evident only in homozygotes of ABCB1*2, ABCG2#IIB, SLCO1B1*15 · 17 in the cisplatin-combination therapy. With combinations of haplotypes/variations of two or more genes, neutropenia incidence increased, but their prediction power for grade 3/4 neutropenia is still unsatisfactory.

Conclusions Certain transporter genotypes additively increased irinotecan-induced neutropenia, but their clinical importance should be further elucidated.

Keywords Irinotecan · Transporter · Genetic polymorphism · Haplotype

Introduction

Irinotecan, an anticancer prodrug, is widely used for treating a broad range of carcinomas including colorectal and lung cancers. However, unexpected severe diarrhea and neutropenia are important clinical side effects from irinotecan treatment. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1], and is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (UGTs), such as UGT1A1, UGT1A7, and UGT1A9, to form an inactive metabolite, SN-38 glucuronide (SN-38G) [2–4]. Irinotecan is also inactivated by CYP3A4 to produce 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC) [5]. Irinotecan and its metabolites are excreted into the bile and urine via the action of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), multiple resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) [6]. Transport of SN-38 from the plasma into the liver is mediated by the organic anion transporting polypeptide C (OATP-C/SLCO1B1) [7]. Most of the previous pharmacogenetic studies on irinotecan have focused on UGT1A1 polymorphisms and have shown clinical relevance of UGT1A1*28, a repeat polymorphism in the TATA box [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA or −40_−39ins TA], to severe toxicities [8–10]. Based on these findings, in 2005, the Food and Drug Administration (FDA) of the United States approved an amendment for the label of Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028) and the clinical use of a genetic diagnostic kit for the *28 allele. In parallel with this advance in the USA, clinical relevance to severe neutropenia of UGT1A1*6 [211G>A (G71R)], another low-activity allele detected specifically in East-Asians, as well as *28 was demonstrated in several studies on Asian patients [11–14]. Accordingly, in June 2008, the Ministry of Health, Labor and Welfare of Japan approved changes to irinotecan labels (Campto and

Topotecin) by adding a caution for the risk of severe toxicities in patients either homozygous or compound heterozygous for UGT1A1*28 and *6 (*28/*28, *6/*6, *28/*6) and the clinical use of a diagnostic kit for UGT1A1*28 and *6. Severe toxicities, however, are found in patients without *6/*6, *28/*28, and *28/*6; therefore, other factors responsible for irinotecan toxicities should be identified.

Several clinical studies have suggested polymorphisms of the drug transporter genes, such as ABCB1, ABCC2, ABCG2, and SLCO1B1, might affect irinotecan pharmacokinetics (PK)/pharmacodynamics (PD) in Caucasian and Asian patients. However, the results obtained from different ethnic populations with various irinotencan regimens are still controversial, and the genetic markers examined also differ [13, 15–26]. We previously identified a number of haplotypes/variations of transporter genes, including ABCB1, ABCC2, ABCG2 and SLCO1B1 in Japanese [12, 26–29], but their clinical significance, either alone or in combination, in irinotecan therapy has not yet been examined.

This study aimed to identify the genetic polymorphisms/variations of ABCB1, ABCC2, ABCG2, and SLCO1B1 which can affect irinotecan PK/PD in Japanese cancer patients. We carefully stratified the patients considering the irinotecan regimen (irinotecan monotherapy or combination therapy with cisplatin) and UGT1A1 genotype (UGT1A1 *6 or *28), and examined additive effects of transporter haplotypes/variations on the area under the time–concentration curves (AUC) of the toxic metabolite SN-38 and on the risk of severe neutropenia.

Patients and methods

Patients

The patients used in this study were the same as those described in a previous paper [12], where details on the eligibility criteria for irinotecan therapy, patient profiles, and irinotecan regimens were described. In this study, 55 patients with irinotecan monotherapy (100 mg/m^2 weekly or 150 mg/m^2 biweekly) and 62 patients with combination therapy of irinotecan (60 mg/m^2 weekly or 70 mg/m^2 biweekly) and cisplatin (60 or 80 mg/m^2, respectively) were included. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants.

Analyses on genetic polymorphisms and PK/PD

Patients' data on genetic variations and haplotypes of UGT1A1, ABCB1, ABCC2, ABCG2 and SLCO1B1 were

previously obtained [12, 26–29]. Regarding *ABCG2*, combination haplotypes were newly defined using the previously reported haplotypes from three linkage disequilibrium (LD) blocks [28]. Patients' PK data on the area under the concentration–time curve (AUC) and toxicities were previously obtained [12].

Association analyses

Associations of transporter genotypes with AUC/dose values for irinotecan, SN-38 and SN-38G, absolute neutrophil count (ANC) nadir, and incidence of grade 3 diarrhea or grade 3/4 neutropenia were investigated. For SN-38 AUC/dose and neutropenia, the patients were stratified by the presence of *UGT1A1*6* or *28* (*UGT+*). Statistical significance (two-sided, $P < 0.1$) was determined by the Mann–Whitney (MW) test or Jonckheere–Terpstra (JT) test for AUC/dose, and by Fisher's exact test and chi-square test (for trend) for incidence of grade 3 and 4 toxicities, using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to SN-38 AUC/dose values and ANC nadir were further determined by multiple regression analysis after logarithmic transformation of the AUC/dose values and ANC nadir counts. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, the ANC at baseline (for neutropenia),

and genetic markers including *UGT1A1*6* or *28* (*UGT+*) and the transporter haplotypes. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.20 using JMP version 7.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Definition of major transporter haplotypes and their selected markers

For screening transporter gene polymorphisms affecting irinotecan PK/PD, major haplotypes and their tagging single nucleotide polymorphisms (SNPs) from *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were selected (Table 1) according to their frequencies (more than 5%) and/or from preliminary results obtained from all patients treated with irinotecan.

For *ABCB1* block 1[26], the haplotype group *BJL*, which consists of *1B* (having −1789G>A), *1J* (having −1789G>A and −371A>G) and *1L* (having −1789G>A and −145C>G), was selected because an association of the marker SNP −1789G>A with lower expression levels of P-gp has been reported [30]. *ABCB1* block 2 *2* was originally defined as haplotypes containing three SNPs, 1236C>T, 2677G>T (A893S) and 3435C>T [31]. Since the *9* haplotype with 1236C>T, 2677G>T (A893S) without 3435C>T [16] showed the same trend for PK/PD as *2* (data not shown), the current study classified the

Table 1 List of major transporter haplotypes and their markers analyzed for Japanese cancer patients

Gene	Haplotype	Tagging SNP	Abbreviation used in this paper	Haplotype frequency	
				Monotherapy $(N = 110)^a$	With cisplatin $(N = 124)^a$
ABCB1	*BJL*[b] (block 1)	−1789G>A		0.182	0.210
	2 group[c] (block 2)	2677G>T(A893S)	B	0.382	0.379
	10 group[d] (block 2)	2677G>A(A893T)		0.182	0.169
	1b (block 3)	IVS27-182G>T		0.200	0.169
ABCC2	*1A*	−1774delG	C	0.373	0.371
	1C/G	3972C>T(I1324I)		0.218	0.266
ABCG2	#*IIB* |*1a*−*2*−*1b*|[e]	421C>A(Q141K), IVS12 + 49G>T	G	0.200	0.274
	#*IIIC* |*1b*−*3*−*1c*|[e]	34G>A(V12M), IVS9-30A>T		0.164	0.097
SLCO1B1	*1b*	388A>G(N130D)		0.373	0.573
	15 · 17	521T>C(V174A)	S	0.191	0.153

[a] Number of chromosome

[b] *BJL* consists of *1B* (having −1789G>A), *1J* (having −1789G>A and −371A>G) and *1L* (having −1789G>A and −145C>G) previously defined [26]

[c] *2* Group includes *2*, *9*, *12* and *14* haplotypes previously defined [26]

[d] *10* Group includes *10* and *13* haplotypes previously defined [26]

[e] Combination of *ABCG2* haplotypes of three blocks [block (−1)–block 1–block 2] previously defined [28]

haplotypes with 2677G>T (A893S), *2, *9, *12 and *14 [ ], as the *2 group (*2 in this paper). Similarly, the *10 group was classified as haplotypes with 2677G>A (A893T), i.e., *10 and *13, since no differences in PK/PD parameters were observed between these haplotypes. The *4, *6, and *8 haplotypes in block 2 [16, 26] showed no significant effect in the current analysis (data not shown). The ABCB1 block 3 *1b haplotype containing IVS27-182G>T was selected because our previous study showed it was associated with an increased renal clearance of SN-38 [ ].

