申請日 西暦　　年　　月　　日

更新日 西暦　　年　　月　　日

〈登録レジメン名称〉

療法

申請グループ＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

代表医師＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

対象がん腫	実施場所	臨床使用分類	投与時患者状態
	□入院のみ □外来のみ □入院，外来 □ □ □	□日常診療 □単施設研究（IRB 認可済） □多施設研究（IRB 認可済） □市販後臨床試験（IRB 認可済） □治験（IRB 認可済） □保険適応外（IRB 認可済）	□初発（1st line） □再発・難治性（2nd line） □再発（3rd line 以降） □術後補助療法 □術前補助療法 □緩和療法

番号	薬剤一般名	投与量 mg/m^2 or mg/kg or mg/body	投与時間 / 速度（どちらか片方を記載）	投与経路（経口 or 静脈 or 皮下 or 動脈）	投与日（例：day1 - 5 or day8 等）
1					
2					
3					
4					
5					
6					
7					
8					

投与期間			
標準投与回数		最大投与回数	
最大投与量の規定			
休薬期間短縮規定			
予想される副作用			
減量基準			
増量基準			
最も重要な文献	タイトル		
	雑誌名		
	年号，巻，ページ		
	試験の種類（いずれかを選択）	ランダム化比較試験のメタアナリシス (1a)，ランダム化比較試験 (1b)， ランダム割り付けを伴わない同時コントロールを伴うコホート研究 (2a)， ランダム割り付けを伴わない過去のコントロールを伴うコホート研究 (2b)， 後方視的研究 (3)，対照群を伴わない研究 (4)，症例報告 (5)	
	総症例数		
	論文の要点 endpoint の結果は必須 PDF をメイルに 添付し提出		

2009.9.7 独立行政法人国立病院機構四国がんセンター　がん化学療法委員会

図5◈ レジメン審査表（四国がんセンター版）

レジメンセットを作成し，処方の際にはセットを呼び出して処方する．個々の薬剤を呼び出し，その都度レジメンを構築することによる過誤を防ぐことができる．

c. 支持療法や溶液の統一

同一薬剤の支持療法（悪心・嘔吐予防，アレルギー予防），レジメンの希釈液，希釈量における診療科ごとのばらつきは委員会の判断で整理統一する．

3）患者教育と指導・支援

外来化学療法においては副作用のアセスメントと初期対応を行うのは患者本人である．胃癌化学療法におけるメインの薬剤は経口内服薬（S-1）であり，服薬管理も行う必要がある．そのため，**抗がん剤による副作用症状の種類とその程度，対処方法や薬剤の管理についての教育・指導が非常に重要**となる．

治療前にはセルフアセスメントや自己管理が可能か，身近に援助をしてくれる人は存在するかの確認を行う．教育・指導は目標を決めて，患者の理解度に合わせて段階的に行う．主には看護師・薬剤師が指導を行うが，専門職へのコンサルトを行うなど医療チームでの支援が望まれる．

4）在宅時の緊急対応の体制

大部分の化学療法は，外来通院で安全に実施可能である．しかし，重篤な副作用や病状の悪化した場合の緊急対応の体制作りは必須である．前述の外来化学療法加算には，緊急事態に備え，患者が入院できる体制が確保されていること，または他の保険医療機関との連携により，緊急時に当該患者が入院できる体制が整備されていることも要件として求められている．

a. 緊急連絡先について

緊急時の連絡先について施設内で統一化し，連絡先について患者に明示しておく（図6）．四国がんセンターでは業務時間内の対応は日中はがん相談支援・情報センターの専属の対応係（看護師，ソーシャルワーカーなど），夜間・休日は当直師長が対応を行う．電話による問診により状態を確認し対応可能であればその場で対応を行う．副作用や病状の程度で医師の対応が必要と判断した場合には，主治医だけでなく各種の専門スタッフにも連絡をとる．

b. 他科や地域医療機関との連携

副作用の中には担当医では対応が困難なものもあり，院内の他科や地域医療機関と事前に連携を行っておくことで迅速な対応が可能となる．

c. 緊急の入院体制

緊急入院に対応できるよう空床を確保し，その状態を把握しておく．

Point

- 外来化学療法には多職種が連携したチーム医療が必須
- システムは委員会により組織的に企画・運営・管理する
- 患者自身による副作用対応・服薬管理が必要となるため教育・指導が重要
- 重篤な副作用や病状の悪化に対応できるシステムの構築が重要

患者に知らせておくべきこと

1．緊急連絡が必要な症状について記載
2．連絡先を明記
　①平日の8時30分～17時15分
　②夜間，休日
　それぞれの電話連絡と対応部署を明記
3．電話連絡時に，何を伝えればいいのか，読めばいいように準備，説明
4．電話を行っていいか迷ったときには電話をするように説明
5．24時間連絡可能であることを説明

連絡先について患者に配布している用紙

化学療法を受けられる方へ

化学療法を受けている間は，治療後の副作用や患者さんの状態が非常に重要です．次に示すことがありましたら，速やかに連絡ください．

- 下痢　治療前に比べて1日の排便回数が　　回以上に増えた場合
  （ストーマの方はストーマからの排出が治療前に比べて増え，日常生活に支障が出る場合）
- 1日6回以上の嘔吐がある場合
- 2～3日間，ほとんど水分も食事も取れない場合
- 38℃以上の熱が2日間以上続く場合
- 会話ができない，意識が朦朧とするなどの症状がある場合
- 胸が苦しい，息苦しい，激しい痛みなどの症状がある場合
- その他，治療前後で全身が悪化していると感じられる場合
- _____
- _____
- _____

［病院への連絡先］
平日：8：30から17：15　がん相談支援・情報センターが対応します
000-000-0000（直通）
平日夜間と土日の終日：当直師長が対応します　000-000-1111（代表）

＜以下をお伝えください＞

私は_____（ご自身のお名前）_____です．
診察券の番号は_____です．
_____科_____医師の外来で抗がん剤化学療法を受けいます．
今困っていることは_____です．

