teractions of treatment with covariates were used to identify predictive factors by assessing whether there was a significant difference in the treatment effect for progression-free survival (hazard ratio for progression or death) between subgroups.

Overall survival was analyzed with the use of methods that were similar to those used for the analysis of progression-free survival. The results of an early analysis are presented; follow-up with respect to overall survival is ongoing. The objective response rate (in the intention-to-treat population) and quality of life and rates of symptom reduction (among all patients with a baseline and at least one post-baseline quality-of-life assessment that could be evaluated) were assessed with the use of a logistic-regression model with the same covariates as those considered for progression-free survival to calculate odds ratios and 95% confidence intervals. Planned subgroup analyses of the objective response rate were performed with the use of methods that were similar to those used for the analysis of progression-free survival.

Adverse events were summarized for all patients who received at least one dose of the assigned study treatment. The incidence rates of 10 specified safety events (5 that were possibly associated with each study treatment) were compared with the use of Fisher's exact test; adjustment for multiple comparisons was performed with the use of the method of Westfall and Young.[24]

RESULTS

PATIENTS AND TREATMENT

From March 2006 through October 2007, a total of 1217 patients from 87 centers in Hong Kong, elsewhere in China, Indonesia, Japan, Malaysia, the Philippines, Singapore, Taiwan, and Thailand were randomly assigned to a study group (Fig. 1). The two groups were well balanced with respect to demographic and baseline characteristics (Table 1). The mean duration of treatment was 6.4 months (median, 5.6; range, 0.1 to 22.8) for gefitinib and 3.4 months (median, 4.1; range, 0.7 to 5.8) for carboplatin–paclitaxel. The median number of treatment cycles in the carboplatin–paclitaxel group was six. At the cutoff date for collection of data (April 14, 2008), a total of 24.5% of the patients in the gefitinib group were continuing to receive the study treatment; all patients in the carboplatin–paclitaxel group had discontinued the drugs. After discontinuation of the assigned treatment at any time during the study, 38.9% of the patients in the gefitinib group received carboplatin–paclitaxel, and 39.5% of the patients in the carboplatin–paclitaxel group received an EGFR tyrosine kinase inhibitor; 10.5% of the patients in the gefitinib group and 14.0% of those in the carboplatin–paclitaxel group received other anticancer treatments.

EFFICACY

The median follow-up period for the analysis of progression-free survival was 5.6 months. The median progression-free survival was 5.7 months in the gefitinib group and 5.8 months in the carboplatin–paclitaxel group, approximately coinciding with crossing of the Kaplan–Meier curves. The 12-month rates of progression-free survival were 24.9% with gefitinib and 6.7% with carboplatin–paclitaxel; a total of 950 patients had progression of disease. The study met its primary objective of demonstrating noninferiority and showed the superiority of gefitinib as compared with carboplatin–paclitaxel for progression-free survival (hazard ratio for progression or death, 0.74; 95% confidence interval [CI], 0.65 to 0.85; $P<0.001$). The probability that a patient would be free of disease progression was greater with carboplatin–paclitaxel in the first 6 months and greater with gefitinib in the following 16 months (Fig. 2A). Progression-free survival was longer in the gefitinib group than in the carboplatin–paclitaxel group in all clinical subgroups; the only clinical factor that affected progression-free survival was age (<65 years: hazard ratio, 0.81; 95% CI, 0.70 to 0.95; $P=0.007$; ≥65 years: hazard ratio, 0.58; 95% CI, 0.45 to 0.76; $P<0.001$; $P=0.03$ for the interaction of treatment with age) (Fig. 1 in the Supplementary Appendix).

A total of 1038 patients (85.3%) gave their consent for biomarker analyses, and 683 patients (56.1%) provided samples. *EGFR* mutation data for 437 patients (35.9%) could be evaluated. Patients with a tissue sample that could be evaluated had demographic characteristics that were similar to those of the overall population (Table 1 in the Supplementary Appendix). Of the 437 samples, 261 (59.7%) were positive for a mutation. Of these 261 samples, 140 (53.6%) had exon 19 deletions, 111 (42.5%) had a mutation at exon 21 (L858R), 11 (4.2%) had a mutation at exon 20 (T790M), and 10 (3.8%) had other mutations; 11 patients had multiple mutations. The proportions of mutations