Based on reports showing possible functional alterations of -1774delG [32] and 3972C>T (I1324I) [18, 24], ABCC2 haplotypes containing those variations were classified as *1A and "*1C and *1G (*1C/G)", respectively, according to our previous definition: *1A, -1774delG; *1C, -24C>T and 3972C>T; *1G, 3972C>T [27]. ABCC2*2 [1246G>A (V417I)] and *1H [2934G>A (S978S)] [ ] showed no statistically significant effects (data not shown).

The ABCG2 combinatorial haplotypes were newly defined as combinations of haplotypes across the three blocks [block (−1)–block 1–block 2] previously reported [ ]. Major combinations in 177 patients were the wild type #1A (frequency = 0.291), #IIB [containing 421C>A (Q141K) and IVS12 + 49G>T] (0.251) and #IIIC [containing 34G>A (V12M) and IVS9-30A>T] (0.107). Note that #IIB and #IIIC are subgroups of block 1 *2 [421C>A (Q141K)] and block 1*3 [34G>A (V12M)], respectively [ ].

The SLCO1B1 haplotypes used were the major haplotypes *1b [containing 388A>G (N130D) without 521T>C (V174A)] [ ] and *15 · 17 [containing 521T>C (V174A)], the functional relevance of which has been reported [ ].

Association of transporter genotypes with AUC values

Since we previously found that some PK parameters, including AUC/dose, Cmax/dose and $t_{1/2}$ for irinotecan and/or its metabolites, as well as incidence of grade 3/4 toxicities were affected by irinotecan regimen [12], the following analyses were conducted using the two groups of patients; i.e., those treated with irinotecan monotherapy (100–150 mg/m² for initial dosage) or by combination therapy with cisplatin (60–70 mg/m² for initial dose of irinotecan). Since SN-38 AUC levels were largely dependent on the UGT1A1 genotype "*6 or *28" [12], the associations of transporter genotypes with SN-38 AUC values were analyzed within the groups stratified by the marker UGT1A1 "*6 or *28" (UGT+); i.e., UGT−/−, UGT+/− and UGT+/+. Since the SN-38 AUC/dose level of one patient with haplotypes ABCB1*2 [2677G>T

(A893S)] and *14 [2677G>T (A893S) and 1345G>A (E448K)] showed an outlying value (indicated as "a" in Fig. 1), this patient was excluded from the statistical analysis. In this study, we preliminarily found that effect of each transporter genotype on irinotecan PK/PD was generally small. However, it was hypothesized that multiple transporter genotypes might act additively as described below. Accordingly, we adopted a statistical significance level of P = 0.1 (two-sided) to pick up candidate polymorphisms for further evaluation of their combined effects.

Figure 1 shows the association of transporter genotypes with SN-38 AUC values in the irinotecan monotherapy. In all patients (ALL), higher values of the SN-38 AUC/dose were observed in the ABCB1*2/*2 [1.64-fold of −/−, P = 0.095 (MW test)] (Fig. 1b) and ABCG2#IIB [1.24-fold of −/−, P = 0.078 (MW test)] genotypes (Fig. 1e) and lower values were observed in the ABCB1*1b (block 3) [0.78-fold of −/−, P = 0.008 (MW test)] (Fig. 1c) genotype. In UGT−/− patients, an increase in SN-38 AUC/dose was observed in the ABCB1 BJL [1.22-fold of −/−, P = 0.073 (MW test)] (Fig. 1a) and ABCG2#IIB [1.21-fold of −/−, P = 0.082, (MW test)] genotypes (Fig. 1e). In UGT (+/− and +/+) patients, an increase in SN-38 AUC/dose in SLCO1B1*15 · 17 (S) [1.59-fold of −/−, P = 0.036 (MW test)] was also observed (Fig. 1f). Multiple regression analysis for the SN-38 AUC/dose (logarithm-transformed values) in the irinotecan monotherapy revealed significant associations of ABCB1*2/*2 (coefficient = 0.212 ± 0.075, P = 0.007), along with UGT+/− (0.113 ± 0.054, P = 0.040) and UGT+/+ (0.225 ± 0.088, P = 0.014) in the final model [R^2 = 0.226, Intercept = 0.281 (log 10^{-3}h m²/L), N = 53].

Regarding other compounds, ABCB1*2/*2 also showed higher irinotecan AUC/dose (1.27-fold) [66.2 (48.2–82.4) [median (25th–75th percentiles)] for *2/*2 vs. 52.2 (40.6–61.9) for −/− and *2/−; P = 0.063 (MW test)] and SN-38G AUC/dose (1.62-fold) [18.0 (14.6–27.7) for *2/*2 vs. 11.1 (7.7–14.2) for −/− and *2/−; P = 0.002 (MW test)]. Conversely, lower irinotecan AUC/dose for ABCB1*10/*10 (0.79-fold) [54.8 (44.4–65.7) for −/− vs. 43.3 (40.6–54.1) for *10/*10; P = 0.062 (JT test)] was detected.

For the combination therapy with cisplatin, an increase of the SN-38 AUC/dose for ABCB1*2/*2 (1.43-fold) in non-UGT+/+ patients (UGT−/− and UGT+/−) (N = 55) [3.57 (2.72–4.19) for *2/*2 vs. 2.51 (1.99–3.28) for −/− and *2/−; P = 0.032 (MW test)], and a decrease for ABCB1*1b (0.80-fold) in UGT−/− patients (N = 35) [2.03 (1.72–2.33) for *1b/− and *1b/*1b vs. 2.55 (2.02–3.31) for −/−; P = 0.026 (MW test)] were observed. Multivariate analysis, however, showed no significant contributions of these transporter haplotypes to the SN-38 AUC/dose values.
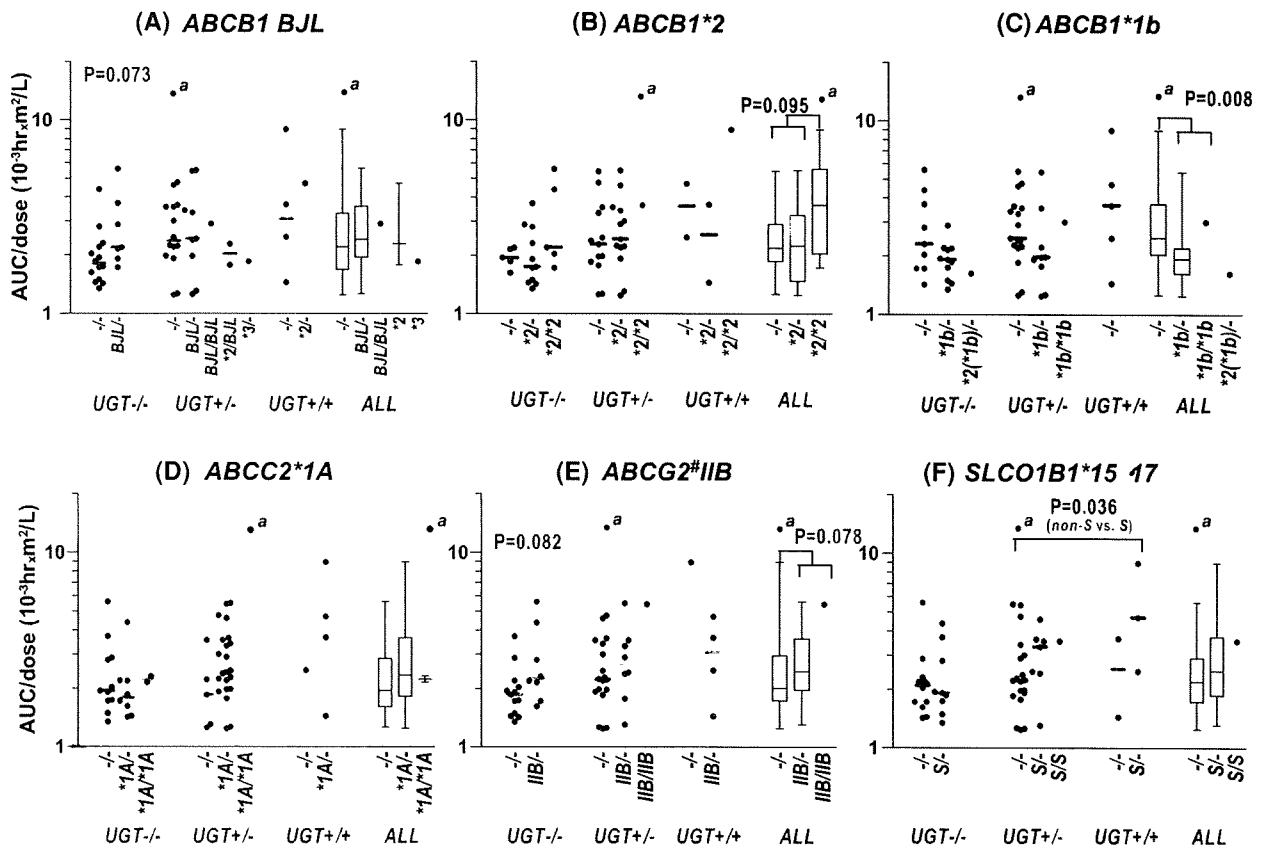
Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). a Excluded from statistical analysis. The bars represent the medians. UGT+ = UGT1A1*6 or *28. a BJL contains −1789G>A, *2 (block 1) = 325G>A (E109K), *3 (block 1) = 304G>A (G102R); b *2 (block 2) contains 2677G>T (A893S); c *1b (block 3) = IVS27-182G>T, *2 (block 3) = 3751G>A (V1251I); d *1A contains −1774delG; e IIB contains 421C>A (Q141K) and IVS12 + 49G>T; f S = SLCO1B1*15 · 17 containing 521T>C (V174A)