図6◈ 在宅時の緊急連絡の体制

文　献

1）「がん外来化学療法マニュアル」（国立がんセンター中央病院，通院治療センター 編），南江堂，2009
2）「外来がん化学療法マニュアル」（佐々木康綱 監修），文光堂，2009
　→上記2書は外来化学療法についてのシステムから実際について大変参考になるマニュアルである．
3）「胃がん標準化学療法の実際」（山口研成 編），金原出版，2007
　→胃癌の化学療法についてチーム医療における実地臨床の役割分担の詳細が述べられている．
4）「抗がん剤レジメン管理ガイド」（国立がんセンター中央病院　薬剤部 編），じほう，2008
　→がん化学療法に必須のレジメン管理についてのすべてが記載されており参考となる．
5）「チームで進めるがん外来化学療法」（藤原康弘 監修），日経メディカル開発，2009
　→地域調剤薬局との連携についても記載されており参考になる．

Profile　**仁科智裕**（Tomohiro Nishina）
四国がんセンター 消化器内科 医員

1996年3月岡山大学卒業，2001年8月より現職．医学博士．日本臨床腫瘍学会がん薬物療法専門医．
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Invest New Drugs
DOI 10.1007/s10637-009-9306-9

PHASE II STUDIES

Phase I/II study of sunitinib malate in Japanese patients with gastrointestinal stromal tumor after failure of prior treatment with imatinib mesylate

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Summary *Purpose:* To establish a recommended sunitinib dosing schedule in Japanese patients with imatinib-resistant/intolerant gastrointestinal stromal tumor (GIST) and to evaluate the efficacy, safety/tolerability, pharmacokinetics, and pharmacodynamics of sunitinib using this schedule. *Patients and methods:* In the phase I part of this open-label phase I/II trial, Japanese GIST patients received 25, 50, or 75 mg/day of sunitinib on Schedule 4/2 (4 weeks on treatment; 2 weeks off treatment) following imatinib failure. In phase II, patients received the recommended (maximum tolerated) dose on this schedule; the primary endpoint was clinical benefit rate (CBR; percent objective responses or stable disease [SD] ≥22 weeks). Additional efficacy, safety, pharmacokinetic, and biomarker analyses were performed. *Results:* In phase I (12 patients), the recommended dose was determined to be 50 mg/day. Sunitinib pharmacokinetics were similar to those observed in studies with Western patients. In the phase II part (36 patients), the CBR was 39% (95% CI: 23–57%; 11% partial responses, 28% SD ≥22 weeks). The most common treatment-related non-hematologic adverse events (AEs) were hand–foot syndrome (86%) and fatigue (67%). A trend towards a correlation between decreases from baseline in plasma soluble KIT levels and improved CB was found. *Conclusions:* The pharmacokinetics observed and clinical outcomes achieved in Japanese GIST patients on sunitinib (50 mg/day, Schedule 4/2) after imatinib failure appeared similar to those of Western patients in previous sunitinib trials. Although some serious AEs were observed, AEs were generally manageable using dose interruption/modification and/or standard medical treatments.

Keywords Sunitinib · GIST · Japanese patients · Pharmacokinetics · Biomarkers

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Introduction

Gastrointestinal stromal tumor (GIST) is a soft-tissue sarcoma that most commonly arises in the stomach or small intestine, and less frequently in the large bowel or other gastrointestinal sites [1,2]. Greater than 80% of GISTs are associated with activating mutations of KIT (stem-cell-factor receptor, CD117) [3,4], and another 5–7% express activating mutations of platelet-derived growth factor receptor alpha (PDGFR-α) [4,5].

Imatinib mesylate—a small-molecule tyrosine kinase inhibitor with selectivity for KIT and PDGFRs—is the current mainstay of treatment for metastatic or unresectable GIST. However, approximately 11–14% of GISTs are initially resistant to imatinib [6,7] and another 40–50% acquire resistance within 18–24 months of initial therapy [7,8].

Sunitinib malate (SUTENT®) is an oral multitargeted tyrosine kinase inhibitor with activity against KIT and PDGFRs, as well as vascular endothelial growth factor receptors (VEGFRs), glial cell line-derived neurotrophic factor receptor (REarranged during Transfection; RET), colony-stimulating factor 1 receptor (CSF-1R), and FMS-like tyrosine kinase-3 receptor (FLT3) (Pfizer Inc., data on file) [9–13]. Sunitinib received multinational approval for the treatment of GIST after failure of imatinib due to resistance or intolerance, based largely on the interim results of an international, randomized, double-blind, placebo-controlled phase III trial [14].

The clinical safety and efficacy of both imatinib and sunitinib in GIST have primarily been established in Western patients residing in the USA or Europe and have not been thoroughly studied in Asian patients. Fifty-six centers in 11 countries participated in the phase III trial of sunitinib in GIST, but only 15 of the 312 patients were of Asian descent (10 and 5 in the sunitinib and placebo groups, respectively). An open-label, phase I/II trial was therefore undertaken to establish a recommended dosing schedule for sunitinib in Japanese GIST patients after imatinib failure and to better evaluate the efficacy and safety of sunitinib in this patient population. In addition, the pharmacokinetic profiles of sunitinib and its active metabolite were assessed, and an initial evaluation of potential biomarkers of sunitinib activity in this patient population was performed.

Patients and methods

Patients

Japanese patients, 20–75 years of age, were required to have histologically proven metastatic or unresectable malignant GIST and confirmed failure of prior imatinib therapy, as demonstrated by disease progression (based on Response Evaluation Criteria in Solid Tumors [RECIST] [15]) or discontinuation of imatinib due to toxicity. Additional eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate cardiac, hepatic, renal, coagulation, and hematologic function. Key exclusion criteria included lack of recovery from the acute toxic effects of previous anticancer therapy or imatinib treatment, discontinuation of imatinib therapy within 2 weeks or of any other approved or investigational drug for GIST within 4 weeks prior to starting sunitinib treatment, clinically significant cardiovascular events or disease in the previous 12 months, diabetes mellitus with clinical evidence of peripheral vascular disease or diabetic ulcers, or a diagnosis of any second malignancy within the previous 5 years. All patients provided written informed consent to participate in the study.