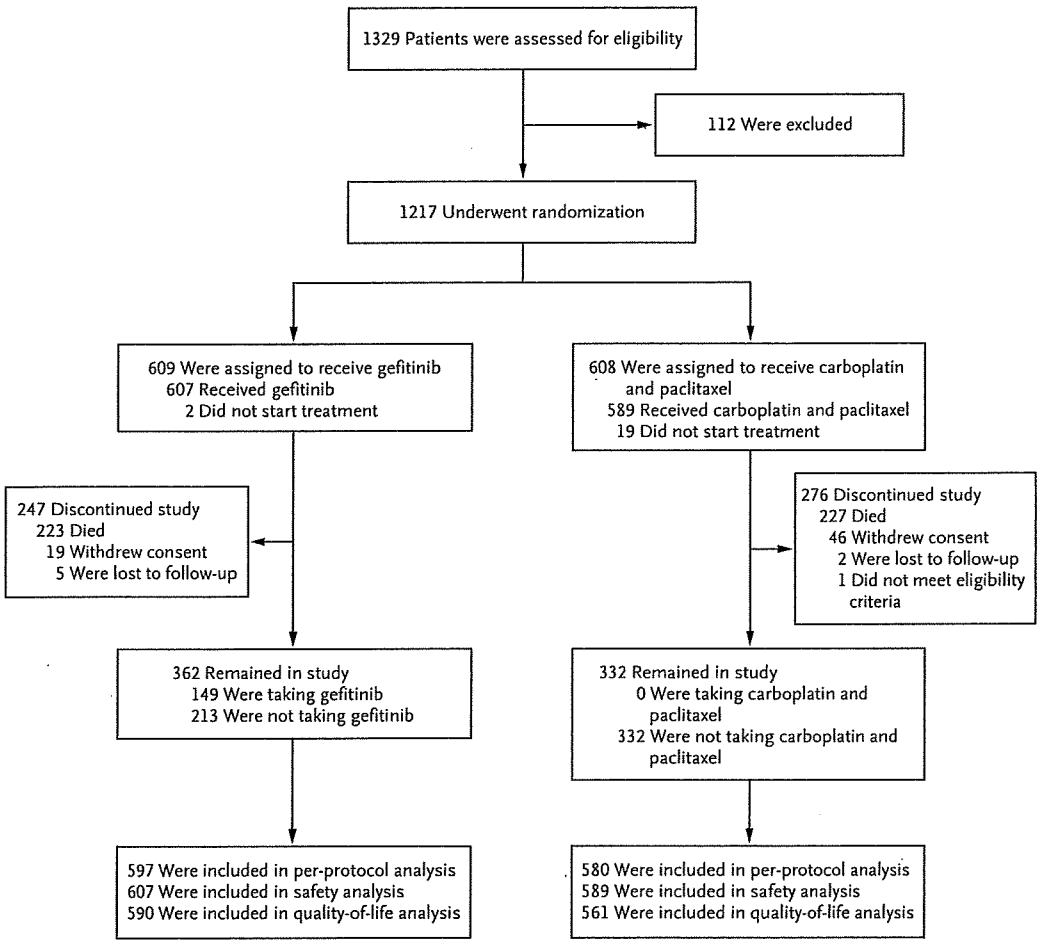

Figure 1. **Screening, Group Assignment, and Inclusion in Analyses.**

All patients who were randomly assigned to a study group were included in the intention-to-treat analysis; all patients with a baseline and at least one post-baseline quality-of-life assessment that could be evaluated were included in the quality-of-life analysis; patients who did not deviate substantially from the inclusion and exclusion criteria at entry or from the protocol were included in the per-protocol analysis; and all patients who received at least one dose of study treatment were included in the safety analysis. Among the 112 patients who were assessed for eligibility but were not assigned to a study group, the main reasons for exclusion were a serum creatinine level that was higher than 1.5 times the upper limit of the reference range or a creatinine clearance of 60 ml per minute or less; newly diagnosed central nervous system metastases that had not yet been definitively treated with surgery or radiation; or an absolute neutrophil count of less than 2.0×10^9 per liter, a platelet count of less than 100×10^9 per liter, or a hemoglobin level of less than 10 g per deciliter. A total of 63 patients who were treated with gefitinib continued to receive gefitinib after disease progression, and 1 patient who was treated with carboplatin–paclitaxel continued to receive carboplatin–paclitaxel after disease progression because the investigator believed that the treatment was providing a benefit.

were well balanced between the two groups (Table 2 in the Supplementary Appendix).

There was a significant interaction between treatment and *EGFR* mutation with respect to progression-free survival (P<0.001). Progression-free survival was significantly longer among patients receiving gefitinib than among those receiving carboplatin–paclitaxel in the mutation-positive subgroup (hazard ratio for progression, 0.48; 95% CI, 0.36 to 0.64; P<0.001) (Fig. 2B) and significantly shorter among patients receiving gefitinib than among those receiving carboplatin–paclitaxel in the mutation-negative subgroup (hazard ratio, 2.85; 95% CI, 2.05 to 3.98; P<0.001) (Fig. 2C). Results in the subgroup with unknown *EGFR*-mutation status (hazard ratio with gefitinib, 0.68; 95%

Table 1. **Demographic and Baseline Characteristics in the Intention-to-Treat Population.***

Characteristic	Gefitinib (N=609)	Carboplatin–Paclitaxel (N=608)
Age — yr		
Median	57	57
Range	24–84	25–84
Sex — no. (%)		
Male	125 (20.5)	127 (20.9)
Female	484 (79.5)	481 (79.1)
Ethnic group — no. (%)†		
Chinese	314 (51.6)	304 (50.0)
Japanese	114 (18.7)	119 (19.6)
Other East Asian‡	179 (29.4)	184 (30.3)
Other	2 (0.3)	1 (0.2)
Smoking history — no. (%)		
Never smoked	571 (93.8)	569 (93.6)
Former light smoker	37 (6.1)	38 (6.2)
Former non–light smoker	1 (0.2)	1 (0.2)
WHO performance status — no. (%)§		
0	157 (25.8)	161 (26.5)
1	391 (64.2)	382 (62.8)
2	61 (10.0)	65 (10.7)
Histologic feature of tumor — no. (%)		
Adenocarcinoma	581 (95.4)	591 (97.2)
Bronchoalveolar carcinoma	27 (4.4)	15 (2.5)
Unknown	1 (0.2)	2 (0.3)
Disease stage at entry — no. (%)		
IIIB	150 (24.6)	144 (23.7)
IV	459 (75.4)	463 (76.2)
Unknown	0	1 (0.2)
Time from diagnosis to randomization — no. (%)		
<6 mo	582 (95.6)	573 (94.2)
≥6 mo	27 (4.4)	34 (5.6)
Unknown	0	1 (0.2)
Disease stage at diagnosis — no. (%)¶		
IA	7 (1.1)	12 (2.0)
IB	2 (0.3)	9 (1.5)
IIA	2 (0.3)	1 (0.2)
IIB	1 (0.2)	6 (1.0)
IIIA	6 (1.0)	3 (0.5)
IIIB	166 (27.3)	163 (26.8)
IV	424 (69.6)	413 (67.9)
Unknown	1 (0.2)	1 (0.2)

* Percentages may not sum to 100 because of rounding.
† Ethnic group was self-reported.
‡ Other East Asian refers to patients who belong to East Asian ethnic groups other than Chinese and Japanese.
§ The World Health Organization (WHO) performance status measures level of activity and is assessed on a scale of 0 to 4, with lower numbers indicating a higher degree of activity.
¶ All patients had Stage IIIB or IV disease at entry.