Effects of transporter genotypes on toxicities in irinotecan monotherapy

Since 80 and 100% of UGT+/+ patients showed grade 3/4 neutropenia in the irinotecan monotherapy and combination therapy with cisplatin, respectively, neutropenia incidence was analyzed only in the non-UGT+/+ population. Two patients were excluded from the analysis; one patient who showed an outlier SN-38 value (indicated as "a" in Fig. 1) and a second patient from the cisplatin-combination therapy group who discontinued irinotecan therapy.

In terms of incidence of grade 3/4 neutropenia in irinotecan monotherapy (Table 2), ABCC2*1A-dependent increases [0, 25.8 and 50.0% for −/−, *1A/− and *1A/*1A, respectively; $P = 0.014$ (chi-square test for trend)] were observed in UGT (−/− and +/−) patients. Higher incidence with ABCG2#IIB was also found in UGT (−/− and +/−) patients [9.5% for −/− and 35.3% for #IIB/− and #IIB/#IIB, respectively; $P = 0.049$ (Fisher's exact test)],

and with SLCO1B1*15 · 17(S) in the UGT+/− patients [15.0, 28.6 and 100% for −/−, S/− and S/S, respectively; $P = 0.076$ (chi-square test for trend)].

Multiple regression analysis for the ANC nadir (logarithm-transformed values) was conducted. The final model [$R^2 = 0.466$, Intercept = 1.088 (log counts/µL), $N = 52$] revealed associations of ABCC2*1A/*1A (coefficient = −0.339 ± 0.088, $P = 0.0004$), ABCG2#IIB (−0.131 ± 0.067, $P = 0.057$) and SLCO1B1*15 · 17 (−0.136 ± 0.066, $P = 0.046$) in addition to UGT+/− (−0.134 ± 0.073, $P = 0.074$) and UGT+/+ (−0.238 ± 0.117, $P = 0.047$) and ANC at baseline (0.541 ± 0.226, $P = 0.021$), but association of ABCB1*2/*2 was not significant (−0.158 ± 0.095, $P = 0.104$).

Although total incidence of grade 3 diarrhea was low (11%), an ABCB1*2-dependent increase was observed [0, 15.4 and 28.6% for −/−, *2/− and *2/*2, respectively; $P = 0.022$ (chi-square test for trend)]. Note that all patients who experienced grade 3 diarrhea had neither the ABCC2*1C/G nor ABCG2#IIIC genotypes.

Table 2 Effects of transporter genotypes on incidences of grade 3/4 neutropenia in Japanese patients treated with irinotecan monotherapy

Gene	Genotype	UGT−/−				UGT+/−				UGT (−/−, +/−)			
		No./total	%	P value		No./total	%	P value		No./total	%	P value	
				Exact[a]	Trend[b]			Exact[a]	Trend[b]			Exact[a]	Trend[b]
ABCB1	B.IL (block 1)[c]												
	−/−	3/14	21.4	>0.1		4/15	26.7	>0.1	>0.1	7/29	24.1	>0.1	>0.1
	+/−	0/7	0.0			2/9	22.2			2/16	12.5		
	+/+					0/1	0.0			0/1	0.0		
	*2 group (block 2)												
	−/−	1/5	20.0	>0.1[d]	>0.1	5/14	35.7	>0.1[d]	>0.1	6/19	31.6	>0.1[d]	>0.1
	+/−	1/11	9.1			0/13	0.0			1/24	4.2		
	+/+	1/5	20.0			1/1	100			2/6	33.3		
	*1b (block 3)[e]												
	−/−	2/9	22.2	>0.1		4/18	22.2	>0.1	>0.1	6/27	22.2	>0.1	>0.1
	+/−	0/11	0.0			2/9	22.2			2/20	10.0		
	+/+					0/1	0.0			0/1	0.0		
ABCC2	*1A												
	−/−	0/11	0.0	>0.1	0.031	0/5	0.0	>0.1		0/16	0.0	0.022	0.014
	+/−	2/8	25.0			6/23	26.1			8/31	25.8		
	+/+	1/2	50.0							1/2	50.0		
ABCG2	#IIB												
	−/−	0/13	0.0	.0.042		3/19	15.8	>0.1	>0.1	3/32	9.4	0.049	0.057
	+/−	3/8	37.5			3/8	37.5			6/16	37.5		
	+/+					0/1	0.0			0/1	0.0		
SLCO1B1	*15 · 17												
	−/−	2/12	16.7	>0.1		3/20	15.0	>0.1	0.076	5/32	15.6	>0.1	>0.1
	+/−	1/9	11.1			2/7	28.6			3/16	18.8		
	+/+					1/1	100			1/1	100		

a Fisher's exact test for (−/−) versus (+/− and +/+)

b Chi-square test for trend

c Three patients bearing *2 (block 1) or *3 (block 1) were excluded

d Fisher's exact test for (−/− and +/−) versus (+/+)

e One patient bearing *2 (block 3) was excluded

Effects on toxicities in combination therapy with cisplatin

Since only four patients (6.0%) experienced grade 3 diarrhea from the cisplatin-combination therapy, association analysis for diarrhea was not done.

Grade 3/4 neutropenia incidence was higher with ABCB1*2 [47.1, 63.3 and 85.7% for −/−, *2/− and *2/*2, respectively; P = 0.073 (chi-square test for trend)] in UGT (−/− and +/−) patients. In UGT−/− patients, a higher incidence was also observed with ABCG2#IIB [55.6, 83.3 and 100% for −/−, #IIB/− and #IIB/#IIB, respectively; P = 0.075 (chi-square test for trend)]. Conversely, the incidence was lower with ABCG2#IIIC [71.4% for −/−, and 25% for #IIIC/− and #IIIC/#IIIC, respectively; P = 0.006 (Fisher's exact test)] in UGT (−/− and +/−)

patients. Notably, all patients homozygous for ABCG2#IIB (N = 5) or SLCO1B1*15 · 17 (N = 1) experienced grade 3/4 neutropenia. The effect of ABCC2*1A on neutropenia was not consistent among the UGT genotypes in contrast to the results from the monotherapy. Multiple regression analysis was not applied to the neutropenia parameters in the cisplatin-combination therapy because, as described in the next section, contributions of minor variations could not be ignored.

Minor genetic variations possibly related to grade 4 neutropenia

We have detected a number of rare non-synonymous variations of the transporter genes to which statistical analysis could not be applied. Since grade 4 neutropenia

Table 3 Minor genetic variations detected in non-UGT+/+ patients who experienced grade 4 neutropenia

ID	Gene	Genetic variation	
		Nucleotide change (amino acid substitution)	Haplotype[a]
b1	ABCB1	304G>C (G102R)	Block 1 *3
b2(B)[b]		1804G>A (D602N)	Block 2 *12
b3(B)[b]		1342G>A (E448K)	Block 2 *14
b4		3043A>G (T1015A)	Block 2 *16
b5		3751G>A (V1251I)	Block 3 *2
c1	ABCC2	1177C>T (R393W)	*7
g1	ABCG2	376C>T (Q126X)	Block 1 *4
g2		1465T>C (F489L)	Block 2 *2
g3		1723C>T (R575X)	Block 2 *5
s1(S)[c]	SLCO1B1	1007C>G (P336R)	
s2		311T>A (M104K)	
u1	UGT1A1	−3279T>G, 1941C>G	#60-#1B (+/+)

[a] Defined in previous papers for ABCB1 [26], ABCC2 [27], ABCG2 [ ] and UGT1A1 [ ]

[b] Linked with ABCB1*2 (B)

[c] Linked with SLCO1B1*15 · 17 (S)

occurred in non-UGT+/+ patients at rates of 8.0% (4/50) in the irinotecan monotherapy and 20% (11/55) in the cisplatin-combination therapy, we investigated possible contributions of these minor transporter variations and another low-activity UGT-haplotype, UGT1A1#60-#1B [ ], to severe neutropenia.