Study design

This was an open-label, single-arm, non-randomized, multicenter, dose-escalation, phase I/II trial performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines. In the phase I part of the study, patients received one 6-week treatment cycle of sunitinib (4 consecutive weeks on treatment, followed by 2 weeks off treatment; Schedule 4/2). Successive cohorts of three to six patients received doses of 25, 50, or 75 mg administered orally once daily in the morning. Patients were enrolled in the subsequent cohort if less than one-third of patients in the initial or preceding cohort had experienced a dose-limiting toxicity (DLT). A DLT was defined as a grade 4 hematologic toxicity of ≥7 days' duration or complicated by fever ≥38°C; a grade 3/4 hematologic toxicity complicated by infection, hemorrhage, or requiring blood product support, including a hematopoietic growth factor; a grade 3/4 non-hematologic event (except for asymptomatic increases in serum amylase or lipase, or nausea/vomiting or diarrhea manageable with antiemetic and antidiarrheal drugs); or evidence of left ventricular dysfunction, defined as development of congestive heart failure or decline in left ventricular ejection fraction (LVEF) by an absolute value of ≥20% from baseline and to less than the lower limit of normal (LLN). Patients experiencing a DLT during the phase I part of the study were withdrawn from sunitinib treatment but could resume dosing if the toxicity resolved and there was evidence of clinical benefit. A primary objective of the phase I part of the study was to determine a recommended dose for the phase II part by identifying the maximally tolerated dose (MTD) of sunitinib, defined as the highest dose below the dose at which the proportion of patients experiencing DLTs was ≥33.3%.

Patients continuing in the phase II part of the study from the phase I part switched to/continued on the recommended dose (50 mg/day) on Schedule 4/2 after it was determined. Newly enrolled patients in the phase II part received the recommended dose on Schedule 4/2. Any drug-related grade 3/4 adverse events developing during the phase II part were managed using standard medical treatments and/or by discontinuing drug temporarily until the event resolved sufficiently, followed by dose reduction by 12.5–25 mg for grade 3/4 non-hematologic events or grade 4 hematologic events. Criteria for permanent drug withdrawal were a need to reduce the sunitinib dose to <25 mg/day, evidence of RECIST-defined disease progression, or evidence of left ventricular dysfunction (as previously defined).

Study endpoints and assessments

The primary endpoints of the phase I part were measures of safety and pharmacokinetic parameters of sunitinib and its principal active metabolite, SU12662. Adverse events were assessed by type, grade, and relationship to study drug, with grading determined using National Cancer Institute Common Toxicity Criteria version 2.0 [16]. Serious adverse events were defined as any untoward medical occurrences that resulted in death, were life-threatening, required or prolonged hospitalization, or resulted in persistent or significant disability/incapacity or a congenital anomaly/birth defect. Safety assessments included vital signs, ECOG performance status, 12-lead electrocardiogram, echocardiogram or multiple-gated acquisition scan, and laboratory analysis of blood and urine. Pharmacokinetic parameters were determined using blood samples collected pre-dose on days 1, 2, 7, 14, 21, 28, and end of treatment (or withdrawal), and post-dose at 1, 2, 4, 6, 8, and 10 h on days 1 and 28 as well as at 24 and 48 h on day 28. Plasma concentrations of sunitinib, SU12662, and total drug (sunitinib + SU12662) were determined using a liquid chromatography/mass spectrometry method with a lower limit of detection of 0.1 ng/ml [17].

The primary endpoint of the phase II part was the clinical benefit rate, defined as the percentage of patients with RECIST-defined objective response (confirmed complete response [CR] or partial response [PR]) or stable disease (SD) ≥22 weeks. Best overall response was evaluated by the investigators and an independent extramural review committee, with the evaluations of the latter group used for the primary efficacy analysis. Secondary efficacy endpoints included objective response rate, disease control rate (proportion of patients with confirmed CR or PR, or SD ≥10 weeks), time to tumor progression (TTP), and progression-free survival (PFS). As in the phase I part, measures of safety were also recorded. In the phase II part, blood samples for pharmacokinetic analysis were collected pre-dose on days 1, 14, and 28 of cycles 1–4 (or cycles 2–4 for patients entering phase II following completion of phase I).

Plasma concentrations of soluble KIT (sKIT), soluble VEGFR-2 (sVEGFR-2), and VEGF were determined using blood samples collected pre-dose on days 1, 14, and 28 of phase I and cycles 1–4 of phase II (cycles 2–4 for patients completing phase I) and evaluated as potential biomarkers for sunitinib activity using quantitative performance-validated enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) run under Good Laboratory Practice conditions as described [18].

Statistical methods

Statistical analyses were performed on three patient populations: DLT-analysis, intention-to-treat (ITT), and per-protocol. The DLT-analysis population, used in phase I of the study, consisted of patients who experienced DLTs or who received ≥85% of the planned total dose. The ITT population, defined as all patients who received at least one dose of study drug, was used as the primary analysis set for efficacy, safety, pharmacokinetic, and biomarker analyses. The per-protocol population consisted of the ITT population after exclusion of patients because of serious deviation from inclusion/exclusion criteria, administration of prohibited concomitant medications, administration of study drug on <75% of the planned dosing days before confirming clinical benefit (CR, PR, or SD≥22 weeks) or cycle 4, or no evaluation of objective tumor response after sunitinib dosing. The per-protocol population was used as a secondary set for analysis of the primary endpoint.

Time-to-event data were assessed using Kaplan–Meier methods. TTP was defined as the period between the day of initial study treatment and the day of initial confirmation of progressive disease (PD). Data from patients who were not confirmed to have PD either during the study or before the initiation of another antitumor therapy were censored at the final confirmation of absence of PD during the study. PFS was defined as the period between the day of initial study treatment and the day of initial confirmation of PD or of death due to any cause. Data from patients who were not confirmed to have PD or to have died during the study or before the initiation of another antitumor therapy were censored at the final confirmation of absence of PD during the study. Descriptive statistics were used to evaluate pharmacokinetic parameters and potential soluble biomarkers. Relationships between changes in plasma levels of biomarkers and the antitumor effects of sunitinib were evaluated using the Wilcoxon rank-sum test.

