study Group: **Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group.** *J Clin Oncol* 2006, **24**(31):4991–4997.
3. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR, Upper Gastrointestinal Clinical Studies Group of the National Cancer Research Institute of the United Kingdom: **Capecitabine and oxaliplatin for advanced esophagogastric cancer.** *N Engl J Med* 2008, **358**(1):36–46.
4. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M: **S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial.** *Lancet Oncol* 2008, **9**(3):215–221.
5. Kang YK, Kang WK, Shin DB, Chen J, Xiong J, Wang J, Lichinitser M, Guan Z, Khasanov R, Zheng L, Philco-Salas M, Suarez T, Santamaria J, Forster G, McCloud PI: **Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial.** *Ann Oncol* 2009, **20**(4):666–673.
6. Ajani JA, Rodriguez W, Bodoky G, Moiseyenko V, Lichinitser M, Gorbunova V, Vynnychenko I, Garin A, Lang I, Falcon S: **Multicenter phase III comparison of cisplatin/S-1 with cisplatin/infusional fluorouracil in advanced gastric or gastroesophageal adenocarcinoma study: the FLAGS trial.** *J Clin Oncol* 2010, **28**(9):1547–1553.
7. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T: **Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients.** *Eur J Cancer* 1998, **34**(11):1715–1720.
8. Koizumi W, Kurihara M, Nakano S, Hasegawa K: **Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 Cooperative Gastric Cancer Study Group.** *Oncology* 2000, **58**(3):191–197.
9. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, Shimma N, Umeda I, Ishitsuka: **Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue.** *Eur J Cancer* 1998, **34**(8):1274–1281.
10. *NCCN Clinical Practice Guidelines for Treatment of Cancer by site.*: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.
11. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK, ToGA Trial Investigators: **Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial.** *Lancet* 2010, **376**(9742):687–697.
12. Ohtsu A, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK: **Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study.** *J Clin Oncol* 2011, **29**(30):3968–3976.
13. Yamada Y, Yamamoto S, Ohtsu A, Suzuki Y, Nasu J, Yamaguchi K, Denda T, Tsuji A, Hara Y, Boku N, Gastrointestinal Oncology Study Group/Japan Clinical Oncology Group: **Impact of dihydropyrimidine dehydrogenase status of biopsy specimens on efficacy of irinotecan plus cisplatin, S-1, or 5-FU as first-line treatment of advanced gastric cancer patients in JCOG9912.** *ASCO Meeting Abstracts*, **27**(15s):4535.
14. Koizumi W, Okayasu I, Hyodo I, Sakamoto J, Kojima H, Clinical Study Group of Capecitabine: **Prediction of the effect of capecitabine in gastric cancer by immunohistochemical staining of thymidine phosphorylase and dihydropyrimidine dehydrogenase.** *Anticancer Drugs* 2008, **19**(8):819–824.
15. Ichikawa W, Takahashi T, Suto K, Hirayama R: **Gene expressions for thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT), and thymidine phosphorylase (TP), not dihydropyrimidine dehydrogenase (DPD), influence outcome of patients (pts) treated with S-1 for gastric cancer (GC).** *J Clin Oncol (Meeting Abstracts) ASCO Meeting Abstracts* 2004, **22**(14_suppl):4050.
16. Napieralski R, Ott K, Kremer M, Specht K, Vogelsang H, Becker K, Müller M, Lordick F, Fink U, Rüdiger Siewert J, Höfler H, Keller G: **Combined GADD45A and thymidine phosphorylase expression levels predict response and survival of neoadjuvant-treated gastric cancer patients.** *Clin Cancer Res* 2005, **11**(8):3025–3031.
17. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J: **New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1).** *Eur J Cancer* 2009, **45**(2):228–247.

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CLINICAL TRIAL REPORT

Metronomic oral combination chemotherapy with capecitabine and cyclophosphamide: a phase II study in patients with HER2-negative metastatic breast cancer