Among the rare variations detected, eleven heterozygous transporter genetic variations and one UGT1A1#60-#1B homozygote were found in non-UGT+/+ patients who experienced grade 4 neutropenia (Table 3). These variations include an amino acid substitution leading to reduced in vitro activity, ABCG2 1465T>C (F489L) [36], and the stop codons, ABCG2 376C>T (Q126X) and 1723C>T (R575X) [ ].

Additive effects of transporter gene haplotypes on neutropenia

Since multiple transporters are involved in irinotecan PK/PD, severity of toxicity might depend on the number and combinations of the low-activity variants, each of which does not effectively affect PD. To examine this possibility, we surveyed relationships between ANC nadirs and combinations of haplotypes associated with grade 3/4 neutropenia (P < 0.1) and the minor variations associated with grade 4 neutropenia (listed in the previous section); the data for selected haplotypes/variations are depicted in Fig. . For the combination therapy with cisplatin (Fig. b), homozygous SLCO1B1*15 · 17 was included,

but ABCC2*1A was excluded since its effect in the cisplatin-combination therapy was not consistent among the UGT genotypes.

In the irinotecan monotherapy, ANC nadirs in most patients with either one or more of ABCG2#IIB, SLCO1B1*15 · 17 and the minor variations were lower than the median ANC nadirs of both UGT−/− and UGT+/− patients without them (None) (Fig. 2a). In particular, the effects were more evident in patients bearing two or more of the selected haplotypes/variations (including the UGT+). Among the patients who experienced grade 3 or 4 neutropenia, 80% of patients had two or more candidate haplotypes/variations in the UGT (−/− and +/−) group (Fig. 2a).

In UGT+/− patients with the cisplatin-combination therapy, ANC nadirs of the patients with ABCB1*2/*2, ABCG2#IIB/#IIB, SLCO1B1*15 · 17/*15 · 17 or any minor variations, and their combinations were lower than the median values of patients without these markers (None), except for one patient with ABCB1*2/*2 and SLCO1B1*15 · 17 (B/B + S/−) (Fig. 2b). Also, in UGT−/− and UGT+/− patients, the effects were more evident in the patients with two or more of the selected haplotypes/variations. Among the patients who experienced grade 4 neutropenia, 82% of patients had two or more candidate haplotypes/variations in the UGT (−/− and +/−) group (Fig. 2b).

It was noted that the additive effect of g1 [ABCG2 376C>T (Q126X)] was not observed in the heterozygotes (g1/−), but was evident in the compound heterozygotes with another ABCG2 genetic polymorphism, #IIB, (G/g1) (Fig. 2a, b).

Regarding the combined effects of the above transporter genotypes on SN-38 AUC values, higher levels were observed in patients with the candidate haplotypes/variations of two or more genes in the monotherapy, but this trend was not always evident in the cisplatin-combination therapy patients (data not shown).

Discussion

In this study, we showed possible additive effects of transporter and UGT1A1 genotypes on irinotecan PK and PD. Since multiple transporters are involved in irinotecan PK, it is likely that a functional alteration of one of the responsible transporters can be compensated by other transporters; thus, changes in PK/PD parameters by transporter genotypes may not always be large. However, the overall elimination rate of irinotecan or its metabolites might be altered under the conditions of simultaneously reduced activities of multiple transporters, higher irinotecan doses, or reduced UGT activity.

(A) Monotherapy
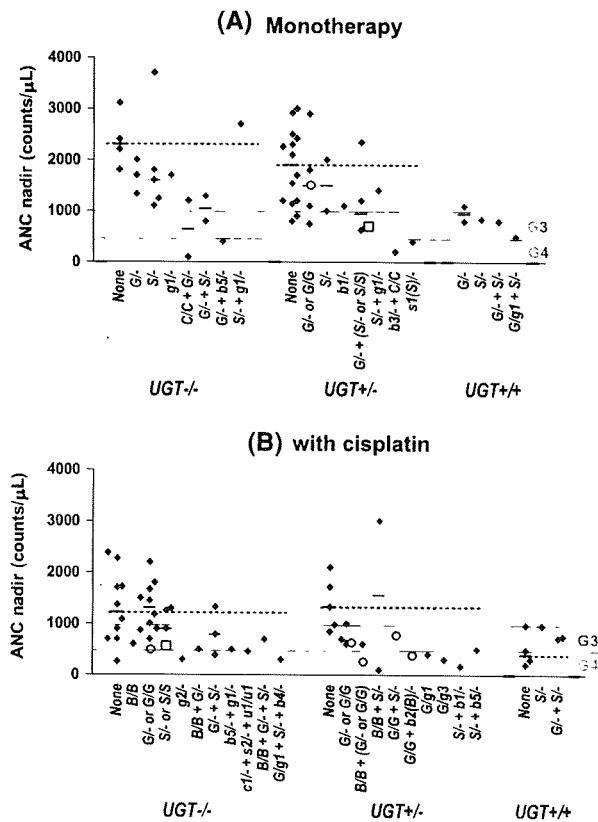
(B) with cisplatin

Fig. 2 Additive effects of transporter haplotypes/variations on ANC nadirs in irinotecan monotherapy (a) and combination therapy with cisplatin (b). $UGT+ = UGT1A1*6$ or $*28$; $B = ABCB1*2$; $C = ABCC2*1A$; $G = ABCG2^{\#}IIB$ (open circle, $^{\#}IIB/^{\#}IIB$); $S = SLCO1B1*15 \cdot 17$ (open square, $*15 \cdot 17/*15 \cdot 17$); $b1$–$u1$ = minor variations listed in Table 3. a None = non-(C, G, S or minors), b None = non-(B, G, S or minors). The bar in each genotype represents the median. The dotted lines in each UGT genotype show the median values of patients without any selected transporter polymorphisms/variations (None). The lines (G3 and G4) represent the border of grade 3 and 4 neutropenia

In the irinotecan monotherapy, the increasing effect of $ABCB1*2/*2$ (block 2) on SN-38 AUC/dose was evident while contributions of $ABCB1$ BJL (block 1), $ABCB1*1b$ (block 3), $ABCG2^{\#}IIB$ and $SLCO1B1*15 \cdot 17$ were not significant in the multivariate analysis. For neutropenia, additive effects were suggested for $ABCC2*1A/*1A$, $ABCG2^{\#}IIB$, $SLCO1B1*15 \cdot 17$, and possibly some minor genetic variations in addition to $UGT1A1*6$ or $*28$ (Fig. 2a). The association of $ABCB1*2$ (block 2) with grade 3 diarrhea was also observed.
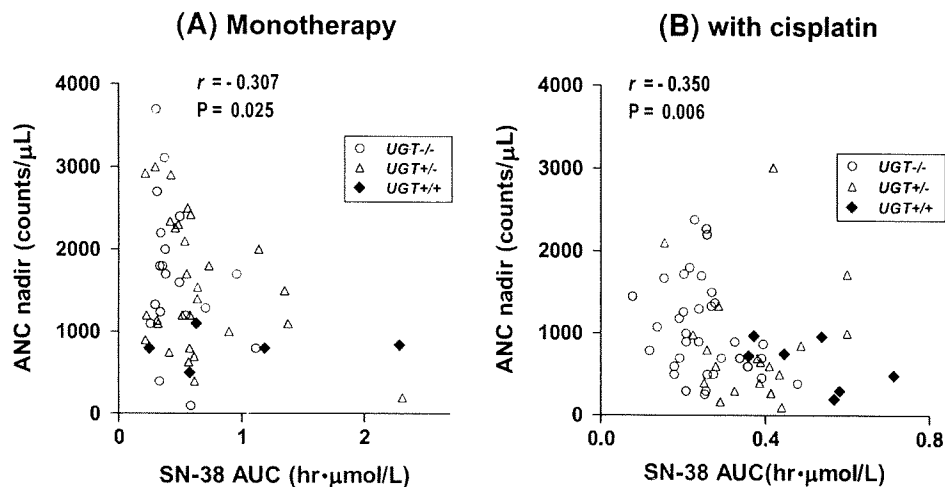
In the combination therapy with cisplatin, an increase in the SN-38 AUC/dose by $ABCB1*2$ and for a decrease by $ABCB1*1b$ were observed, but the multivariate analysis did not show their significant contributions. Regarding neutropenia, additive effects of $ABCB1*2/*2$, $ABCG2^{\#}IIB/^{\#}IIB$, and possibly, $SLCO1B1*15 \cdot 17/*15 \cdot 17$ and some minor variations were suggested (Fig. 2b).

