TABLE 5. Estimated Pharmacokinetic Parameters of Vandetanib[a]

	Clearance (L/h)	C_{max} (ng/ml)	Steady-state Exposure (ng/h/ml)	Half-life (d)	Accumulation Ratio
Median	10.2	1282	29,469	6.2	8.87
Minimum value	4.04	740	16,685	3.4	4.89
Maximum value	17.98	3018	74,257	13.8	19.85

[a] Simulated PK parameters if all patients (n = 51) were administered 300 mg vandetanib once a day for 56 d.

TABLE 6. Summary of Plasma Angiogenesis Biomarker Levels by Best Overall RECIST Response

Biomarker	Best Response (RECIST)	Median (range; n)		
		Baseline	Day 29	Day 57
VEGF (pg/ml)	PR	22.3 (0–264.2; n = 6)	73.2 (0–164.4; n = 6)	80.9 (28.7–183.7; n = 6)
	SD	37.0 (0–227.7; n = 16)	79.4 (38.5–281.6; n = 16)	97.4 (19.0–238.7; n = 16)
	PD	63.7 (0–897.7; n = 21)	121.0 (10.7–477.9; n = 21)	93.6 (63.9–343.2; n = 5)
	Total	51.5 (0–897.7; n = 43)	82.8 (0–477.9; n = 43)	95.5 (19.0–343.2; n = 27)
Tie-2 (ng/ml)	PR	23.5 (16.6–29.1; n = 6)	22.6 (19.8–38.8; n = 6)	23.3 (17.2–37.0; n = 6)
	SD	26.9 (6.0–33.6; n = 16)	27.4 (12.3–45.4; n = 16)	28.5 (23.3–52.4; n = 16)
	PD	28.5 (18.2–43.3; n = 21)	30.7 (18.0–56.3; n = 21)	30.2 (20.7–36.0; n = 5)
	Total	27.4 (6.0–43.3; n = 43)	29.2 (12.3–56.3; n = 43)	27.5 (17.2–52.4; n = 27)
VEGFR-2 (pg/ml)	PR	7406.5 (5564–9868; n = 6)	6418.5 (4878–8030; n = 6)	6001.5 (4846–7156; n = 6)
	SD	7577.5 (5622–8687; n = 16)	6819.5 (4666–8630; n = 16)	6450.5 (5024–8372; n = 16)
	PD	7861.0 (4981–11391; n = 21)	6910.0 (3763–11136; n = 21)	6710.0 (4131–8606; n = 5)
	Total	7721.0 (4981–11391; n = 43)	6881.0 (3763–11136; n = 43)	6563.0 (4131–8606; n = 27)

PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; Tie-2, soluble angiopoietin receptor; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2.

tion for five patients (300 mg, n = 4; 100 mg, n = 1); none of these QTc prolongation events met the protocol criteria for QTc prolongation by subsequent central review.

No significant abnormalities in any clinical laboratory variables were observed except for an increase of alanine aminotransferase (ALT increased) with CTC grade 3 in three patients in the vandetanib 300 mg arm.

Pharmacokinetic Results

The observed vandetanib plasma concentrations at day 28 for each evaluable patient are shown in Figure 3. These data were fitted to a one-compartment model to estimate population PK parameter values for the 300 mg arm, which were found to adequately characterize the observed plasma concentrations over time, and there was a good correlation between the individual predicted and observed data (data not shown). Estimates of the half-life ranged from 3.4 to 13.8 days, with a median population value of 6.2 days. The estimated time to PK steady state was approximately 1 month. Simulations from the PK characteristics of this patient population suggest a median steady-state exposure of approximately 29,500 ng/h/ml for a 300 mg dose administered once a day and an estimated median C_{max} of 1282 ng/ml (range, 740 to 3018 ng/ml). The estimated population PK parameters for the 300 mg arm are summarized in Table 5.

Tumor Biomarkers

Twenty-seven tumor samples were available for analysis and 12 of these were evaluable for determination of *EGFR* gene copy number by fluorescence in situ hybridization. Four of 12 evaluable patients had high *EGFR* gene copy number (best overall RECIST response of SD and PD, both n = 2) whereas the remaining eight patients did not (best overall RECIST response: PR, n = 1; SD, n = 3; and PD, n = 4). Nine of 27 samples were successfully sequenced for *EGFR* exons 19–21. In addition, 21 of 27 samples had successful ARMS analysis for L858R and the most common exon 19 deletion mutation (746–750). A confirmed mutation (exon 19 deletion [746–750]) was observed in a female nonsmoker from the 200 mg arm with adenocarcinoma and a high *EGFR* gene copy number (best RECIST response of PD). Of the remaining tumor samples, 21 had no *EGFR* mutation (by DNA sequencing or ARMS analysis), and in five cases, the *EGFR* mutation status could not be determined (not evaluable by either DNA sequencing or ARMS). Tumor samples were obtained from three patients who achieved a PR. Two of these tumor samples had no *EGFR* mutation and one had an unconfirmed result by direct DNA sequencing and ARMS assay of *EGFR* exons 19–21.