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Abstract
Purpose Metronomic combination chemotherapy with the oral fluoropyrimidine doxifluridine/5′-deoxy-5-fluorouridine (5 -DFUR) and oral cyclophosphamide (C) showed promising efficacy in a single-arm study. The oral fluoropyrimidine capecitabine was designed to deliver 5-fluorouracil preferentially to tumors, potentially improving efficacy over doxifluridine. We conducted a phase II multicenter study to evaluate an all-oral XC combination in patients with HER2-negative metastatic breast cancer (MBC).
Materials and methods Patients received capecitabine 828 mg/m^2 twice daily with cyclophosphamide 33 mg/m^2 twice daily, days 1–14 every 3 weeks. The primary endpoint was overall response rate (ORR). Secondary endpoints included progression-free survival (PFS), overall survival (OS), and safety.
Results Between May 2007 and April 2009, 51 patients were enrolled and 45 were included in the efficacy analysis. The median follow-up was 18.1 months. ORR was 44.4% and stable disease (≥24 weeks) was achieved in 13.4%, resulting in a 57.8% clinical benefit response rate. Median PFS was 12.3 months (95% confidence interval: 8.9–18.9 months). Median PFS was 10.7 months in triple-negative disease and 13.2 months in estrogen-receptor positive, HER2-negative disease. The 1- and 2-year OS rates were 86 and 71%, respectively. Median OS has not been reached. Grade 3 adverse events comprised leukopenia (26%), neutropenia (16%), and decreased hemoglobin (2%). There was no grade 3 hand-foot syndrome.
Conclusions Oral XC is an effective first- or second-line therapy for MBC, demonstrating high activity in both luminal A and triple-negative disease with few severe side effects. This metronomic oral combination chemotherapy could be beneficial for the treatment of HER2-negative MBC.

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Introduction

Recent advances in chemotherapy for breast cancer have produced remarkable results in the adjuvant setting [1], but less improvement in the metastatic setting [2]. Regimens including anthracyclines and taxanes are widely used, but metastatic breast cancer (MBC) remains an incurable disease, although a small proportion of patients may achieve long-term disease-free survival [3, 4]. Therefore, the main goal of treatment for MBC is to prolong survival and maintain quality of life (QOL). Standard chemotherapy regimens, based on the concept of maximum tolerated dose (MTD), for front line can achieve relatively high response rates, but do not satisfy the needs of patients in terms of survival and QOL because of the short duration of clinical benefit and the detrimental impact of treatment-related toxicity.

In contrast to standard chemotherapy, low-dose metronomic (LDM) chemotherapy describes the prolonged administration of relatively low doses of cytotoxic agents at short, regular intervals without extended breaks. This therapeutic strategy has become widely recognized following the discovery that some cytostatic agents administered using an LDM schedule have significant antiangiogenic activity. Studies have shown that chronic administration of low-dose chemotherapy, including cyclophosphamide, methotrexate, and other agents, produces apoptosis of endothelial cells in the tumor microvasculature, resulting in impairment of repair processes and a reduction in the level of viable circulating endothelial progenitor cells [5]. LDM chemotherapy may provide a strategy to achieve long-term disease control by maintaining tumor dormancy and potentially extending survival, with only mild side effects [6]. The antiangiogenic activity of LDM chemotherapy may also contribute to a decreased susceptibility to drug resistance [7–9]. However, the clinical efficacy of LDM chemotherapy and the optimal regimen has yet to be established.

Capecitabine is a precursor of 5-fluorouracil (5-FU), which is converted to 5′-deoxy-5-fluorouridine (5′-DFUR) in the presence of carboxylesterase and cytidine deaminase mainly in the liver, and then to 5-FU in the presence of thymidine phosphorylase (TP), a strong antiangiogenic factor identical to platelet-derived endothelial cell growth factor [10]. TP, the key enzyme mediating the final activation step, is present at significantly higher concentrations in the tumor than in other tissues, leading to preferential delivery of capecitabine to 5-FU on the tumor site with limited impact on non-tumor tissue. In xenograft models, TP is upregulated by several chemotherapeutic agents, including taxanes, cyclophosphamide, and mitomycin [11, 12]. These findings provide a compelling rationale for combining capecitabine with potentially synergistic anticancer drugs. For example, the combination of capecitabine with either docetaxel or paclitaxel has been evaluated extensively in MBC and demonstrated considerable activity with a modest impact on toxicity [13, 14].

Based on previously reported preclinical and clinical results [15, 16], we evaluated a metronomic oral combination chemotherapy regimen of doxifluridine and cyclophosphamide. The regimen demonstrated encouraging efficacy, which we considered may be further improved by replacing doxifluridine with capecitabine [17]. Capecitabine combined with oral cyclophosphamide was shown to be feasible and tolerable in a pilot phase I study [18]. We therefore conducted a single-arm phase II study of the capecitabine/cyclophosphamide (XC) regimen as treatment for HER2-negative MBC.