Thus, in both regimens, the associations of $ABCB1*2$ (block 2) with higher SN-38 AUC/dose levels and toxicities (diarrhea or neutropenia), and additive effects of $ABCG2^{\#}IIB$ and $SLCO1B1*15 \cdot 17$ with $UGT1A1*6$ or $*28$ on neutropenia were observed. The current study also suggests that combination genotypes with two or more genes could have a greater effect on neutrophil count reduction than a single gene, indicating a quantitative property of multiple genetic factors affecting phenotype. These findings could partly explain a large interindividual variation in irinotecan toxicities within each UGT genotype.

In this study, influences of the transporter genotypes on SN-38 AUC/dose did not always correlate to an influence on neutropenia as observed in the combination therapy with cisplatin and in the case of $ABCB1*2$ (block 2) in the monotherapy. Although weak negative correlations were observed between the SN-38 AUC level and ANC nadir, the SN-38 AUC values of patients who exhibited grade 3/4 neutropenia (ANC nadir < 1,000 counts/μL) were fairly diverse, especially in the combination therapy with cisplatin (Fig. 3). It is likely that the extent of toxicities depends not only on systemic exposure levels of the active metabolite for which hepatic UGT activity is a large contributor, but also on the elimination from the target cells (neutrophil progenitor cells or enterocytes) where transporter function might be more critical.

Our previous study showed the association of $ABCB1$ block 2 $*2$ [1236C>T, 2677G>T (A893S) and 3435C>T] with lower renal clearance of irinotecan and its metabolites [16]. The current data obtained in the irinotecan monotherapy also suggest higher AUC/dose for irinotecan, SN-38G, and SN-38 with $ABCB1*2/*2$. Since a high affinity of P-gp for irinotecan is known, lower elimination rate of irinotecan could also result in higher plasma levels of its metabolites. Other studies have also suggested associations of the haplotype 1236T–2677T (corresponding to our $*2$ group in this study) with a reduced excretion rate of P-gp substrates [37] and SN-38 [25], and associations of the haplotype 2677T–3435T (corresponding to our $*2$ group in this study) with paclitaxel-induced neutropenia [38].

For $ABCC2$, $ABCC2$ −1774delG, a tagging SNP of $*1A$, was reported to be associated with low promoter activity and cholestatic or mixed-type hepatitis [32]. Patients with $ABCC2*1A/*1A$ together with $ABCB1*2/*2$ or $ABCG2^{\#}IIB$ showed higher values of SN-38 AUC (Fig. 1) and neutropenia in the monotherapy (Fig. 2a), but these trends were not evident in the UGT−/− patients treated with cisplatin-combination therapy (data not shown). Thus, the effects of $ABCC2$ might be dependent on combinations with other genetic and non-genetic factors. Conflicting clinical outcomes of $ABCC2$ 3972C>T, a marker of $*1C/G$, were reported to cause higher AUC of irinotecan and its

(A) Monotherapy

(B) with cisplatin

Fig. 3 Correlations between SN-38 AUC and ANC nadir in patients in irinotecan monotherapy (a) and combination therapy with cisplatin (b). r Spearman's rank correlation coefficient

metabolites in Caucasians treated with irinotecan monotherapy [18] and to lower the incidence of grade 3 diarrhea in Koreans treated with a combination therapy of irinotecan and cisplatin [24]. In the current study, no significant association of $ABCC2*1C/G$ on PK/PD was observed in the monotherapy. Although a high incidence of grand 3/4 neutropenia was observed in patients with $ABCC2*1C/G$ in the combination therapy with cisplatin, most patients also had $ABCG2^{\#}IIB$ (data not shown); thus, the effect of $ABCC2*1C/G$ remains obscure.

For $ABCG2$, the current study examined the association with the combinatorial haplotypes consisting of the three previously defined block haplotypes [28]. $ABCG2^{\#}IIB$ contains the non-synonymous SNP 421C>A (Q141K), which was detected at higher frequencies in Asians and was reported to cause reduced expression of BCRP in vitro [36, 39–41]. In clinical studies, the association of 421C>A (Q141K) with higher plasma levels of diflomotecan was shown in Caucasians [42]. However, an association of this SNP with irinotecan PK/PD had not been shown [19, 24]. An association of 421C>A (Q141K) alone with irinotecan PK/PD was not significant in our hands (data not shown), but $^{\#}IIB$ containing both 421C>A (Q141K) and IVS12 + 49G>T showed a moderate association with neutropenia. It is unclear whether the additional SNP IVS12 + 49G>T itself or another unknown linked SNP is causative for the reduced function. $ABCG2^{\#}IIIC$ contains a non-synonymous SNP 34G>A (V12M) which has no influence on BCRP expression or activity in vitro [36, 39–41]. Our study showed no influence of $ABCG2^{\#}IIIC$ on the SN-38 AUC/dose levels and neutropenia in the irinotecan monotherapy (data not shown), but did show a decreasing trend in grade 3/4 neutropenia in the combination therapy with cisplatin. In contrast, a report on Korean patients

suggested the association of $ABCG2$ 34G>A (V12M) with a higher incidence of grade 3 diarrhea in a combination therapy of irinotecan and cisplatin [24].

Among $SLCO1B1$ polymorphisms, 521T>C (V174A), a tagging SNP of $*15 \cdot 17$, was demonstrated to reduce in vitro SN-38 influx [7], and clinical studies in Asians also showed its relevance to a higher SN-38 AUC and severe neutropenia in combination therapy of irinotecan with cisplatin [22–24]. Our results support these previous findings. Note that our $*15 \cdot 17$ mainly consists of $*17$ [containing −11187G>A, 521T>C (V174A) and 388A>G (N130D)].

Taken together, the clinical data on transporter genotypes show variability among the studies. The reasons for these conflicting findings might be partly attributed to the ethnic differences in transporter genotypes and the regimens used. In addition, non-genetic factors, such as disease status and inflammation [43, 44], hepatic or renal function [45], and co-administered or pre-administered drugs, may also influence the clinical outcome.

The current study suggests combined effects of multiple haplotypes/variations on neutropenia. From clinical aspects of irinotecan therapy, the benefit of additional genotyping of transporters to predict severe toxicities should be clarified. Regarding grade 3 and 4 neutropenia, positive prediction values for two or more candidate genotypes including UGT (+) (Fig. 2) were 46 and 89% in the monotherapy and the cisplatin-combination therapy, respectively, which are low compared with $UGT+/+$ (80 and 100%, respectively). Regarding grade 4 neutropenia, positive predictive values for these candidate genotypes were 15 and 41% in the monotherapy and the cisplatin-combination therapy, respectively, while for $UGT+/+$, they were 0 and 43%, respectively. Further studies using a

larger population size are needed to further elucidate the roles of these candidate markers.

In conclusion, the current study suggests there are additive effects for several transporter genotypes on the SN-38 AUC level and the reduction of neutrophil counts in irinotecan therapy. The clinical benefits of additional genotyping of these candidate markers should be further delineated.

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わが国における切除不能再発大腸癌(MCRC)に対する化学療法；最近の動向

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は じ め に

1990年代では，切除不能再発大腸癌の化学療法は，best supportive careに比べて数ヵ月の延命が期待できる程度であった。しかし2000年以降，従来用いられていたフルオロウラシル(5-FU) に加えて，イリノテカン(CTP-11) やオキサリプラチン(L-OHP) といった新規抗癌剤が開発され，さらに近年，ベバシズマブ(Bev)，セツキシマブ(Cet) などの分子標的治療薬の導入により，切除不能再発大腸癌の化学療法はめまぐるしく発展し，治療成績は大きく改善した。わが国でも2007年にBevが，2008年にはCetがそれぞれ承認されたことで，この5剤のkey drugを実地臨床でいかに使いこなしていくのかが今後の重要な課題となっている。

本稿では，近年，切除不能大腸癌の治療戦略として重要視されている "Continuum of Care" の概念[1]および経口抗癌剤の位置づけにつき概説し，これら海外のエビデンスを国内実地臨床にどのように受け入れるかを論じてみたい。

I. Continuum of care model

大腸癌化学療法は，フルオロピリミジン，L-OHP，CPT-11に加え，Bev，抗EGFR抗体のCetやパニツムマブ(Pan) の5種類の薬剤が使用できるようになり，生存期間が2年を超えることが期待できるようになった。これは10年前と比べて2倍以上の生存期間である。一方で，現時点でこれら有効な薬剤をどのような順番で，あるいはどのような組み合わせで使用すると効果が最大限に期待できるのかのコンセンサスはない。また初回治療として併用療法を行った場合には，その副作用の