CI, 0.58 to 0.81; P<0.001) (Fig. 2D) were similar to those for the overall population.

The objective response rate in the overall population was significantly higher with gefitinib than with carboplatin–paclitaxel (43.0% vs. 32.2%; odds ratio, 1.59; 95% CI, 1.25 to 2.01; P<0.001) (Table 3 in the Supplementary Appendix) and numerically or statistically greater with gefitinib in all clinical subgroups. The objective response rate was 71.2% with gefitinib versus 47.3% with carboplatin–paclitaxel in the mutation-positive subgroup (P<0.001) and 1.1% (one patient) versus 23.5%, respectively, in the mutation-negative subgroup (P=0.001) (Table 3 in the Supplementary Appendix).

Overall survival in this early analysis (450 patients [37.0%] died, with follow-up ongoing) was similar between the two groups in the overall population (hazard ratio for death in the gefitinib group, 0.91; 95% CI, 0.76 to 1.10) (Fig. 2A in the Supplementary Appendix). Median survival was 18.6 months among patients receiving gefitinib and 17.3 months among patients receiving carboplatin–paclitaxel. After observing the results with respect to progression-free survival, we performed an analysis of overall survival according to mutation status, although this analysis included only 81 deaths in the mutation-positive subgroup and 94 in the mutation-negative subgroup. The hazard ratios with gefitinib were 0.78 (95% CI, 0.50 to 1.20) in the mutation-positive subgroup and 1.38 (95% CI, 0.92 to 2.09) in the mutation-negative subgroup (Fig. 2B and 2C in the Supplementary Appendix).

Significantly more patients in the gefitinib group than in the carboplatin–paclitaxel group had a clinically relevant improvement in quality of life, as assessed by scores on the FACT-L questionnaire (odds ratio, 1.34; 95% CI, 1.06 to 1.69; P=0.01) and by scores on the TOI (odds ratio, 1.78; 95% CI, 1.40 to 2.26; P<0.001) (Fig. 3). Rates of reduction in symptoms, as assessed on the basis of the LCS scores, were similar between patients who received gefitinib and those who received carboplatin–paclitaxel (odds ratio with gefitinib, 1.13; 95% CI, 0.90 to 1.42; P=0.30) (Fig. 3). Results according to mutation status are provided in Figure 3 in the Supplementary Appendix.

SAFETY AND ADVERSE-EVENT PROFILE

Table 2 lists the most common adverse events. Gefitinib, as compared with carboplatin–paclitaxel, was associated with a lower rate of grade 3 or 4