Patients and methods

Patients

Patients eligible for inclusion in the study were aged >20 years at time of enrollment and had histologically or cytologically confirmed advanced or recurrent breast cancer that was measurable according to Response Evaluation Criteria in Solid Tumors (RECIST) and determined to be HER2-negative by immunohistochemistry (IHC 0 or 1+) or fluorescence in situ hybridization (FISH negative). Patients with unknown HER2 status were eligible. All patients were required to have a life expectancy of at least 6 months, an Eastern cooperative oncology group performance status (ECOG PS) of 0–2 (except for patients with pain of PS 3 caused by bone metastasis), sufficient organ function to allow safety evaluation, and to be capable of receiving oral therapy. Eligible patients had received no more than 1 prior chemotherapy regimen, no previous treatment with the combination with doxifluridine and cyclophosphamide or capecitabine-containing therapy, and were required to have no carry-over effects from previous treatments. Prior radiotherapy to the target measurable lesion was not permitted. The study was approved by the ethics committees at participating institutions, and all patients provided written informed consent.

Study design

Capecitabine (828 mg/m^2 twice daily) and cyclophosphamide (33 mg/m^2 twice daily) were both administered orally, days 1–14, followed by a 7 day drug free interval (days 15–21). Treatment was continued for at least 6 cycles or disease progression. Delay in treatment cycle due to

toxicity was allowed if the interval was ≤14 days, otherwise the patient was required to discontinue study treatment.

During the study, drug dosage was adjusted in patients experiencing treatment-related adverse events of grade 2 or higher intensity, graded according to Common Terminology Criteria for Adverse Events (CTCAE) v 3.0 [19]. At the first occurrence of a grade 2 event, treatment was interrupted until resolution to grade 1 or 0 and resumed at the original dose. Recurrences of grade 2 events were managed by treatment interruption followed by a 25% dose reduction. If grade 3 or 4 toxicity occurred, treatment was interrupted and continued with a 25 or 50% dose reduction, respectively. If the same grade 2 toxicity occurred for a third time, treatment was interrupted until the adverse event resolved to grade 0–1 and then continued at 50% of the original dose. At the third occurrence of a given toxicity (grade 3 severity), treatment was discontinued and the patient withdrawn from the study.

Study endpoints

The primary endpoint was overall response rate (ORR), and secondary endpoints were progression-free survival (PFS), overall survival (OS), clinical benefit response (CBR) defined as complete response (CR) plus partial response (PR) plus long-term (≥24 weeks) stable disease (LSD), and safety.

Assessment of response rate and adverse events

Tumor response was assessed according to RECIST version 1.0 [20]. Evaluation of response was performed after every 2 cycles during the treatment. Adverse events were graded according to CTCAE v 3.0 [19].

Statistical analysis

Assuming an ORR of 50% and a threshold ORR of 30%, based on the literature [13, 14, 21], a sample size of 43 patients was required to give 80% power with $\alpha = 0.05$. Therefore, the target sample size was 50 patients over a 1-year period, allowing for dropouts and inclusion of non-evaluable patients.

Analyses of efficacy and safety were performed in the per-protocol set (PPS) population. The PPS population comprised subjects fulfilling the study inclusion criteria. PFS was estimated by the Kaplan–Meier method.

Results

Between May 2007 and April 2009, 51 patients were enrolled. The median duration of follow-up was 18.1 months. The baseline characteristics of patients are shown in Table 1. The median age of patients was 61 years (range, 32–82) and the majority (38 of 51) of patients had PS 0. Efficacy was evaluated in 45 patients excluding ineligible patients including 1 patient with HER2-positive breast cancer and 5 patients with no target region. Safety was evaluated in 51 patients.

Table 1 Patient characteristics ($n = 51$)

Median age, years (range)	61 (32–82)
PS (ECOG): 0/1/2/unknown	38/9/3/1
Tumor histological types[a]: scirrhous/solid-tubular/papillotubular carcinoma/other	21/12/14/4
HER2 status[b]: positive/negative/unknown	1/42/8
ER status: positive/negative/unknown	32/18/1
PgR status: positive/negative/unknown	29/21/1
Triple negative (ER-, PgR-, and HER2-negative)	10
Surgical operation for primary breast cancer: yes/no	44/7
Post-operative radiation therapy: yes/no	25/26
Prior adjuvant treatment[c]	
Anthracyclines	23
Taxanes	17
Anthracyclines and taxanes	17
Hormone therapy	31
Others (CMF, 5′DFUR, UFT, 5-FU)	13
Number of prior chemotherapy regimens for MBC: 0/1	41/10
Prior MBC treatment	
Anthracyclines[d]	6
Taxanes	3
Hormone therapy	17
Others (doxifluridine)	2