ために生活の質を犠牲にせざるを得ないところがある。そこで，近年の大腸癌化学療法の治療戦略として，これまでの増悪するまで同じレジメンで治療を継続し，増悪後は非交叉耐性の薬剤を使用するといった1st-あるいは2nd-line治療という考え方から，患者個々の治療のゴール設定や治療経過に合わせて，たとえば併用療法を単剤投与に変えて維持療法を設定したり，完全な休薬期間を設けたり，あるいは著効し手術可能な例には積極的に手術に移行するなど，患者個別の状態に応じて臨機応変に治療戦略の設定を修正することが必要となってきている。このような場合に注意しなければならない重要なポイントは，FOLFOX療法で問題となる蓄積性感覚性末梢神経障害への対応；"Stop & Go strategy"と[2)3)]，あるレジメンに不応となった後にも，後治療として用いる薬剤に相乗効果を有する薬剤を継続して併用することで，効果が増強することの2点がある。前者の"Stop & Go strategy"は次項にて詳述する。後者に関しては，たとえばBOND-1試験において[1)]，CPT-11に不応になった患者に対して，Cetを単独で使用した治療群と，CetにCPT-11を併用した群を比較したところ，無増悪生存期間(PFS)では，1.5ヵ月に対して4.1ヵ月(p<0.001)，奏効率も11%に対して23%と(p=0.007)，いずれも併用群の方が良好であった。よってCPT-11での増悪後にもCPT-11をCetに併用した方が治療成績は良好であることが示されている。また5-FUとCPT-11を併用した治療(IFL)に不応となった場合に，次治療としてL-OHPとFOLFOX療法との比較試験が行われ，FOLFOX療法の方がPFSおよび奏効率は良好であった[5)]。以上より，従来の交叉耐性のない薬剤に変更し次治療を行うというセオリーは切除不能進行大腸癌では用いられず，"Continuum of Care"の概念に沿って大腸癌に有効な薬剤を適材適所で使用することが肝要である[1)]。

II．Chemotherapy-Holidays

近年，大腸癌に有効な薬剤を適材適所で使用することにより，生存期間が2年を超えることが多くなってきたが，L-OHPの蓄積性末梢神経障害やCPT-11の下痢や倦怠感などの薬物有害反応は，長期化した治療期間中においては患者のQOLへの影響が大きい。これらの有害反応は治療を中止することで可逆的である。L-OHP併用療法においては，奏効しているにもかかわらず，神経毒性の増悪のために治療中止を余儀なくされることが少なからずみられる。このような知見から，chemothrapy-holidayという概念が導き出され，蓄積性の薬物有害反応を減らし，QOLを向上するとともに患者にとっての利便性を高めることが期待できるとしている。この概念はOPTIMOX-1試験により実証され[2)]，L-OHPを使用しない期間を設けることで，末梢神経障害が緩和されることが示された。FOLFOXをPDとなるまで使用した場合と，FOLFOXを3ヵ月間投与した後にsLV5FUで6ヵ月間維持療法を行い，その後FOLFOXを再導入する(OPTIMOX-1法："Stop & Go strategy")方法との比較試験が行われた。OPTIMOX-1の方が，通常のFOLFOX法に比べて末梢神経障害の頻度が少ない傾向にあり，効果は全生存率(OS)，PFS，奏効率でほぼ同等であった。OPTIMOX-2試験は[3)]，FOLFOXを3ヵ月間投与後に3ヵ月間完全に化学療法を休薬するか(chemo-free interval)，腫瘍サイズが治療開始前のサイズとほぼ同等になったところでFOLFOXを再導入する方法(OPTIMOX-2)と，OPTIMOX-1法との比較試験である。ここではOPTIMOX-2はOPTIMOX-1に比べて，PFSやOSでむしろ悪化する傾向がみられた。以上の結果より，FOLFOX療法をより末梢神経障害を軽減し，かつ，より長期的に使用するためには，OPTIMOX-2のように完全に休薬するのではなく，OPTIMOX-1のようにL-OHPのみを休薬しsLV5FUで維持療法を行うことが推奨される。現時点では，どのタイミングで維持療法に移行するかは，①治療前にあらかじめ規定されたサイクル数に到達した時点，②最も腫瘍縮小が得られた時点，③長期間SDが得られた時点，④たとえば神経毒性がgrade 2に達した時点，などが考えられるが，またどのような時にFOLFOXを再導入するのがいいか，といったことは明らかになっておらず，今後の検討が必要である。

III．抗EGFR抗体の効果予測因子としてのKRAS

大腸癌患者の約40%の腫瘍にKRASの遺伝子変異が存在し，抗EGFR抗体耐性に関連していることが知られている。Cet単剤およびPan単剤[6)]とBSCとの比較試験，CRYSTAL試験[7)]，OPUS試験[8)]，EVEREST試験において[9)]，KRAS遺伝子変異の有無に分けて治療効果のretrospectiveな解析が行われ，
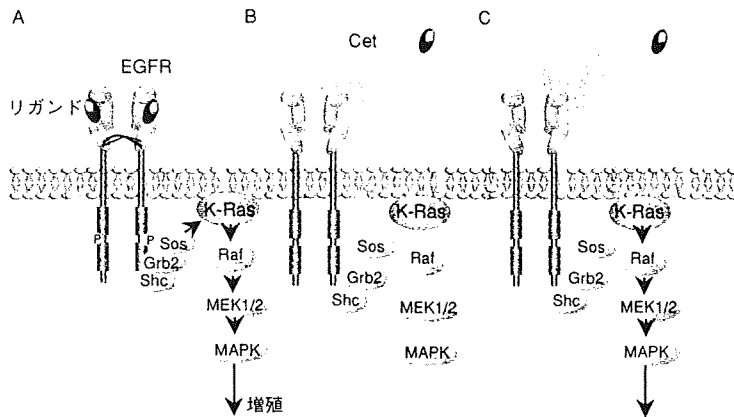
図. KRAS変異の有無による抗EGFR抗体の作用

(文献[10]を一部改変)

いずれもKRAS野生型の患者では，抗EGFR抗体併用群が，非併用群に比べてPFSおよび奏効率が優れた結果であった。一方，変異型の患者では抗EGFR抗体併用療法群は非併用療法群に対して，PFSおよび奏効率ともに良好な結果は得られなかった。またgrade 3/4の副作用は，野生型と変異型では大きな差はなかった。以上より，KRAS変異型の患者において抗EGFR抗体の有用性は示されなかった。その耐性機序は以下のように考えられている。図に示すように[10]，通常のEGFRシグナル経路では，リガンドがEGFRに結合することによりRas/MAPK経路が活性化されるが(A)，抗EGFR抗体がEGFRに結合することによりRas/MAPK経路は活性化されず，結果として腫瘍細胞の増殖抑制とアポトーシスの誘導などをもたらす(B)。一方，KRAS変異が存在すると，EGFRからのシグナルがなくてもMAPK経路は活性化されてしまうために，抗EGFR抗体がEGFRに結合しても，この経路を

不活化できない(C)。

このようにKRAS変異の有無により，抗EGFR抗体の効果予測が可能となり，欧米では抗EGFR抗体治療前にKRAS遺伝子検査を行うことが強く推奨されている[11]。国内でもKRAS遺伝子検査の保険承認に向け検討が進んでいるところであり，早期承認が望まれるところである。

Ⅳ. 経口抗癌剤

2009年9月にカペシタビン(Cap)が切除不能大腸癌に適応拡大となり，L-OHPやBevとの併用が可能となった。Cap単剤では5-FU/LV静注療法(Mayo Clinic regimen)との比較試験が2つ行われ[12,13]，Cap群の方が奏効率は高かったもののTTPやOSは同等であった。両試験ともCap群の方が，好中球減少，口内炎，悪心，脱毛は軽度であるものの，手足症候群と高ビリルビン血症は強かった。以上より，Cap単剤と5-FU/LV静注療法の同等性が