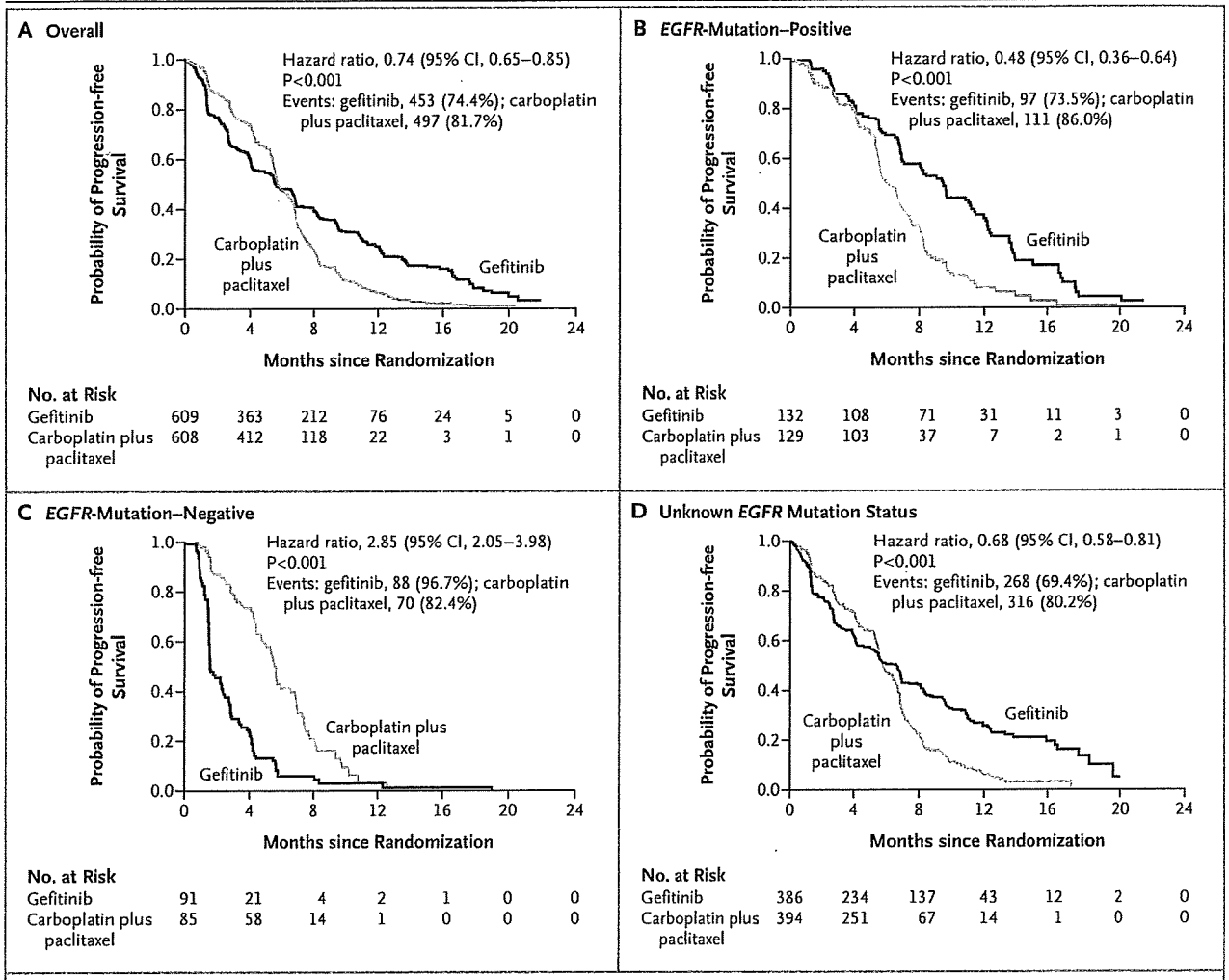

Figure 2. **Kaplan–Meier Curves for Progression-free Survival.**

Kaplan–Meier curves for progression-free survival are shown for the overall population (Panel A), patients who were positive for the *EGFR* mutation (Panel B), patients who were negative for the *EGFR* mutation (Panel C), and patients with unknown *EGFR* mutation status (Panel D). Analyses were performed on the basis of the intention-to-treat population. With respect to the overall population, results of the supportive secondary analyses (including a log-rank test, which is valid under the null hypothesis even when hazards are not proportional, and analysis in the per-protocol population) were consistent with the result of the primary analysis. Hazard ratios were calculated with the use of a Cox proportional-hazards model, with the WHO performance status (0 or 1, or 2), smoking history (nonsmoker or former light smoker), and sex as covariates. EGFR denotes epidermal growth factor receptor.

adverse events, as defined according to the Common Terminology Criteria for Adverse Events (28.7% vs. 61.0%), a lower rate of adverse events leading to discontinuation of the drug (6.9% vs. 13.6%), and a lower rate of dose modification due to toxic effects (16.1% vs. 35.2% for carboplatin and 37.5% for paclitaxel). Adverse events leading to death occurred in 3.8% of the patients treated with gefitinib and in 2.7% of the patients treated with paclitaxel–carboplatin; serious adverse events, including death, occurred in 16.3% and 15.6% of patients in the two groups, respectively; and serious adverse events leading to hospitalization occurred in 13.8% and 13.1% of patients in the two groups, respectively. The incidences of rash or acne, diarrhea, and elevated liver aminotransferase levels were significantly higher with gefitinib than with carboplatin–paclitaxel, whereas the incidences of neurotoxic effects, nausea and vomiting, and hematologic toxic effects were significantly higher with carboplatin–paclitaxel (Table 4 in the Supplementary Appendix). Interstitial-lung-disease events (i.e., the acute respiratory distress syndrome, interstitial lung disease, pneumonitis, or radiation

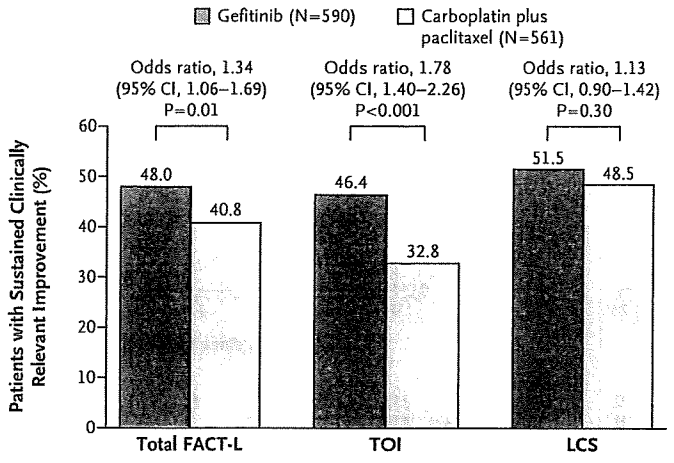

Figure 3. **Rates of Improvement in Scores for Quality for Life and Symptoms.**
Calculations were performed on the basis of all patients with a baseline and at least one post-baseline quality-of-life assessment that could be evaluated. P values were calculated with the use of logistic regression, with the WHO performance status (0 or 1, or 2), smoking history (nonsmoker or former light smoker), and sex as covariates. Clinically relevant improvement was predefined as an improvement of six points or more in scores on the Functional Assessment of Cancer Therapy–Lung (FACT–L, in which scores range from 0 to 136, with higher scores indicating better quality of life) and Trial Outcome Index (TOI, in which scores range from 0 to 84, with higher scores indicating better quality of life) or an improvement of two points or more in scores on the lung-cancer subscale (LCS) of the FACT–L (in which scores range from 0 to 28, with higher scores indicating fewer symptoms), with the higher scores maintained for at least 21 days.

pneumonitis) occurred in 16 patients treated with gefitinib (2.6%), 3 of whom died, and in 8 patients treated with carboplatin-paclitaxel (1.4%), 1 of whom died.

DISCUSSION

Platinum-based combination chemotherapy, such as carboplatin–paclitaxel, is the standard first-line therapy for advanced non–small-cell lung cancer.[25,26] The results of this trial showed that gefitinib by itself is superior to carboplatin–paclitaxel in a selected population of East Asian patients.