PS, performance status; *ECOG*, Eastern co-operative oncology group; *HER2* human epidermal growth factor receptor type 2; *ER*, estrogen receptor; *PgR*, progesterone receptor; *CMF*, cyclophosphamide, methotrexate, 5-flourouracil; *5′-DFUR*, doxifluridine; *UFT*, tegafur uracil; *5-FU*, 5-flourouracil; *MBC*, metastatic breast cancer

[a] According to the pathological classification of the Japanese breast cancer societyl

[b] One HER2-positive patient was excluded from our analysis

[c] Some patients received more than 1 chemotherapy regimen

[d] One patient received epirubicin and paclitaxel

Efficacy

Among the 45 patients evaluable for efficacy, 4 achieved a CR and 16 achieved a PR, giving an ORR of 44.4%. SD and PD were reported 11 and 7 patients, respectively. LSD was reported in an additional 6 patients, and therefore, the CBR (CR + PR + LSD) was 57.8% (Table 2). A subanalysis of clinical response according to hormone-receptor status showed that the CR and PR rates among patients

Table 2 Response to the treatment ($n = 45$)[a]

	CR	PR	ORR (%)	CBR (%)
All patients	4	16	44.4	57.8
Hormone receptor ($n = 44$)[b]				
ER-positive ($n = 28$)	2	11	46.4	64.3
ER-negative ($n = 17$)	2	5	41.2	47.1
PgR-positive ($n = 26$)	3	8	42.3	53.8
PgR-negative ($n = 19$)	1	8	47.4	63.2
Triple negative (ER-, PgR-, and HER2-negative) ($n = 9$)	0	4	44.4	55.6
Prior anthracyclines; Adjuvant+MBC ($n = 23$)	3	6	39.1	52.2
Prior taxanes; Adjuvant + MBC ($n = 13$)	2	4	46.2	53.8
Major metastatic organ[c]				
Organ (liver and lung) ($n = 33$)	3	12	45.5	57.6
Bone ($n = 7$)	0	5	71.4	85.7
Soft tissue only (lymph node and skin) ($n = 10$)	1	7	40.0	50.0
Hand-foot syndrome				
Yes ($n = 24$)	2	9	45.8	62.5
No ($n = 21$)	2	6	38.1	47.6

CR, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; *NE*, not evaluable; *ORR*, overall response; *CBR*, clinical benefit rate (CR+PR+LSD); *LSD*, long-term (≥24 weeks) stable disease; *ER*, estrogen receptor; *PgR*, progesterone receptor: *HER2*, human epidermal growth factor receptor type 2; *MBC*, metastatic breast cancer

[a] Evaluable patient excluding no target region

[b] One patient, unknown

[c] Patients may have metastases at more than 1 major organ site

with estrogen receptor (ER)-positive disease were 2 of 28 and 11 of 28, respectively, producing an ORR of 46.4%, while a further 5 patients achieved LSD, resulting in a CBR of 64.3%. In comparison, 4 of 9 patients with triple-negative disease achieved a PR (ORR 44.4%) and 1 patient achieved LSD resulting in a CBR of 55.6%.

PFS curves for the overall population and according to hormone-receptor status are shown in Fig. 1. The median PFS for the overall population was 12.3 months (95% confidence interval [CI]: 8.9–18.9 months). The median PFS in 34 patients with ER- and/or progesterone receptor (PgR)-positive disease was 13.2 months (95% CI: 8.9–23.7 months), while in 10 patients with ER- and PgR-negative and HER2-negative (triple-negative) disease, it was 10.7 months (95% CI: 3.9–20.0 months). A subanalysis of efficacy according to use of prior chemotherapy in the adjuvant or metastatic disease settings showed that median PFS was 9.3 months (95% CI: 5.1–18.9 months) in 24 patients previously treated with anthracycline-containing therapy and 13.9 months (95% CI: 8.5–23.7 months) in 22 anthracycline-naïve patients (figure not shown). Median OS has not been reached (Fig. 2). The 1- and 2-year cumulative OS rate were 86% (95% CI: 76–96%) and 71% (95% CI: 54–88%), respectively.

Safety

Safety was evaluated in 51 patients (Table 3). The most commonly reported adverse events of grade 3 or higher intensity were leukopenia in 13 patients (25%), neutropenia in 8 (16%), decreased hemoglobin in 1 (2%), and alkaline phosphatase elevation in 1 (2%). Hand-foot syndrome (HFS) of any grade was reported in 27 patients (53%); however, the severity was only grade 1 or 2 in all cases. No patient experienced grade 3 HFS. Grade 1 alopecia was reported in 1 patient (2%). No patient was withdrawn from the study because of adverse events.