示され，利便性を考慮にいれ，静注5-FU＋LV療法はCapに置換されるようになった。FOLFOXやFOLFIRIでは，5-FU持続静注投与のために，中心静脈ポート留置が必要になるが，5-FU持続静注をカペシタビンに置換できれば，中心静脈ポート留置が不要になり，患者にとって利便性向上につながる。そこでFOLFOX/FOLFIRIとCapとL-OHP/CPT-11併用(XELOX/XELIRI)との比較試験が行われてきた。XELOXはFOLFOXと比較した試験のメタ解析[14]の結果より，有効性の指標である奏効率，PFS，OSは若干XELOXの方が悪い傾向にあるが，ほぼ同等である。すなわちXELOXはFOLFOXに比較してpalliative chemotherapyとしてはほぼ同等とみなされている。また有害事象に関しては，好中球減少はFOLFOXで強い傾向にあるが，血小板減少・下痢・手足症候群ではXELOXの方が強い傾向にある。ただ，現時点では，XELOXにおけるCapの至適投与量は確定しておらず，また副作用出現につき人種間較差もある[15]。日常生活で葉酸を摂取する生活スタイルのためか，あるいは代謝酵素の人種間格差のためか，米国人では，西欧人やアジア人に比べて，Capおよび急速静注5-FU＋LV療法において副作用が増強する傾向にある。よって投与量やスケジュールに関しては，米国のデータをそのまま国内に外挿することには注意が必要である。国内で行われたXELOXおよびXELOX＋BeV併用の第Ⅱ相試験では[16]，L-OHP 130mg/m² 1日目およびCap 2,000mg/m²/日(朝夕2回14日間内服)を1コースとして3週ごとに繰り返すレジメンで行われた。64例が登録され，奏効率72%，PFS

11.0ヵ月と良好な成績が得られた。ま
た薬物有害反応に関しても，Grade 3
〜4の下痢は3.1％，grade 3〜4の好中
球減少は15.5％であり，日本人におい
てCap 2,000mg/m²/日のXELOXは忍容
性に優れていることが示された。した
がってCapの薬物有害反応が強くでる
傾向にある米国の臨床試験の結果をも
とにCapの投与量設定（1,700mg/m²/
日）をすると[17]，日本人にとっては用
量不足になる可能性があり注意を要する。

　経口抗癌剤の利点は，持続静注法よ
りも外来通院回数が少なく，点滴時間
の短縮やポートが不要となることから，
身体的自由度が増し，患者の利便性が
向上することにある。その反面，患者
の内服コンプライアンスを高めなけれ
ば，効果的な治療は期待できない。し
たがって患者に内服方法とその副作用
対処法を十分に指導することによって，
患者の自己管理能力を向上させること
が治療上重要となる。

　今後，経口抗癌剤とL-OHPおよび
Bevとの併用が保険で適応拡大された
ことで，実地臨床においては利便性を
重要視し，経口抗癌剤をベースにした
併用療法が汎用されると予想されるが，
経口抗癌剤であるからといって決して
すべての副作用が軽減するわけではな
い。外来受診日が減り，患者自身が服
薬管理をしなければならない分，
FOLFOX/FOLFIRIなどの静注療法よ
りも，きめ細かな管理と患者教育が必
要になることを忘れてはならない。

お わ り に

　2008年9月に抗EGFR抗体であるCet
が承認され，また2009年9月に経口
フッ化ピリミジンとL-OHPとBevとの
併用療法が，保険適応拡大され，転移
性大腸癌における化学療法は海外とほ
ぼ同様の治療が行える状況になった。
あとは抗EGFR抗体の効果予測因子で
あるKRAS遺伝子検査の大腸癌への保
険適応拡大を待つばかりである。この
ようななかで，個々の患者の状態や希
望に合わせて，有効な治療法を安全か
つ確実に投与することが，われわれ臨
床医の使命である。

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ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Patterns of Local Recurrence in Rectal Cancer: A Single-Center Experience

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ABSTRACT A cohort of patients operated at the National Cancer Center Hospital in Tokyo for rectal carcinoma, at or below the peritoneal reflection, was reviewed retrospectively. The purpose was to study the risk factors for local relapse and the patterns of local recurrence. Three hundred fifty-one patients operated between 1993 and 2002 for rectal carcinoma, at or below the peritoneal reflection, were analyzed. One hundred forty-five patients, with preoperatively staged T1 or T2 tumors without suspected lymph nodes, underwent total mesorectal excision (TME). Lateral lymph node dissection (LLND) was performed in suspected T3 or T4 disease, or when positive lymph nodes were seen; 73 patients received unilateral LLND and 133 patients received bilateral LLND. Of the 351 patients 6.6% developed local recurrence after 5 years. TME only resulted in 0.8% 5-year local recurrence. In lymph-node-positive patients, 33% of the unilateral LLND group had local relapse, significantly more ($p = 0.04$) than in the bilateral LLND group with 14% local recurrence. Local recurrence in the lateral, presacral, perineal, and anastomotic subsites was lower in the bilateral LLND group as compared with in the unilateral LLND group. We conclude that, in selected patients, surgery without LLND has a very low local recurrence rate. Bilateral LLND is more effective in reducing the chance of local recurrence than unilateral LLND. Either surgical approach, with or without LLND, requires reliable imaging during work-up.

For rectal cancer, surgery is the principal treatment in order to cure. Total mesorectal excision (TME) removes the primary tumor with its surrounding mesorectum as an intact package, preventing residual tumor cells in the mesorectum from developing into local recurrence.[1,2] In advanced lesions neoadjuvant (chemo)radiotherapy can downstage tumors, but good surgical quality is still essential in order to achieve total clearance of tumor cells.[3]

The Japanese concept of surgical treatment of rectal cancer has evolved from anatomical studies in which three lymphatic flow routes were identified.[4,5] The upper route is along the superior rectal artery to the inferior mesenteric artery; the lateral route reaches from the middle rectal artery to the internal iliac and obturator basins; and the downward route extends to the inguinal lymph nodes. The upper and lateral routes were shown to be the main two routes of rectal cancer spread, with the peritoneal reflection as the limitation between the two lymphatic areas.[6] Consequently, lateral lymph node dissection (LLND) was developed in Japan in order to resect the tumor with the primary locoregional lymph node basins beyond the mesorectal plane.[7] LLND has resulted in better survival and lower recurrence rates than conventional surgery.[8,9]

A problem is that the lateral lymph node routes are anatomically close to the pelvic autonomic nerve plexus, requiring challenging surgery to preserve these during LLND.[10] In order to prevent damage to autonomic nerves, nowadays case-oriented policy is practised in Japan, adopting LLND only in advanced disease at or below the peritoneal reflection.

The aim of this study is to evaluate the treatment of rectal cancer between 1993 and 2002 at the National Cancer Center Hospital (NCCH), looking at patterns of local recurrence and the risk factors for local recurrence.

PATIENTS AND METHODS

Patients

From 1993 to 2002, 923 patients were operated for confirmed primary adenocarcinoma of the rectum at the National Cancer Center Hospital (NCCH) in Tokyo. Surgery was performed according to the guidelines of the Japanese Research Society for Cancer of the Colon and Rectum.[11,12] The rectum was defined as located below the lower border of the second sacral vertebra. The peritoneal reflection is the most important landmark in defining the location of the tumor, and *low* rectal carcinoma is defined as a tumor of which the major part is located at or below the reflection.[13]

For this analysis the following patients were excluded: metastasis at the time of surgery ($n = 134$) and in situ carcinoma ($n = 22$). Of the remaining 767 patients, only patients with rectal carcinoma at or below the peritoneal refection were selected, resulting in 360 patients.

Neoadjuvant chemotherapy was given to some patients with suspicion of stage T4 disease ($n = 3$) in other hospitals, before referral to the NCCH. Neoadjuvant radiotherapy was not routinely given, so no patients received preoperative radiotherapy. Sometimes in the case of positive lymph nodes, adjuvant radiotherapy ($n = 5$) or chemoradiotherapy ($n = 1$) was given. The nine patients who received neoadjuvant chemotherapy and adjuvant (chemo)radiation were excluded, leaving 351 patients for analysis.

Methods

Until 2002 preoperative evaluation at the NCCH consisted of computed tomography (CT) imaging and endoscopic ultrasonography for all patients. Based on preoperative imaging and intraoperative findings, standard total mesorectal excision (TME) was performed in T1 or T2 stage disease without suspected lymph nodes. Lateral lymph node dissection (LLND) was added to TME in stage T3 or T4 rectal cancer at or below the peritoneal reflection, or when positive mesorectal lymph nodes were suspected. Unilateral LLND was performed when the tumor was located lateral in the low rectum, bilateral LLND when the tumor was located centrally. When the lateral lymph nodes were 1 cm or larger on preoperative imaging or intraoperative findings, bilateral extended lymph node dissection was performed, consisting of dissection of the complete internal iliac artery and the autonomic nerve system. When there was no suspicion on positive lateral lymph nodes, autonomic nerve preservation (ANP) was carried out.