Results

Phase I results

Patient disposition and identification of recommended dose A total of 12 patients were enrolled in the phase I part of the study: three in the 25-mg cohort, six in the 50-mg cohort, and three in the 75-mg cohort. None of the initial three patients in the 25-mg cohort or the subsequent six patients in the 50-mg cohort experienced any DLTs in the first cycle of sunitinib treatment. However, two of the three patients in the 75-mg cohort experienced DLTs leading to termination of treatment after 12 and 15 days at that dose, and the third patient's treatment was subsequently terminated on day 7 as per recommendation of the Independent Safety Data Monitoring Committee. One of the patients' DLTs included grade 3 fatigue, anorexia, hypophosphatemia, and skin reaction, while the other patient's DLTs were grade 3 decreased platelet count (complicated by a need for blood product support) and grade 3 increased aspartate transaminase (AST). Therefore, 50 mg/day was identified as the MTD and as the recommended dose for use on Schedule 4/2 in the phase II part of the trial. All three patients who received 75 mg/day of sunitinib during the phase I part resumed dosing during cycle 2 of the phase II part at the recommended dose of 50 mg/day (the two patients who had experienced DLTs resumed after 18 and 28 days, respectively). There were no other dosing interruptions or any dose reductions due to toxicity during the phase I part of the trial, although initiation of cycle 2 was delayed by 14 days in one patient on 50 mg/day due to adverse events.

Pharmacokinetics Plasma concentration–time profiles and pharmacokinetic parameters for sunitinib, SU12662, and total drug are presented in Fig. 1 and Table 1, respectively. Exposure (maximum concentration [C_{max}] and area under the concentration–time curve from 0 to 24 h [AUC_{0-24}]) to sunitinib, SU12662, and total drug increased approximately linearly with dose on day 1 (25, 50, and 75 mg) and day 28 (25 and 50 mg). By day 28, AUCs for sunitinib, SU12662, and total drug were approximately 4, 11, and 5 times as high as those on day 1, respectively. Sunitinib was absorbed slowly after administration, with a median time to C_{max} of 6–10 h. Trough plasma concentrations of sunitinib and SU12662 appeared to reach steady state by 7–14 and 14–21 days after administration, respectively (data not shown). Trough concentrations of sunitinib and SU12662 at steady state (on day 28) closely correlated with the corresponding AUC_{0-24} and C_{max} values (r^2=0.80~0.90). The oral clearance (CL/F) of sunitinib did not show dose-dependency (data not shown).

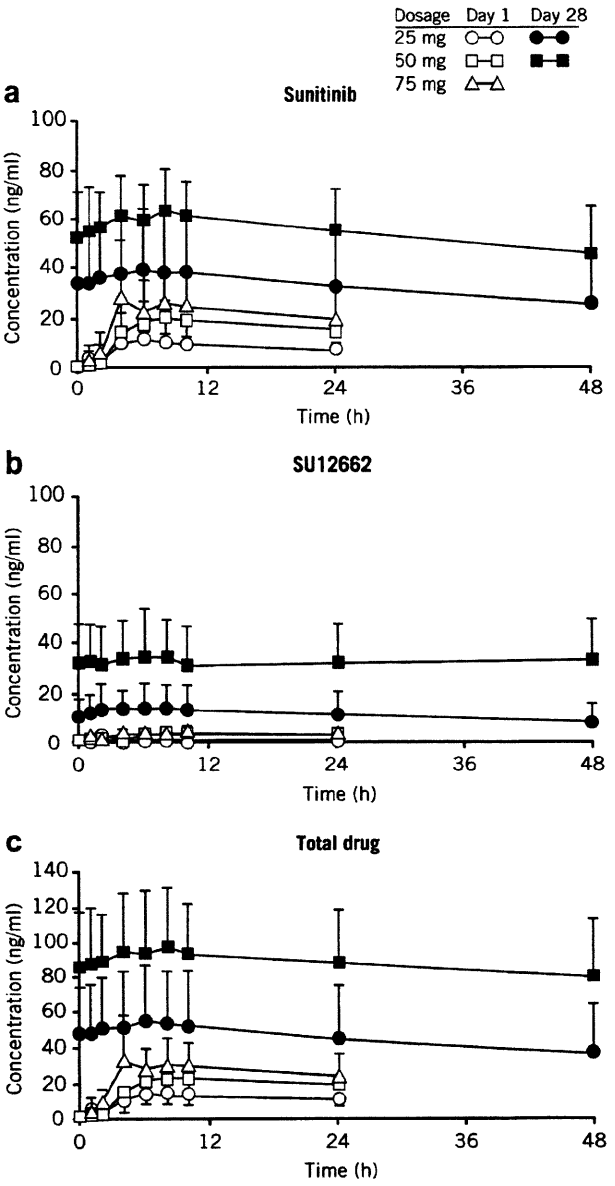

Fig. 1 Mean plasma concentration–time profiles of **a** sunitinib, **b** its active metabolite SU12662, and **c** total drug (sunitinib plus SU12662) by treatment cohort on days 1 and 28 in the phase I part of the study

Phase II results

Patient baseline characteristics, disposition, and study drug exposure Thirty-six patients were enrolled in the phase II part of the study and received sunitinib 50 mg/day on Schedule 4/2, three of whom initially received sunitinib 25 mg/day and three of whom initially received sunitinib 75 mg/day during the phase I part of the study. The ITT population comprised all 36 patients, while the per-protocol

Table 1 Pharmacokinetic parameters (mean ± standard deviation) of sunitinib, SU12662, and total drug by treatment cohort in the phase I part of the study

Parameter		Sunitinib			SU12662			Total drug		
		25 mg (n=3)	50 mg (n=6)	75 mg (n=3)	25 mg (n=3)	50 mg (n=6)	75 mg (n=3)	25 mg (n=3)	50 mg (n=6)	75 mg (n=3)
Day 1	C_{max}	12.1	22.8	32.3	2.0	4.1	4.8	14.1	26.7	37.0
	(ng/ml)	±4.9	±6.4	±20.8	±1.3	±0.9	±2.5	±6.1	±7.4	±22.1
	AUC_{0-24}	199	374	508	30.9	70.0	91.1	230	444	599
	(ng·h/ml)	±89.4	±68.9	±259	±20.6	±14.4	±45.3	±108	±82.8	±287
	T_{max}[b] (h)	6 (4–8)	7 (6–24)	8 (4–10)	6 (4–8)	9 (6–24)	10 (4–10)	6 (4–8)	7 (6–24)	8 (4–10)
Day 28[a]	C_{max}	39.5	69.3	–	15.2	38.8	–	54.0	105	–
	(ng/ml)	±25.0	±18.9		±10.2	±15.9		±32.2	±35.1	
	AUC_{0-24}	858	1,406	–	324	772	–	1,183	2,178	–
	(ng·h/ml)	±600	±364		±223	±358		±734	±702	
	T_{max}[b] (h)	10 (6–10)	6 (1–24)	–	4 (2–8)	3 (0–48)	–	6 (4–8)	6 (0–24)	–