Discussion

Although standard chemotherapy may eradicate breast cancer micrometastases and improve the cure rate of patients with breast cancer in the adjuvant setting, such an approach is inevitably unsuccessful for overt metastatic cancers even when dose-intensive regimens are administered, as evidenced by the failure of high-dose chemotherapy strategies in clinical trials [22]. The limitations of standard chemotherapy may be related to the mechanism of action of anticancer agents and the dynamics of tumor growth. The cytotoxicity of the majority of anticancer drugs is attributable to direct DNA damage and disruption of DNA replication, especially in proliferating cells. However, based on the assumption that proliferating cells comprise only a minor proportion of the tumor and the proliferation period is very short [23], it is unlikely that bulky metastatic tumors could be eradicated by standard chemotherapy regimens administered using short-period, intermittent schedules. Furthermore, it is not practical to administer standard chemotherapy successfully for prolonged periods because of severe cumulative toxicities, which requires relatively long treatment-free recovery

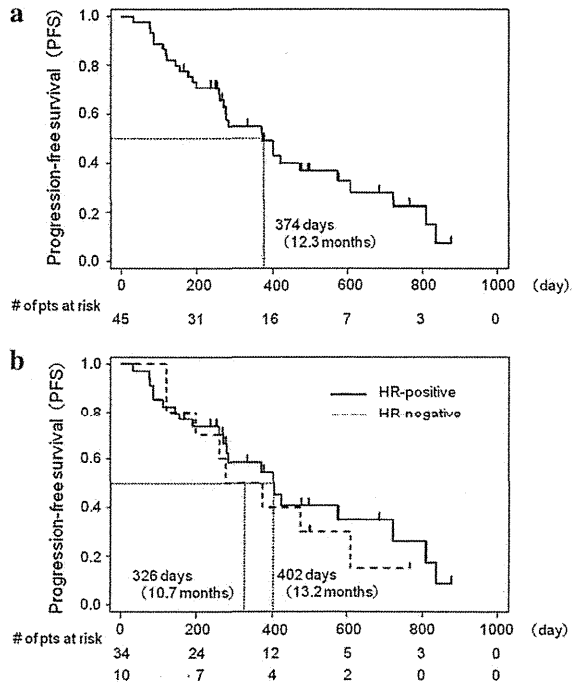

Fig. 1 Progression-free survival (PFS). **a** PFS for 45 patients treated with oral capecitabine/cyclophosphamide therapy and evaluable for efficacy. Median PFS was 12.3 months (95% CI; 8.6–18.9 months). **b** PFS analyzed according to hormone-receptor (HR) status. HR-positive ($n = 34$) was defined as estrogen receptor (ER)-positive and/or progesterone receptor (PgR)-positive. Median PFS for patients with HR-positive disease was 13.2 months (95% CI; 8.9–23.7 months; *solid line*). HR-negative ($n = 9$) was defined as triple-negative breast cancer (ER-, PgR-, and HER2-negative) and its median PFS was 10.7 months (95% CI; 3.9–20.0 months; *dotted line*)

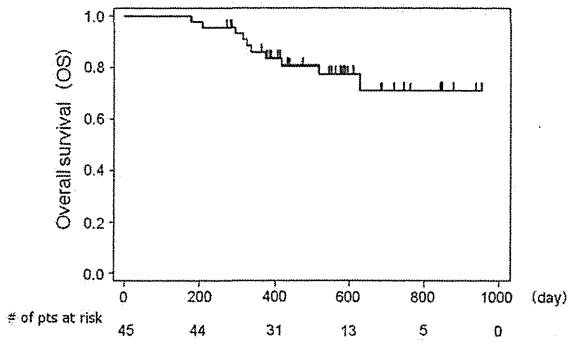

Fig. 2 Overall survival (OS). Median OS was not reached. The 1- and 2-year cumulative OS rate was 86% (95% CI: 76–96%) and 71% (95% CI: 54–88%), respectively

periods that allow regrowth of the tumor [6]. The potential limitations of standard chemotherapy are particularly relevant in the treatment of patients with slowly growing

Table 3 Toxicity ($n = 51$)