As initial treatment of non–small-cell lung cancer in East Asian nonsmokers or former light smokers with pulmonary adenocarcinoma, gefitinib, as compared with carboplatin–paclitaxel, prolonged progression-free survival, increased the objective response rate, reduced toxic effects, and improved quality of life. The overall benefit was driven primarily by the subgroup of patients with *EGFR* mutations; in this subgroup, patients treated with gefitinib, as compared with those treated with carboplatin–paclitaxel, had a remarkably high objective response rate (71.2%) and prolonged progression-free survival (hazard ratio for progression or death, 0.48; 95% CI, 0.36 to 0.64; P<0.001). In the subgroup of patients without *EGFR* mutations, the objective response rate with gefitinib was 1.1%, and progression-free survival favored chemotherapy (hazard ratio with gefitinib, 2.85; 95% CI, 2.05 to 3.98; P<0.001). These contrasting outcomes probably explain the change over time in treatment effect for progression-free survival in the overall population. The initial superiority of carboplatin–paclitaxel was attributed to the benefit that the *EGFR*-mutation–negative subgroup received from chemotherapy but not from gefitinib, whereas prolonged progression-free survival in the *EGFR*-mutation–positive subgroup explained the subsequent improvement favoring gefitinib. Crossing of the Kaplan–Meier curves did not occur in the mutation-positive subgroup or the mutation-negative subgroup.

Lynch et al. found specific *EGFR* mutations that correlated with tumor response to gefitinib.[7] In the Iressa Survival Evaluation in Lung Cancer trial (ISEL; ClinicalTrials.gov number, NCT00242801), the objective response rate for gefitinib-treated patients was 37.5% among the 16 patients with a tumor bearing an *EGFR* mutation as compared with 2.6% among the 116 patients without a mutation.[27] Our trial confirms the predictive value of *EGFR* mutations for the responsiveness of pulmonary adenocarcinoma to gefitinib as compared with carboplatin–paclitaxel. The difference in the rates of objective response between gefitinib-treated patients with an *EGFR* mutation and those without an *EGFR* mutation (71.2% vs. 1.1%) was remarkable. The rate of an objective response to first-line gefitinib in our study is similar to rates reported in other studies in which patients were selected according to *EGFR*-mutation status, including patients in Western countries.[10,12,28] Sequist et al. screened patients (who were selected on the basis of clinical characteristics) for an *EGFR* mutation and reported an objective response rate of 54.8% among 31 gefitinib-treated patients who were positive for an *EGFR* mutation, only 2 of whom were Asian.[12] However, in our study, objective response rates among patients without an *EGFR* mutation were lower than expected, given the results of previous studies.[16,29] One possible explanation is our use of ARMS, a more sensitive technique for detecting *EGFR* mutations.[21,22] When Zhu et al. used ARMS to reanalyze 148 samples

Table 2. **Adverse Events.***

Adverse Event	Gefitinib (N=607)		Carboplatin–Paclitaxel (N=589)	
	All Adverse Events	CTC Grade 3, 4, or 5	All Adverse Events	CTC Grade 3, 4, or 5
	number (percent)			
Rash or acne†	402 (66.2)	19 (3.1)	132 (22.4)	5 (0.8)
Diarrhea	283 (46.6)	23 (3.8)	128 (21.7)	8 (1.4)
Dry skin	145 (23.9)	0	17 (2.9)	0
Anorexia†	133 (21.9)	9 (1.5)	251 (42.6)	16 (2.7)
Pruritus†	118 (19.4)	4 (0.7)	74 (12.6)	1 (0.2)
Stomatitis†	103 (17.0)	1 (0.2)	51 (8.7)	1 (0.2)
Asthenic conditions†	102 (16.8)	2 (0.3)	259 (44.0)	11 (1.9)
Nausea	101 (16.6)	2 (0.3)	261 (44.3)	9 (1.5)
Paronychia	82 (13.5)	2 (0.3)	0	0
Vomiting	78 (12.9)	1 (0.2)	196 (33.3)	16 (2.7)
Constipation	73 (12.0)	0	173 (29.4)	1 (0.2)
Alopecia	67 (11.0)	0	344 (58.4)	0
Neurotoxic effects†	66 (10.9)	2 (0.3)	412 (69.9)	29 (4.9)
Myalgia	47 (7.7)	3 (0.5)	186 (31.6)	10 (1.7)
Arthralgia	39 (6.4)	1 (0.2)	113 (19.2)	6 (1.0)
Neutropenia‡				
Any	NA	22 (3.7)	NA	387 (67.1)
Febrile	1 (0.2)	1 (0.2)	17 (2.9)	17 (2.9)
Anemia‡	NA	13 (2.2)	NA	61 (10.6)
Leukopenia‡	NA	9 (1.5)	NA	202 (35.0)

* Calculations were based on 1196 patients who received at least one dose of the study treatment. The Common Terminology Criteria (CTC) grade is defined on the basis of the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Events are included if they occurred in at least 10% of patients in either treatment group, either while the patients were receiving treatment or during the 28-day follow-up, and if there was at least a 5% difference between groups. There were other adverse events that occurred in few patients and that may or may not have been related to the study drug. NA denotes not available.

† This is a group term (sum of high-level and preferred terms, according to the definitions in the *Medical Dictionary for Regulatory Activities*).