AUC area under the concentration–time curve, C_{max} maximum concentration, T_{max} time to C_{max}

[a] Day 28 data were not collected for the 75-mg cohort due to early termination of this cohort following occurrence of dose-limiting toxicities

[b] Median (range)

population consisted of 30 patients. Six patients, comprising all of the patients in the 75-mg/day cohort and three in the 50-mg/day cohort, were excluded from the per-protocol population due to insufficient dosing.

Baseline patient characteristics, patient disposition, and exposure to study drug over both phases of the study are summarized in Table 2. Tumor progression was the primary reason for termination of imatinib therapy in 92% of patients, with the other 8% having discontinued due to imatinib intolerance. At the time of data cutoff, patients had received a median of four cycles of sunitinib (range: 2–12) at a median dose of 50 mg/day and dose intensity of 89%. Sixteen patients (44%) had dose reductions, 15 (42%) due to adverse events. Twenty-two patients (61%) discontinued sunitinib treatment, two (6%) due to adverse events and 20 (56%) due to PD. Adverse events causing discontinuation of sunitinib treatment were grade 2 decreased LVEF (one patient) and grade 4 decreased neutrophil count (one patient) that persisted despite dose reduction to 25 mg.

Efficacy The clinical benefit rate (percent objective responses or SD ≥22 weeks) based on extramural assessment of the ITT population (the primary endpoint) was 39% (95% CI: 23–57) and 40% (95% CI: 23–59) in the 50-mg cohort (Table 3). Analysis of the per-protocol population yielded similar results (data not shown). Based on extramural assessment, four patients (all in the 50-mg cohort) exhibited a RECIST-defined objective response (all PRs), yielding an objective response rate of 11% in the ITT population and 13% in the 50-mg cohort. The disease control rate (percent objective responses or SD ≥10 weeks) was 61% and 57% in the ITT population and 50-mg cohort, respectively. The median TTP was 28.3 weeks (95% CI: 22.0–39.3) in the ITT population and 27.9 weeks (95% CI: 22.0–39.3) in the 50-mg cohort; TTP and PFS were equivalent (data not shown).

Safety/tolerability All 36 patients in the ITT population experienced at least one adverse event that was considered to be treatment-related. Most adverse events were mild to moderate in intensity: 84% of all treatment-related adverse events were grade 1/2. Toxicities experienced in the study were generally manageable and reversible through careful dosing interruption, dose modification, and/or standard medical treatment. Among treatment-related non-hematologic adverse events, the most common events of any grade reported were hand–foot syndrome (86%); fatigue (67%); and diarrhea, anorexia, and skin discoloration (64%; Table 4), and the most common grade 3 events reported were hand–foot syndrome (31%) and hypertension (25%). No grade 4 events were reported among the non-hematologic adverse events listed in Table 4, and among all treatment-related non-hematologic events, only one grade 4 event (nephrotic syndrome) was reported. This event resolved after a dosing interruption of 52 days and dose reduction from 37.5 mg to 25 mg.

Hematologic and non-hematologic laboratory abnormalities are also presented in Table 4. Two incidents of grade 4 hematologic laboratory abnormalities were reported: one reduced hemoglobin concentration and one reduced neutrophil count; the former resolved after a dosing interruption of 14 days and dose reduction to 37.5 mg; the latter resulted in discontinuation, as mentioned above. The most

Table 2 Summary of patient demographics, imatinib treatment history, disposition, and study drug exposure across the phase I and II parts of the study through data cutoff

Characteristic	Treatment cohort			
	25 mg (n=3)	50 mg (n=30)	75 mg (n=3)	Total (N=36)
Median age (range), years	36 (33–54)	56 (41–74)	66 (50–68)	56 (33–74)
Sex, n (%)				
Male	2	19 (63)	3	24 (67)
Female	1	11 (37)	0	12 (33)
Median weight (range), kg	56 (48–64)	51 (40–79)	56 (51–58)	52 (40–79)
ECOG PS, n (%)				
0	3	18 (60)	2	23 (64)
1	0	12 (40)	1	13 (36)
Tumor immunohistochemistry, n (%)				
KIT-positive	3	29 (97)	3	35 (97)
CD34-positive	2	16 (53)	2	20 (56)
Imatinib treatment history				
Median duration of treatment (range), months	31 (19–32)	26 (2–46)	32 (22–38)	26 (2–46)
Primary reason for termination, n (%)				
Tumor progression	3	27 (90)	3	33 (92)
Intolerance	0	3 (10)	0	3 (8)
Most common metastatic sites, n				
Liver	3	22	3	28
Peritoneum	3	16	3	22
Lung	1	1	1	3
Ascites	2	1	0	3
Sunitinib treatment				
Median number of cycles completed (range)	10 (3–12)	4 (2–10)	5 (3–5)	4 (2–12)
Median daily dose (range), mg	25 (25–31)	50 (33–50)	52 (43–59)	50 (25–59)
Median dosing days/cycle (range)	28 (27–28)	26 (20–28)	20 (14–22)	26 (14–28)
Median dose intensity (range), %	100 (95–124)	89 (47–100)	44 (39–49)	89 (39–124)
Discontinuations, n (%)	1	18 (60)	3	22 (61)
Due to an adverse event	0	2 (7)	0	2 (6)
Due to PD	1	16 (53)	3	20 (56)
Dose reductions, n (%)	0	13 (43)	3	16 (44)
Due to an adverse event	0	13 (43)	2	15 (42)

ECOG PS Eastern Cooperative Oncology Group performance status, *PD* progressive disease

common non-hematologic laboratory abnormalities were increased AST levels (72%) and decreased albumin levels (61%). The most common grade 3/4 non-hematologic laboratory abnormalities were increased lipase (19%), increased uric acid (19%), and increased AST (11%). None of the cases of increased lipase were associated with additional signs or symptoms of pancreatitis.