Accurate documentation of lymph node status and localization is obtained because all lymph nodes are harvested and recorded from the fresh specimen. The definition of mesorectal lymph nodes is pararectal location or in the direction of the mesentery. Lateral lymph nodes are located along the iliac or obturator arteries.

Follow-up of all patients consisted of thorax, abdominal, and pelvic CT imaging every 6 months. Median follow-up of patients alive was 7.9 years.

All patients who developed local recurrence, defined as any recurrence of rectal cancer in the lesser pelvis, were identified. Local recurrence was diagnosed clinically, radiologically or histologically.

For all locally recurrent patients the available preoperative images and the images at the time of discovery of the local recurrence were retrieved. A specialized oncologic radiologist (R.G.H.B.-T.) reviewed the images. Examining the images, the site of the local recurrence was determined. The sites were classified into the following regions: lateral, presacral, perineal, anterior or anastomotic. The same borders for the respective sites were used as defined by Roels et al.[14] When no images were available, the location of recurrence was classified using the radiology reports and clinical data. In one patient insufficient information was provided to determine the location of recurrence with certainty.

Statistical Analysis

Statistical analysis was performed using the SPSS package (SPSS 12.0 for Windows; SPSS Inc., Chicago, IL) and R version 2.5.1. T-tests and chi-square tests were used to compare individual variables. Survival and cumulative recurrence incidences were estimated using the Kaplan–Meier method. Differences between the groups were assessed using the log-rank test. All p-values were two-sided and considered statistically significant at 0.05 or less. For local recurrence, cumulative incidences were calculated accounting for death as competing risk.[15] Similarly, cumulative incidences were calculated for subsite of local recurrence, with death and other types of local recurrence as competing risks, and for cancer-specific survival, with death due to other causes as competing risk. Multivariate analyses of local recurrence and overall survival were performed by first testing the effect of covariates in a univariate Cox regression. Covariates with trend-significant effects (p-value < 0.10) were then selected for multivariate Cox regression. The following variables were studied for local recurrence and overall survival: age, sex, operative procedure, degree of lateral lymphadenectomy, T-stage, mesorectal lymph node N-stage, lateral lymph node positivity, maximum tumor diameter, differentiation, and autonomic nerve preservation.

RESULTS

Clinicopathology

Patient characteristics and treatment details are listed in Table 1. Of the 351 studied patients, 145 had standard TME surgery without LLND, 73 underwent unilateral LLND, and 133 patients received bilateral LLND. LLND was performed in significantly younger patients and more often in combination with a non-sphincter-saving procedure, compared with patients who had not undergone an LLND. The tumors in the LLND patients had higher T- and N-stages and were significantly larger. Comparing the clinicopathological characteristics between the unilateral and the bilateral LLND, no significant differences were found, except that unilateral LLND was more often combined with autonomic nerve preservation (ANP).

Mean lymph node harvest was 21 LNs in standard TME (Table 1). After unilateral LLND the mean number of recovered LNs was 38, and after bilateral LLND this was 45 ($p = 0.004$).

Table 2 shows the outcomes of lymph node involvement for all 351 patients, stratified by T-stage. Overall lymph node involvement was 42%, and lateral lymph node

TABLE 1 Clinicopathological characteristics

	No LLND (n = 145)	Unilateral LLND (n = 73)	Bilateral LLND (n = 133)	p*	p**
Sex ratio (M:F)	96:49 (66:34)	47:26 (64:36)	86:47 (65:35)	0.95	0.97
Mean age (years)	61	57	57	0.03	0.98
Operation					
Sphincter-saving	112 (77)	36 (49)	63 (47)		
Not sphincter-saving	33 (23)	37 (51)	70 (53)	<0.001	0.79
Adjuvant chemotherapy					
No	139 (96)	67 (92)	121 (91)		
Yes	6 (4)	6 (8)	12 (9)	0.24	0.85
T-stage					
T1	52 (36)	3 (4)	3 (2)		
T2	47 (32)	27 (37)	37 (28)		
T3	46 (32)	40 (55)	83 (62)		
T4	0 (0)	3 (4)	10 (8)	<0.001	0.37
Meso LN positive					
0	102 (70)	44 (60)	64 (48)		
1–3	30 (21)	19 (26)	39 (29)		
>4	13 (9)	10 (14)	30 (23)	0.003	0.28
Lat LN positive					
No	–	62 (85)	109 (82)		
Yes	–	11 (15)	24 (18)	–	0.59
ANP					
No	3 (2)	2 (3)	17 (13)		
Yes	142 (98)	71 (97)	116 (87)	<0.001	0.02
Differentiation					
Well	75 (52)	27 (37)	50 (38)		
Moderate	67 (46)	44 (60)	75 (56)		
Poor	2 (2)	2 (3)	8 (6)	0.18	0.29
Tumor size					
0–4 cm	106 (73)	31 (42)	42 (32)		
>4 cm	39 (27)	42 (58)	91 (68)	<0.001	0.12
Diss. LN (mean)	21	38	45	<0.001	0.004

Values in parentheses are percentages

* p value between no LLND, unilateral LLND, and bilateral LLND

** p value between unilateral LLND and bilateral LLND

Meso mesorectal; *Lat* lateral; *LN* lymph node; *ANP* autonomic nerve preservation

TABLE 2 Lateral lymph node dissection and lymph node status, stratified by T-stage

Stage	LLND		LNI		LNI	LLNI
T1: 58	No LLND	52 (90%)	N0	47	8/58 = 14%	1/58 = 2%
			Upper pos	5		
	LLND	6 (10%)	N0	3		
			Upper pos, lat neg	2		
			Upper neg, lat pos	0		
			Upper pos, lat pos	1		
T2: 111	No LLND	47 (42%)	N0	33	32/111 = 29%	7/111 = 6%
			Upper pos	14		
	LLND	64 (58%)	N0	46		
			Upper pos, lat neg	11		
			Upper neg, lat pos	2		
			Upper pos, lat pos	5		
T3: 169	No LLND	46 (27%)	N0	22	97/169 = 57%	19/169 = 11%
			Upper pos	24		
	LLND	123 (73%)	N0	50		
			Upper pos, lat neg	54		
			Upper neg, lat pos	5		
			Upper pos, lat pos	14		
T4: 14	No LLND	0 (0%)	N0	–	12/14 = 86%	8/14 = 57%
			Upper pos	–		
	LLND	14 (100%)	N0	1		
			Upper pos, lat neg	4		
			Upper neg, lat pos	0		
			Upper pos, lat pos	8		
Total: 351		207/351 = 59%*			149/351 = 42%	35/351 = 10%

LLND lateral lymph node dissection; *LNI* lymph node involvement (upper and lateral lymph nodes); *LLNI* lateral lymph node involvement; Upper, upper lymph nodes; *Lat* lateral lymph nodes; *pos* positive; *neg* negative

* Percentage of patients submitted to LLND

involvement was 10%. Jump metastases (mesorectal lymph nodes negative and lateral lymph nodes positive) occurred in 3% (7/207) of the patients with LLND.

Local Recurrence

At time of last follow-up 23 of the total of 351 patients had developed local recurrence (6.6% 5-year local recurrence rate). In the patients who had not undergone LLND, only one patient (0.8%) had local recurrence at the site of the anastomosis. In the unilateral LLND group, 12 of the 73 patients (5-year 15.4%) had local relapse. This was more than in the bilateral LLND group, with 10 of 133 local recurrences (5-year 8.3%). In N+ patients (Fig. 1), the difference between the uni- and bilateral LLND (32.8% versus 14.2%, respectively) was significant ($p = 0.04$).

In multivariate analysis (Table 3) including uni- and bilateral LLND patients, lateral lymphadenectomy, mesorectal lymph node N-stage, and lateral lymph node positivity were independent risk factors for local recurrence.

Compared with patients with bilateral LLND the relative risk for local recurrence was 4.0 for unilateral LLND patients.

Table 4 reports the sites of the local recurrences for the uni- and bilateral LLND groups. The rate of lateral recurrence in the unilateral LLND patients was 5.6%, and in the bilateral LLND patients was 3.3%. It was noticed that the three patients who developed lateral local recurrence on the ipsilateral side after unilateral LLND had lower lymph node harvest (mean 28 LNs) than the patients who developed no lateral recurrence after unilateral LLND (mean 38 LNs). However, the number of patients is too low to draw any firm conclusion from this finding.

Distant Recurrence and Survival

At local recurrence diagnosis 40% of the unilateral LLND patients and 60% of the bilateral LLND patients had distant metastases. One year after local recurrence diagnoses these figures were 70% and 80% in the uni- and bilateral LLND patients, respectively.