	All grades (%)	≥Grade 3 (%)
Hematological toxicity		
Leukopenia	36 (70.6)	13 (25.5)
Neutropenia	20 (39.2)	8 (15.7)
Decreased hemoglobin	37 (72.5)	1 (2.0)
Thrombocytopenia	7 (13.7)	0 (0)
Non-hematological toxicity		
Nausea	10 (19.6)	0 (0)
Vomiting	3 (5.9)	0 (0)
Diarrhea	3 (5.9)	0 (0)
Stomatitis	6 (11.8)	0 (0)
Dysgeusia	2 (3.9)	0 (0)
Anorexia	12 (23.5)	0 (0)
Fatigue	10 (19.6)	0 (0)
Hyperpigmentation	16 (31.4)	0 (0)
Dizziness	5 (9.8)	0 (0)
Palpitations	3 (5.9)	0 (0)
HFS	27 (52.9)	0 (0)
Alopecia	1 (2.0)	0 (0)
Liver dysfunction		
AST	20 (39.2)	0 (0)
ALT	13 (25.5)	0 (0)

All enrolled patients were included in the safety analysis

HFS, hand-foot syndrome; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase

breast cancers, such as the ER-positive, HER2-negative (luminal A) subtype. In this clinical scenario, conventional standard chemotherapy regimens may be less effective in slowly growing than in more rapidly growing breast cancer subtypes. It is apparent, therefore, that standard chemotherapy has inherent limitations for the treatment of MBC. Alternatively, it has been suggested that continuous, chronic administration of anticancer drugs (metronomic chemotherapy) may be required for the additional effective treatment of bulky metastatic breast tumors. If this hypothesis is correct, then the introduction of metronomic chemotherapy provides a new paradigm to overcome the shortcomings of conventional standard chemotherapy for patients with MBC.

Metronomic chemotherapy is based on more frequent administration of low-dose cytotoxic agents compared with conventional standard chemotherapy and is designed to prevent tumor angiogenesis. The potential of metronomic chemotherapy was first demonstrated in animal models a decade ago [8], and the efficacy of this approach has been confirmed in the clinic [6, 7]. Although variable outcomes have been achieved with metronomic chemotherapy, clinical studies have shown that this new treatment strategy represents an interesting alternative for the management of

patients with MBC [16]. Accumulating evidence suggests that the efficacy of metronomic chemotherapy is not only attributable to its antiangiogenic activity. Potential new mechanisms of action also include restoration of the anticancer immune response and the induction of "tumor dormancy," which may contribute to prolongation of survival [24].

The results from the present study showed that metronomic chemotherapy comprising an all-oral combination of capecitabine and cyclophosphamide achieved an acceptably high response rate of 44.4%, CBR of 57.8%, and sufficiently long PFS of 12.3 months for patients with HER2-negative MBC. The median OS was not reached at the time of reporting, and the 1-year cumulative OS rate was as high as 86%.

These results are comparable in terms of the magnitude of clinical benefit to those achieved with other standard chemotherapeutic regimens, although the characteristics of patients included in studies were different. In phase III clinical trials in MBC, single-agent treatment with docetaxel or paclitaxel produced ORRs in the range 14–43% and time to progression of 3.5-7.0 months [25]. In comparison, the results of our study, ORR 44.4% and PFS 12.3 months, appear to be relatively favorable.

The side effects of XC chemotherapy were mild, and the administration schedule was feasible. There were no nonhematological side effects occurring at an intensity of grade 3 or higher. HFS was reported in 53% of patients, but all cases were categorized as grade 1 or 2. Long-term treatment was tolerable, and the major reason for discontinuation was tumor progression.

It is thought that synergistic activity between capecitabine and cyclophosphamide, probably associated with TP activation by cyclophosphamide, contributes at least in part to these results. The promising efficacy seen in the present study may also be attributable to the lower risk of toxicity and immunosuppression associated with both capecitabine and cyclophosphamide than standard polychemotherapy regimens [26]. In addition, combining these two agents is rational based on their non-overlapping dose-limiting toxicities (HFS and liver toxicity with capecitabine and hematological toxicity with cyclophosphamide) and complementary mechanisms of anticancer action, with capecitabine having activity against cyclophosphamide-resistant cancer cells [15] and cyclophosphamide-inhibiting tumor neovascularization.

These results suggest that metronomic chemotherapy with XC offers many advantages over standard parenterally administered chemotherapy. The convenience of oral administration increases treatment options for many patients with MBC while the lack of severe side effects helps patients to maintain their QOL. An additional advantage is that oral XC should reduce the costs associated with the treatment of MBC because it does not require hospital admission, rescue treatments such as granulocyte-colony stimulating factor, or other supportive care for gastrointestinal symptoms. In addition, mild and gradual decreases in bone marrow function permit extended intervals between hematologic monitoring.