In addition to hypertension, cardiovascular adverse events included a prolongation of the QTc interval to 450–<480 ms in two patients and a maximum change of 30–<60 ms from baseline QTc interval in two patients. These were not clinically significant and resolved without treatment changes. LVEF was below the LLN in three patients, two of whom experienced an absolute ≥20% decrease from baseline, which resulted in discontinuation in one patient, as mentioned above. The LVEF decrease in this latter patient ultimately abated after discontinuation. This patient also experienced cardiomyopathy, which was diagnosed by echocardiogram and magnetic resonance imaging after discontinuation and resolved after completion of the study. Two patients in the study experienced hypothyroidism (grade 1).

Nine patients (25%) experienced one or more serious treatment-related adverse events (which were abdominal pain, cardiomyopathy, gastric ulcer, hand–foot syndrome, hemorrhage, hypoglycemia, hypoproteinemia, myalgia, nephrotic syndrome, perianal abscess, reduced platelet

Table 3 Clinical response to sunitinib treatment across the phase I and II parts of the study through data cutoff

Response parameter	Treatment cohort			
	25 mg (n=3)	50 mg (n=30)	75 mg (n=3)	Total (N=36)
Tumor response, n (%)				
PR	0	4 (13)	0	4 (11)
SD	3 (100)	15 (50)	3 (100)	21 (58)
≥10 weeks	3 (100)	13 (43)	2 (67)	18 (50)
≥22 weeks	1 (33)	8 (27)	1 (33)	10 (28)
Objective response rate	0	13	0	11
95% CI		4–31		3–26
Disease control rate[a]	100	57	67	61
95% CI	–	37–75	–	44–77
Clinical benefit rate[b]	33	40	33	39
95% CI	–	23–59	–	23–57

PR partial response, *SD* stable disease

[a] Disease control rate, percent PRs + SD ≥10 weeks

[b] Clinical benefit rate, percent PRs + SD ≥22 weeks

count, and reflux esophagitis). All were reported in one patient each, except reduced platelet count, which was experienced by two patients. None of these events led to discontinuation, although the patient who experienced cardiomyopathy had already discontinued treatment due to LVEF decrease as described above. No treatment-related grade 5 events were reported in the study.

Pharmacokinetics Among patients receiving sunitinib 50 mg/day, median trough concentrations of sunitinib, SU12662, and total drug ranged from 42.3 to 59.5 ng/ml, 18.7 to 29.7 ng/ml, and 62.4 to 84.9 ng/ml, respectively, while on treatment (days 14 and 28) in cycles 1–4. Following 2 weeks off treatment, pre-dose total drug concentrations in cycles 2–4 were low, but measurable in many patients (medians: 3.0–4.0 ng/ml). Trough concentrations of sunitinib and SU12662 varied over time in a manner similar to those of total drug, corresponding to periods on and off treatment, and repeated dosing was not found to result in accumulation of plasma drug levels across four treatment cycles (data not shown).

Biomarkers Plasma levels of sKIT, sVEGFR-2, and VEGF changed in response to treatment, but only sKIT appeared to show sustained changes (data not shown). During sunitinib dosing, plasma concentrations of VEGF increased while concentrations of sVEGFR-2 decreased, but plasma concentrations of both of these biomarkers tended to return to near-baseline levels after the 2-week off-treatment period. Conversely, plasma concentrations of sKIT showed a trend for sustained decrease across both on-treatment and off-treatment periods.

Percent changes in VEGF, sVEGFR-2, and sKIT levels from baseline were compared among patient groups categorized by best overall response. There was no apparent difference in percent change of VEGF and sVEGFR-2 among the patient groups at any time point. Results for cycle 4, day 28 are shown in Table 5. Patients with an objective response and those achieving clinical benefit (objective responses or SD ≥22 weeks) showed a trend of decline in sKIT levels across cycles 1–4, while patients without objective responses exhibited only modest decreases or increases in sKIT levels (p values for clinical benefit versus no clinical benefit for cycle 4, day 28: sKIT, 0.238; VEGF, 0.459; sVEGFR-2, 0.484).

Discussion

The results of this trial of sunitinib in Japanese GIST patients following imatinib failure were highly consistent with those obtained in a number of previous sunitinib trials. The phase I part of the study yielded the same recommended dosing schedule (50 mg/day on Schedule 4/2) as that identified in prior studies of sunitinib in largely Western patients with a variety of tumor types [19–21]. In the phase II part of the study, sunitinib demonstrated similar efficacy to that previously reported in a double-blind, placebo-controlled, phase III trial also involving predominantly Western patients with imatinib-resistant/-intolerant GIST [14]. In the 50-mg cohort in the current study, 13% and 40% of patients experienced objective responses (PRs) or clinical benefit from sunitinib, respectively. By way of

Table 4 Toxicities occurring across the phase I and II parts of the study through data cutoff