‡ Data are from the laboratory reports of 599 patients who were taking gefitinib and 577 who were taking carboplatin–paclitaxel. Events were included if there was a worsening in the laboratory value (absolute neutrophil count in the case of neutropenia, hemoglobin in the case of anemia, and white-cell count in the case of leukopenia) from baseline to CTC grade 3 or 4.

that had previously been classified as negative for an *EGFR* mutation, they found 11 new samples with exon 19 mutations.[30] Another possible explanation is that studies that showed higher response rates among mutation-negative patients were not always conducted in previously untreated patients. Mutation-negative status that is determined in a diagnostic sample obtained at the time of the initial presentation may change during subsequent tumor progression or during the course of chemotherapy.[31]

Our findings suggest that, whenever possible, *EGFR*-mutation status should be determined before the initial treatment of pulmonary adenocarcinoma. Ethnic origin, smoking status, and histologic findings help to identify patients who have a high likelihood of having an *EGFR* mutation; in this study, 59.7% of the tumors in a clinically selected population had *EGFR* mutations, as compared with 12.1% and 14.8% in the unselected populations in the ISEL and Iressa in NSCLC Trial Evaluating Response and Survival versus Taxotere (INTEREST; NCT00076388) studies, respectively.[2,27]

The efficacy of gefitinib seen in this study was coupled with lower incidences of alopecia, nausea, vomiting, neurotoxic symptoms, and myelosuppression than those seen with carboplatin–paclitaxel. Among 607 patients who received gefitinib

and who were included in the safety analysis, interstitial-lung-disease events developed in only 16 (2.6%), 3 of whom (0.5%) died.

In summary, this study shows that first-line therapy with gefitinib as compared with carboplatin–paclitaxel prolongs progression-free survival, increases the objective response rate, and improves quality of life among clinically selected patients with non–small-cell lung cancer. The presence of an *EGFR* mutation was a robust predictor of improved progression-free survival with gefitinib, as compared with carboplatin–paclitaxel, and of the benefit of gefitinib with respect to the objective response rate, indicating that patients in whom an *EGFR* mutation has been identified will benefit most from first-line therapy with gefitinib.

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APPENDIX

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ORIGINAL ARTICLE

Additive effects of drug transporter genetic polymorphisms on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients

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Abstract
Purpose Effects of genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in addition to "*UGT1A1*28* or **6*" on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients were investigated.
Methods Associations between transporter haplotypes/variations along with *UGT1A1*28* or **6* and SN-38 area under the time–concentration curve (AUC) or neutropenia were examined in irinotecan monotherapy (55 patients) and irinotecan–cisplatin-combination therapy (62 patients).
Results Higher SN-38 AUC values were observed in *ABCB1* 2677G>T (A893S) (**2* group) for both regimens. Associations of grade 3/4 neutropenia were observed with *ABCC2* −1774delG (**1A*), *ABCG2* 421C>A (Q141K) and IVS12 + 49G>T (*#IIB*) and *SLCO1B1* 521T>C (V174A) (**15* · *17*) in the irinotecan monotherapy, while they were

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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 [26], 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 [16].

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)] [27] 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 [28]. Major combinations in 177 patients were the wild type #*IA* (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 [28].

The *SLCO1B1* haplotypes used were the major haplotypes *1b [containing 388A>G (N130D) without 521T>C (V174A)] [33] and *15 · 17 [containing 521T>C (V174A)], the functional relevance of which has been reported [34].

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/m^2 for initial dosage) or by combination therapy with cisplatin (60–70 mg/m^2 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 m^2/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.