It has been suggested that chemotherapy is less effective for patients with ER-positive, HER2-negative tumors than for ER-negative tumors [27–30]. It is notable that in our study, the oral combination of capecitabine and cyclophosphamide produced similar response rates in ER-positive and ER-negative MBC, although this observation is based on a retrospective subgroup analysis. The ORR, CBR, and median PFS reported in our study were 46.4 versus 41.2%, 64.3 versus 47.1%, and 13.2 versus 10.7 months for patients with ER-positive and ER-negative MBC, respectively. In a previous report, oral combination chemotherapy with doxifluridine and cyclophosphamide achieved superior results in patients with ER-positive than ER-negative MBC [16]. Additionally, the results of that study showed a trend toward superior ORR in patients with a longer disease-free interval than in those with a shorter disease-free interval for MBC. They showed that the response rates according to disease-free intervals of $\leq$2 years, 2–5 years, and >5 years were 50, 64, and 68%, respectively (the differences were not significant) [16]. On the basis that ER-positivity and a longer disease-free interval characterize less aggressive, slowly proliferating breast cancers, which may be less responsive to chemotherapy than ER-negative tumors and/or those with a shorter disease-free interval [31], it is hypothesized that metronomic chemotherapy as used in the present study may be better suited than conventional regimens for patients with slowly growing tumors or luminal A breast cancer.

In conclusion, the oral combination of capecitabine and cyclophosphamide was shown to be a very feasible and convenient regimen with mild side effects and substantial efficacy in patients with HER2-negative MBC regardless of ER status. The XC regimen may fulfill several requirements for the ideal metronomic treatment. This metronomic chemotherapy regimen may offer an additional new option for patients with MBC, especially for those with ER-positive, HER2-negative (luminal A) breast cancer. Additional research to confirm these promising results is warranted.