Adverse event/laboratory abnormality	Treatment cohort					
	25 mg (n=3)	50 mg (n=30)			75 mg (n=3)	Total (N=36)
	Any grade[a] n	Grade 1/2 n (%)	Grade 3 n (%)	Any grade[a] n (%)	Any grade[a] n	Any grade[a] n (%)
Treatment-related non-hematologic adverse events ≥25%[b]						
Hand–foot syndrome	2	17 (57)	9 (30)	26 (87)	3	31 (86)
Fatigue	2	19 (63)	1 (3)	20 (67)	2	24 (67)
Diarrhea	2	19 (63)	0 (0)	19 (63)	2	23 (64)
Anorexia	1	19 (63)	1 (3)	20 (67)	2	23 (64)
Skin discoloration	0	21 (70)	0 (0)	21 (70)	2	23 (64)
Stomatitis	2	17 (57)	1 (3)	18 (60)	2	22 (61)
Nausea	1	13 (43)	0 (0)	13 (43)	2	16 (44)
Hypertension	0	7 (23)	7 (23)	14 (47)	2	16 (44)
Dysgeusia	1	11 (37)	0 (0)	11 (37)	2	14 (39)
Rash	1	12 (40)	0 (0)	12 (40)	1	14 (39)
Gingivitis	0	12 (40)	0 (0)	12 (40)	0	12 (33)
Abdominal pain	2	9 (30)	0 (0)	9 (30)	0	11 (31)
Cheilitis	1	9 (30)	0 (0)	9 (30)	1	11 (31)
Edema	0	9 (30)	0 (0)	9 (30)	1	10 (28)
Pigmentation disorder	0	7 (23)	0 (0)	7 (23)	2	9 (25)
Hematologic laboratory abnormalities						
Neutrophils	3	15 (50)	11 (37)	27 (90)[c]	3	33 (92)[c]
Leukocytes	3	21 (70)	5 (17)	26 (87)	3	32 (89)
Platelets	2	21 (70)	6 (20)	27 (90)	3	32 (89)
Hemoglobin	2	9 (30)	10 (33)	20 (67)[c]	3	25 (69)[c]
Lymphocytes	1	11 (37)	9 (30)	20 (67)	2	23 (64)
Non-hematologic laboratory abnormalities ≥40%[b]						
AST	2	19 (63)	3 (10)	22 (73)	2	26 (72)
Albumin	1	19 (63)	0 (0)	19 (63)	2	22 (61)
Total bilirubin	1	13 (43)	0 (0)	13 (43)	3[c]	17 (47)[c]
Alkaline phosphatase	1	9 (30)	3 (10)	12 (40)	3	16 (44)
ALT	0	12 (40)	2 (7)	14 (47)	1	15 (42)
Hyperglycemia	1	10 (33)	1 (3)	11 (37)	3	15 (42)
Phosphate	2	10 (33)	0 (0)	10 (33)	3	15 (42)

ALT alanine aminotransferase, *AST* aspartate aminotransferase

[a] No grade 4 events were reported among the treatment-related non-hematologic adverse events listed; the only grade 4 events reported among those listed were one reduced hemoglobin concentration and one reduced neutrophil count in the 50-mg cohort and one increased total bilirubin in the 75-mg cohort, as noted

[b] Based on the total population

[c] Includes one grade 4 event

comparison, the objective response and clinical benefit rates were 7% and 24%, respectively, in the phase III trial [14]. Likewise, the median TTP for the 50-mg cohort in the current study was 27.9 weeks, compared with 27.3 weeks in the phase III trial [14]. Except for ethnicity and the smaller sample size in the current study, patients in the two studies were generally comparable in terms of demographic characteristics, GIST histology, and duration of prior imatinib treatment and primary cause for discontinuation. The benefit derived from sunitinib by Japanese patients after imatinib failure is important because there are no other approved and effective second-line treatments for GIST: sunitinib remains the only approved treatment multinationally for patients with GIST after imatinib failure.

Sunitinib-related adverse events experienced by patients across both phases of the current study were predominantly

Table 5 Change from baseline of soluble protein concentrations (cycle 4, day 28) versus tumor response[a]

Soluble protein	Percent change from baseline, median (minimum, maximum)				
	PR (n=4)	SD ≥22 weeks (n=9)	Clinical benefit[b] (n=13)	SD <22 weeks + PD (n=18)	p value[c]
VEGF	365 (142, 473)	449 (49, 889)	385 (49, 889)	351 (52, 814)	0.459
sVEGFR-2	−51 (−65, −32)	−53 (−66, −20)	−53 (−66, −20)	−42 (−69, −4)	0.484
sKIT	−52 (−76, −46)	−12 (−46, 59)	−24 (−76, 59)	2 (−66, 386)	0.238

PR partial response, *PD* progressive disease, *SD* stable disease, *sKIT* soluble KIT, *sVEGFR-2* soluble VEGF receptor-2, *VEGF* vascular endothelial growth factor

[a] Last observation carried forward

[b] Clinical benefit, PR + SD ≥22 weeks

[c] Clinical benefit versus SD <22 weeks + PD, Wilcoxon rank-sum test

mild to moderate in severity; were manageable and reversible through dosing interruption, dose modification, and/or standard medical treatments; and seldom led to treatment withdrawal. Only two patients (6%) discontinued treatment due to an adverse event, consistent with previous reports of low treatment discontinuation rates due to adverse events with sunitinib therapy [14]. On the other hand, serious adverse events were reported in 25% of patients. Although these events did not result in discontinuations, this result suggests that patients should be monitored carefully. Overall, however, the safety profile observed in this study was similar to that reported in the phase III study, with fatigue and skin and gastrointestinal disorders representing the most frequent adverse events [14]. Moreover, no new adverse events were reported in this study compared with previous studies.

The pharmacokinetic results obtained in the present study were also consistent with those obtained in previous studies. In the phase I part of the study, exposure to sunitinib 50 mg on Schedule 4/2 was similar to that reported in a study of sunitinib in Western patients with various types of solid tumors [19] on day 1 (C_{max}: 22.8 versus 27.7 ng/ml; AUC_{0-24}: 374 versus 420 ng·h/ml), and day 28 (C_{max}: 69.3 versus 72.2 ng/ml; AUC_{0-24}: 1,406 versus 1,296 ng·h/ml). On the other hand, SU12662 exposure was somewhat higher in the current study than in the earlier one on day 1 (C_{max}: 4.1 versus 4.1 ng/ml; AUC_{0-24}: 70 versus 64 ng·h/ml) and day 28 (C_{max}: 38.8 versus 33.7 ng/ml; AUC_{0-24}: 772 versus 592 ng·h/ml). However, SU12662 comprised only 23–37% of total drug on day 28, resulting in total-drug exposures calculated to be approximately 15% higher in the current study, which is well within the range of exposures seen in Western patients. Median trough plasma drug concentrations obtained in the 50-mg cohort in the phase II part of the current study were above the preclinically determined effective plasma concentration of 50 ng/ml [10] throughout dosing and similar to those obtained in the phase III GIST study [14] (total drug: 62.4–84.9 versus 64.8–86.3 ng/ml, respectively). As in the phase III study, repeated dosing did not result in accumulation of sunitinib across several cycles of treatment. Taken together, these results suggest that sunitinib pharmacokinetics are comparable in Asian and Western GIST patients, consistent with the results of other analyses [22,23], and that sunitinib may be dosed similarly in both populations. Additionally, as shown in the phase I part of the study, there was a close correlation (r^2=0.80~0.90) between trough concentrations of sunitinib and SU12662 and AUC_{0-24} and C_{max} values at steady state, suggesting that trough concentration may be a useful marker of exposure.