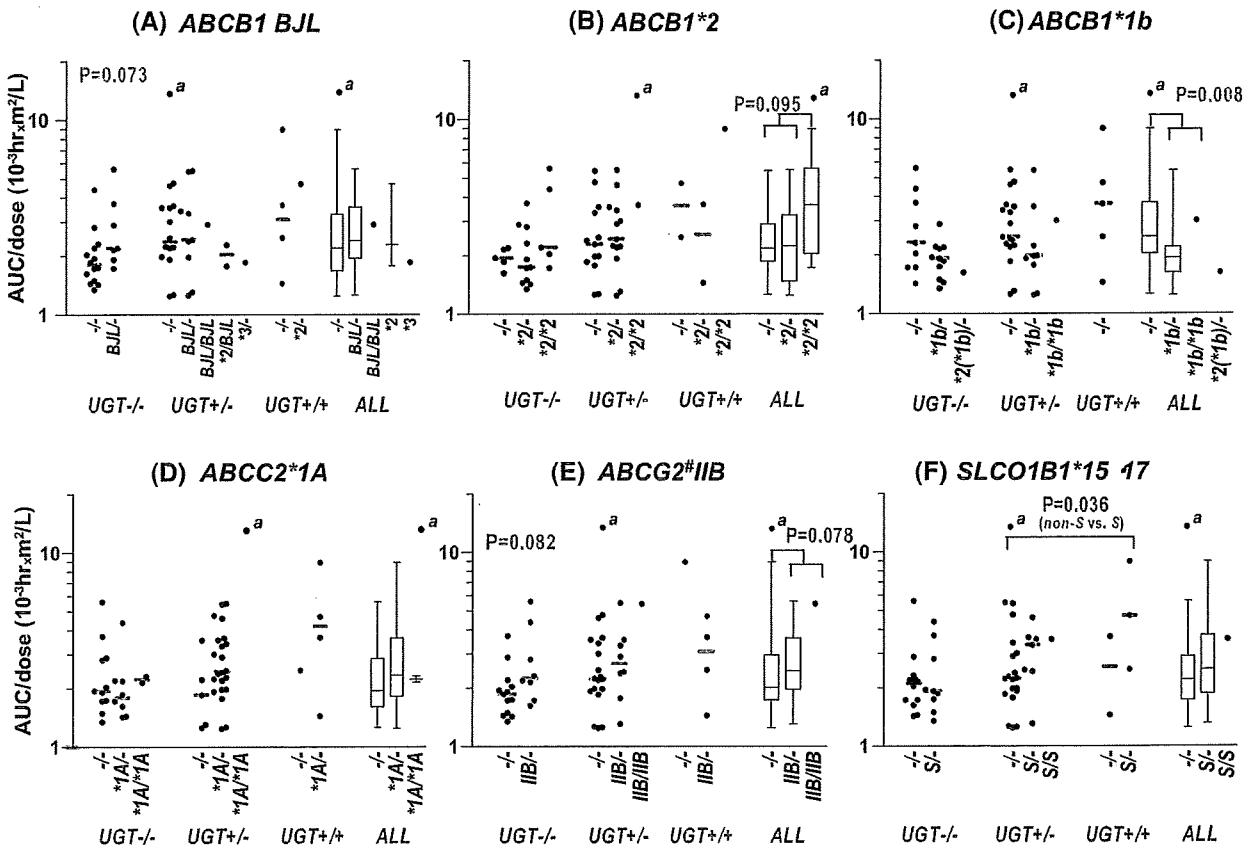

Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). *a* Excluded from statistical analysis. The *bars* represent the medians. *UGT+* = *UGT1A1*6* or **28*. **a** *BJL* contains −1789G>A, *2 (block 1) = 325G>A (E109K), **3* (block 1) = 304G>A (G102R); **b** *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	*BJL* (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* [28] and *UGT1A1* [35]

[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* [35], 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) [28].

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. 2. For the combination therapy with cisplatin (Fig. 2b), 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.

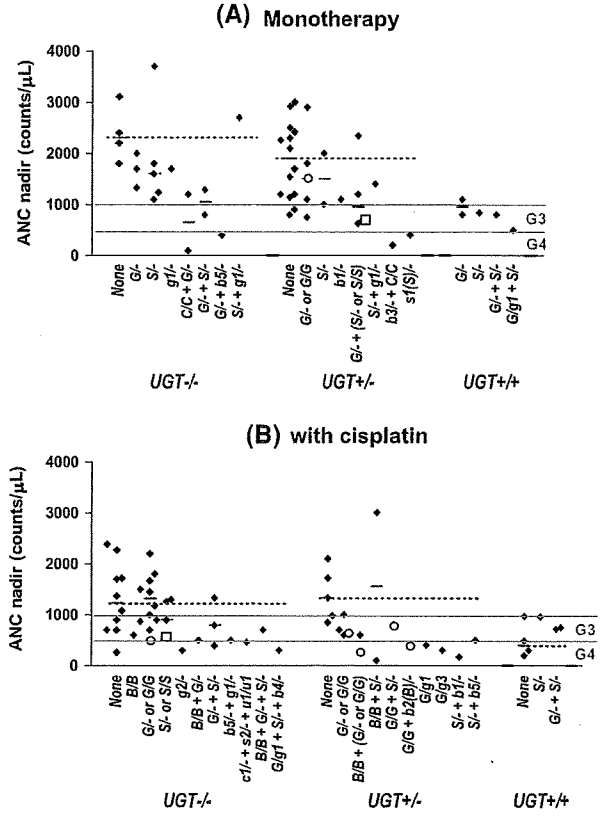

Fig. 2 Additive effects of transporter haplotypes/variations on ANC nadirs in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *UGT+ = UGT1A1*6* or **28*; *B = ABCB1*2*; *C = ABCC2*1A*; *G = ABCG2#IIB* (*open circle*, *#IIB/#IIB*); *S = SLCO1B1*15 · 17* (*open square*, **15 · 17/*15 · 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 · 17* were not significant in the multivariate analysis. For neutropenia, additive effects were suggested for *ABCC2*1A/*1A*, *ABCG2#IIB*, *SLCO1B1*15 · 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 · 17/*15 · 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 · 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

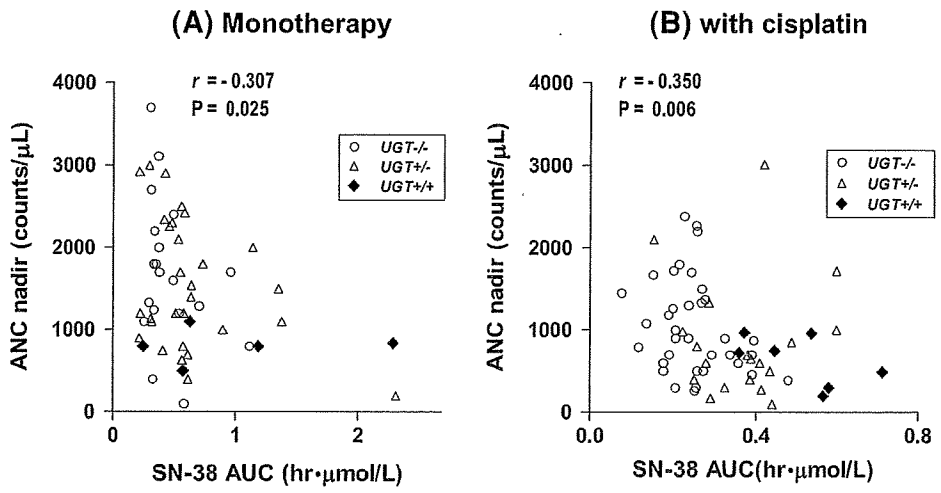

Fig. 3 Correlations between SN-38 AUC and ANC nadir in patients in irinotecan monotherapy (a) and combination therapy with cisplatin (b). *r* Spearman's rank correlation coefficient

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 · 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 · 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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Phase I study of TLR9 agonist PF-3512676 in combination with carboplatin and paclitaxel in patients with advanced non-small-cell lung cancer