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References

1. Early Breast Cancer Trialist Collaborative Group (2005) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687–1717
2. Mauri D, Polyzos NP, Salanti G, Pavlidis N, Ioannidis JP (2008) Multiple-treatments meta-analysis of chemotherapy and targeted therapies in advanced breast cancer. J Natl Cancer Inst 100:1780–1791
3. Rahman ZU, Frye DK, Smith TL, Asmar L, Theriault RL, Buzdar AU, Hortobagyi GN (1999) Results and long term follow-up for 1581 patients with metastatic breast carcinoma treated with standard dose doxorubicin-containing chemotherapy: a reference. Cancer 85:104–111
4. Hortobagyi GN (2002) Can we cure limited metastatic breast cancer? J Clin Oncol 20:620–623
5. Mancuso P, Colleoni M, Calleri A, Orlando L, Maisonneuve P, Pruneri G, Agliano A, Goldhirsch A, Shaked Y, Kerbel RS, Bertolini F (2006) Circulating endothelial-cell kinetics and viability predict survival in breast cancer patients receiving metronomic chemotherapy. Blood 108:452–459
6. Pasquier E, Kavallaris M, André N (2010) Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 7:455–465
7. Kamen BA, Rubin E, Aisner J, Glatstein E (2000) High-time chemotherapy or high time for low dose. J Clin Oncol 18:2935–2937
8. Hanahan D, Bergers G, Bergsland E (2000) Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J Clin Invest 105:1045–1047
9. Browder T, Butterfield CE, Kräling BM, Shi B, Marshall B, O'Reilly MS, Folkman J (2000) Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 60:1878–1886
10. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W, Heldin CH (1989) Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338:557–562
11. Sawada N, Ishikawa T, Fukase Y, Nishida M, Yoshikubo T, Ishitsuka H (1998) Induction of thymidine phosphorylase activity and enhancement of capecitabine efficacy by taxol/taxotere in human cancer xenografts. Clin Cancer Res 4:1013–1019
12. Endo M, Shinbori N, Fukase Y, Sawada N, Ishikawa T, Ishitsuka H, Tanaka Y (1999) Induction of thymidine phosphorylase expression and enhancement of efficacy of capecitabine or 5′-deoxy-5-fluorouridine by cyclophosphamide in mammary tumor models. Int J Cancer 83:127–134
13. O'Shaughnessy J, Miles D, Vukelja S, Moiseyenko V, Ayoub JP, Cervantes G, Fumoleau P, Jones S, Lui WY, Mauriac L, Twelves C, Van Hazel G, Verma S, Leonard R (2002) Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. J Clin Oncol 20:2812–2823
14. Blum JL, Dees EC, Chacko A, Doane L, Ethirajan S, Hopkins J, McMahon R, Merten S, Negron A, Neubauer M, Ilegbodu D, Boehm KA, Asmar L, O'Shaughnessy JA (2006) Phase II trial of capecitabine and weekly paclitaxel as first-line therapy for metastatic breast cancer. J Clin Oncol 24:4384–4390
15. Endo M, Fujimoto-Ouchi K, Matsumoto T, Tanaka Y, Ishitsuka H (1997) Efficacy of combination chemotherapy of cyclophosphamide and 5′-deoxy-5-fluorouridine in a mammary tumor xenograft model, MX-1. Jpn J Cancer Chemother 24:1295–1301
16. Yoshimoto M, Tada K, Tokudome N, Kutomi G, Tanabe M, Goto T, Nishimura S, Makita M, Kasumi F (2003) The potential for oral combination chemotherapy of 5′-deoxy-5-fluorouridine, a 5-FU prodrug, and cyclophosphamide for metastatic breast cancer. Br J Cancer 89:1627–1632
17. Ebi H, Sigeoka Y, Saeki T, Kawada K, Igarashi T, Usubuchi N, Ueda R, Sasaki Y, Minami H (2005) Pharmacokinetic and pharmacodynamic comparison of fluoropyrimidine derivatives, capecitabine and 5′-deoxy-5-fluorouridine (5′-DFUR). Cancer Chemother Pharmacol 56:205–211
18. Ohno S, Mitsuyama S, Tamura K, Nishimura R, Tanaka M, Hamada Y, Kuroki S, Kyushu Breast Cancer Study Group (2007) Dosage of capecitabine and cyclophosphamide combination therapy in patients with metastatic breast cancer. Anticancer Res 27:1009–1013
19. Common Terminology Criteria for Adverse Events (CTCAE v3.0) (2004) 2003 (Japanese translation JCOG/JCSP version, 2004) Int J Clin Oncol 9(Suppl III):1–82 (in Japanese)
20. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205–216
21. Kusama M, Nomizu T, Aogi K, Yoshimoto M, Horikoshi N, Tabei T, Noguchi S, Miura S, Yoshimura N, Kimura M, Toyama K, Shin E (2010) Phase II study of 4-weekly capecitabine monotherapy in advanced/metastatic breast cancer. Breast Cancer 17:233–240
22. Banna GL, Simonelli M, Santoro A (2007) High-dose chemotherapy followed by autologous hematopoietic stem-cell transplantation for the treatment of solid tumors in adults: a critical review. Curr Stem Cell Res Ther 2:65–82
23. Epstein RJ (1999) Drug-induced DNA damage and tumor chemosensitivity. J Clin Oncol 8:2062–2084
24. Goss PE, Chambers AF (2010) Does tumour dormancy offer a therapeutic target? Nat Rev Cancer 10:871–877
25. O'Shaughnessy J (2005) Extending survival with chemotherapy in metastatic breast cancer. Oncologist 10:20–29
26. Tanaka Y, Eda H, Fujimoto K, Tanaka T, Ishikawa T, Ishitsuka H (1990) Anticachectic activity of 5′-deoxy-5-fluorouridine in a murine tumor cachexia model, colon 26 adenocarcinoma. Cancer Res 50:4528–4532
27. Huober J, von Minckwitz G, Denkert C, Tesch H, Weiss E, Zahm DM, Belau A, Khandan F, Hauschild M, Thomssen C, Högel B, Darb-Esfahani S, Mehta K, Loibl S (2010) Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study. Breast Cancer Res Treat 124:133–140
28. Hall C, Krishnamurthy S, Lodhi A, Mosalpuria K, Kuerer HM, Meric-Bernstam F, Bedrosian I, Hunt KK, Lucci A (2010) Disseminated tumor cells in biologic subtypes of stage I-III breast cancer patients. Ann Surg Oncol 17:3252–3259
29. Toi M, Nakamura S, Kuroi K, Iwata H, Ohno S, Masuda N, Kusama M, Yamazaki K, Hisamatsu K, Sato Y, Kashiwaba M, Kaise H, Kurosumi M, Tsuda H, Akiyama F, Ohashi Y, Takatsuka Y, Japan Breast Cancer Research Group (JBCRG) (2008) Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival. Breast Cancer Res Treat 110:531–539