Greater antiangiogenic effects as well as continued sensitivity of some imatinib-resistant KIT mutants have been postulated as possible explanations for sunitinib activity in GISTs resistant to imatinib. In-vitro studies using KIT constructs have demonstrated that sunitinib is capable of inhibiting the kinase activity of KIT mutants resistant to imatinib, including those commonly associated with secondary resistance [24–26]. Although patient numbers were small, a trend towards sustained decreases in plasma sKIT with sunitinib treatment was found in the current study, which correlated with improved outcomes (particularly objective responses). However, how these changes relate to antiangiogenic effects versus direct actions on mutant KIT receptors is not known. That the largest decreases were observed in patients with objective responses suggests that tumor cell loss may contribute to the decreases, although this could result from either antiangiogenic or direct antitumor effects. A correlation between decreased plasma levels of sKIT and sunitinib activity has also been reported among GIST patients who participated in the phase III trial [27], as well as in patients with metastatic breast cancer [28]. Likewise, a correlation between plasma sKIT decreases and response to imatinib in GIST has also been reported [29]. However, more work needs to be done to validate biomarkers that may be used to predict GIST response to sunitinib or other tyrosine kinase inhibitors.

In summary, the results from the present study suggest that Japanese GIST patients obtain comparable benefit from

sunitinib after failure of imatinib as did patients in the international phase III study. In addition, the results indicate that sunitinib may be dosed similarly in Asian and Western patients, and that adverse events can generally be managed by dosing interruptions, dose modifications, and/or the use of standard medical treatments. Although the present study was small and requires verification in larger controlled trials, these results provide guidance to clinicians treating Asian GIST patients after imatinib failure due to disease progression or intolerance.

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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 #*1A* (frequency = 0.291), #*1B* [containing 421C>A (Q141K) and IVS12 + 49G>T] (0.251) and #*1C* [containing 34G>A (V12M) and IVS9-30A>T] (0.107). Note that #*1B* and #*1C* 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#1B* [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#1B* [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.

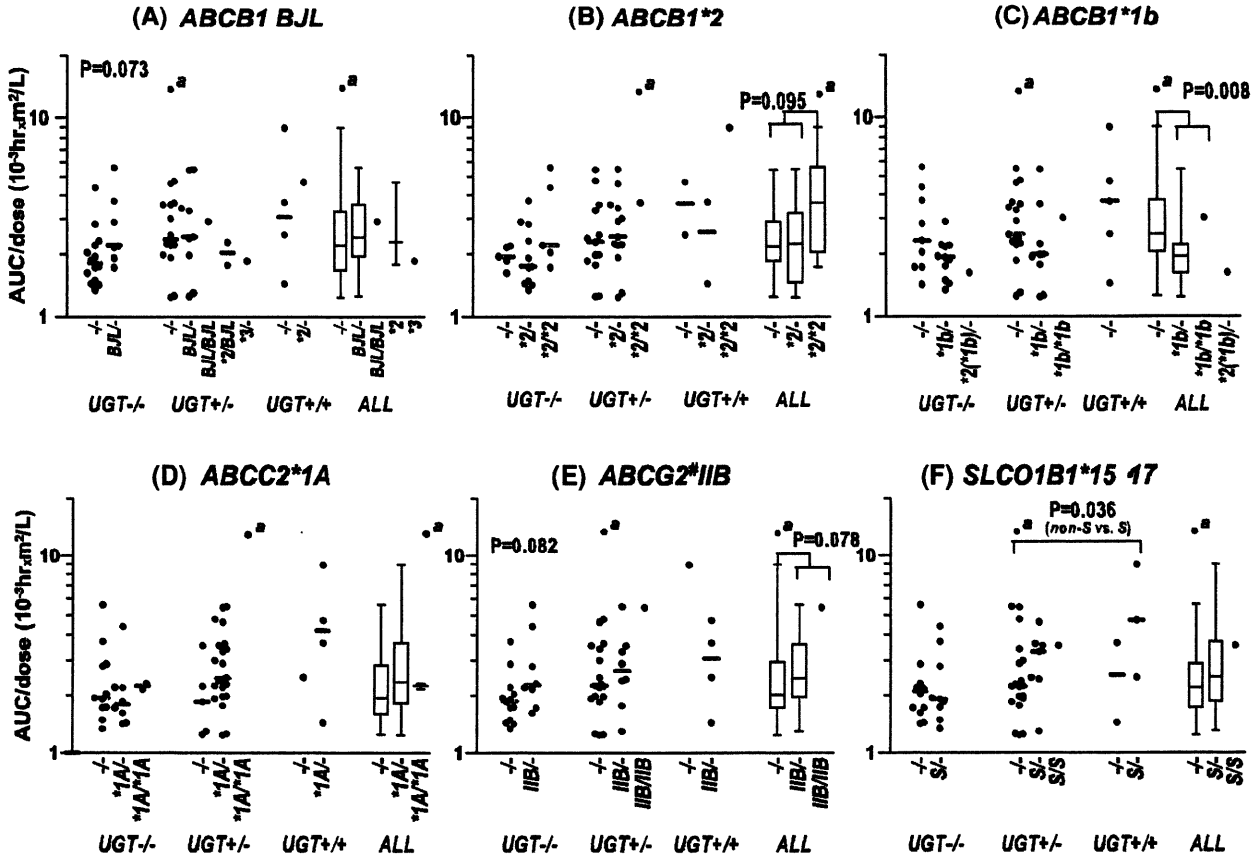

Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). *a* Excluded from statistical analysis. The *bars* represent the medians. *UGT+* = *UGT1A1*6* or **28*. **a** *BJL* contains −1789G>A, *2 (block 1) = 325G>A (E109K), **3* (block 1) = 304G>A (G102R); **b**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

[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-#IB* [35], to severe neutropenia.

Among the rare variations detected, eleven heterozygous transporter genetic variations and one *UGT1A1#60-#IB* 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.