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This phase I, open-label study investigated the Toll-like receptor 9 agonist, PF-3512676, in combination with carboplatin and paclitaxel in Japanese patients with advanced, non-small-cell lung cancer (NSCLC). Patients (n = 12) with treatment-naive stage IIIB or IV NSCLC received single-agent PF-3512676 subcutaneously once during the first 7 days (monotherapy phase) in three escalating dose levels (0.1, 0.2, and 0.4 mg/kg) followed by a combination phase during which patients received 0.1 or 0.2 mg/kg PF-3512676 subcutaneously on days 8 and 15 of each 3-week cycle of carboplatin (area under the curve, 6 mg × min/mL) and paclitaxel (200 mg/m^2). Safety and pharmacokinetics of PF-3512676 were assessed during monotherapy and combination therapy phases. PF-3512676 was tolerable as monotherapy or in combination with chemotherapy in patients with NSCLC. Most common treatment-related, non-hematologic adverse events (AEs) throughout the study were injection-site reactions (n = 12, 100%) and flu-like symptoms (n = 11, 91.7%) that were each grade 1 or 2 in all but one patient. All patients experienced neutropenia and leukopenia (≥grade 3 in 11 [91.7%] and seven [58.3%] patients, respectively). One patient in dose level 2 had a dose-limiting toxicity: grade 3 rash and grade 3 increase in γ-glutamyltransferase during combination therapy. Mean PF-3512676 half-life ranged from 4.8 to 21.6 h (longer with higher doses). Four (33%) patients had objective responses (one complete response, three partial responses), and seven (58%) patients achieved stable disease. PF-3512676 as monotherapy and in combination with chemotherapy had an acceptable safety profile in Japanese patients with treatment-naive NSCLC. (*Cancer Sci* 2010; 101: 188–195)

Worldwide, lung cancer accounts for 1.3 million deaths per year, and cancers of the lung, trachea, and bronchus are the leading cause of cancer-related death in Japanese men. Non-small-cell lung cancer (NSCLC) accounts for approximately 80% of lung cancers,[1] and the vast majority (~70%) of cases of NSCLC are locally advanced or metastatic at diagnosis.[2] The current standard first-line treatment for patients with stage IIIB or IV NSCLC and good performance status is doublet chemotherapy with a platinum agent (e.g. carboplatin or cisplatin) in combination with paclitaxel, docetaxel, gemcitabine, or vinorelbine.[3,4] This treatment is associated with objective response rates of approximately 20% to 40% and median survival of 8 to 10 months, which is not considered satisfactory to patients.[4,5] Therefore, development of more effective treatment regimens is warranted for the unmet medical needs of patients with advanced NSCLC.

Toll-like receptors (TLRs) are a family of specialized immune receptors that induce protective immune responses upon detection of highly conserved pathogen-expressed molecules. To date, 10 different TLRs have been identified in humans.[6,7] Each TLR binds one or more distinct pathogen-expressed molecules and can function as an immune system 'alarm signal,' leading to initiation of appropriate host immune defenses.[6,8] In humans, TLR9 is expressed primarily by plasmacytoid dendritic cells (pDCs) and B cells. It recognizes unmethylated cytosine-phosphate-guanine (CpG) dinucleotide sequences commonly found in bacterial and viral DNA.[9] TLR9 can also be stimulated using synthetic oligodeoxynucleotides (ODNs) containing one or more unmethylated CpG dinucleotide motifs. This stimulation leads to activation of type 1 helper T cell (T_H1)-like innate immunity, including upregulated production of interleukin (IL)-6, IL-12p40, interferon-alpha (IFN-α), and IFN-inducible chemokines such as interferon-γ-inducible protein 10 (IP-10).[10] Innate immune activation with a TLR9 agonist may enhance tumor antigen presentation and promote an antitumor immune response.

PF-3512676 (formerly known as CpG 7909) is a TLR9 agonist that has been tested in clinical trials for the treatment of patients with several types of cancer.[11] This synthetic CpG ODN can induce potent innate and adaptive immune T_H1 responses, and to a lesser extent, T_H2 immune responses in murine models.[9–12] Preclinical evidence supporting the use of PF-3512676 in lung cancer was provided by studies with a murine Lewis lung cancer model in which mice treated with PF-3512676 in combination with paclitaxel had significantly prolonged survival compared to mice treated with either drug given alone ($P < 0.0001$).[13] This preclinical evidence, combined with the promising clinical activity of PF-3512676 in other types of advanced cancer, supported investigation in patients with NSCLC. In non-clinical studies in mice investigating efficacy of PF-3512676 plus paclitaxel in the metastatic Renca renal cell carcinoma (RCC) models, survival following treatment with PF-3512676 was longer with regional divided dosing and weekly administration compared with temporal divided dosing using twice-weekly administration. Furthermore, in early clinical studies, elevations of IP-10 observed after dosing with PF-3512676 returned to baseline levels after about 1 week. Therefore, PF-3512676 was administered weekly with rotation of administration sites in clinical studies. Chemotherapy and PF-3512676 were not co-administered because chemotherapy was intended to cause decomposition of tumor cells and release of tumor antigens. PF-3512676 was administered after chemotherapy so that pDCs activated through the TLR9 pathway might present these antigens, thus increasing the number of antigen-specific, cytotoxic T cells.

The safety of PF-3512676 has been studied in more than 800 subjects, including more than 400 cancer patients. The extensive human clinical experience demonstrates that PF-3512676 is safe and well tolerated. In phase I studies in Western patients, 0.0025 to 0.81 mg/kg PF-3512676 weekly subcutaneous doses have

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