Blood Biomarkers

Median plasma levels of VEGF showed a trend to increase during the study period irrespective of clinical out-

come. In contrast, plasma levels of VEGFR-2 showed a trend to decrease over the same period, whereas plasma Tie-2 levels did not seem to change (Table 6). Baseline plasma VEGF levels appeared to be lower in patients who experienced clinical benefit following vandetanib treatment: PR (median 22.3 pg/ml, $n = 6$) and SD (median 37.0 pg/ml, $n = 16$) versus PD (median 63.7 pg/ml, $n = 21$). Patients with a low (below median) baseline plasma VEGF level had a longer TTP (median, 24.1 week) than those with a high (above median) baseline VEGF level (median, 8.3 weeks) (Figure 4). No clear relationship was apparent between baseline levels of plasma Tie-2 and VEGFR-2 and tumor response.

DISCUSSION

The primary objective of this phase IIa study was to assess the ORR to three doses of vandetanib (100, 200, and 300 mg/d) in Japanese patients with advanced or recurrent NSCLC. These doses of vandetanib were selected based on the outcomes of a Japanese phase I study where it was observed that vandetanib was well tolerated up to a dose of 300 mg and objective tumor responses were observed in 4 of 9 patients with NSCLC at doses of either 200 or 300 mg.[11]

In this study, objective tumor responses were observed at all three doses of vandetanib. The ORR in the 100, 200, and 300 mg arms was 17.6% (3 of 17 patients), 5.6% (1 of 18 patients), and 16.7% (3 of 18 patients), respectively. The DCR and TTP were similar across the three dose arms. It was noted that 50% (9 of 18) of the patients in the 200 mg arm had failed two previous chemotherapy regimens, compared with 23.5% (4 of 17 patients) and 22.2% (4 of 18 patients) in the 100 and 300 mg arms, respectively. It is possible that these differences contributed to the lower ORR observed in the 200 mg arm, although the number of patients in each dose arm was too small to allow any definitive conclusions to be made.

Vandetanib was well tolerated at 100, 200, and 300 mg dose levels in this study. Overall, AEs were generally mild and manageable with symptomatic treatment, dose interruption or reduction. In addition, the AE profile was consistent with that determined during phase I evaluation in patients with advanced solid tumors[10,11] and phase II monotherapy data in NSCLC.[12] Furthermore, the AE profile was also consistent with that reported previously for agents that inhibit the VEGFR[17,18] or EGFR[4,19] signaling pathways. In general, no apparent dose dependence was noted in the incidence of the common AEs in this study except for asymptomatic QTc prolongation (24%, 56%, and 44% for the 100, 200, and 300 mg dose arms, respectively), an event that was manageable by dose interruption/reduction.

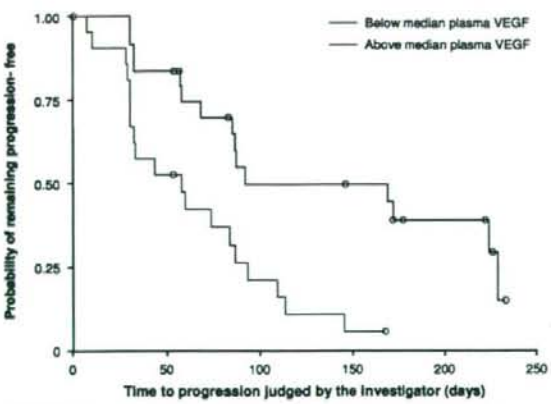

FIGURE 4. Kaplan–Meier curve of low (below median) versus high (above median) baseline plasma VEGF and time to progression.

A notable feature of this study, and the phase II program for vandetanib in NSCLC, is that patients with squamous cell histology or stable brain metastases were permitted to enter the trials. Both of these factors have been associated with an increased risk of bleeding, including severe life-threatening hemoptysis in NSCLC patients with squamous histology in a randomized phase II study of bevacizumab with carboplatin and paclitaxel.[20] These events have also been reported with other inhibitors of VEGF/VEGFR signaling, such as sunitinib and sorafenib.[17,18] Importantly, no CNS hemorrhage AEs or hemoptysis attributable to vandetanib were reported in this study.

The PK profile in this NSCLC patient population was consistent with that seen previously during Phase I evaluation in Japanese and USA/Australian patients with a range of solid tumors.[10,11]

In patients with NSCLC, specific *EGFR* mutations are associated with increased sensitivity to EGFR tyrosine kinase inhibitors,[21,22] and a better survival outcome with gefitinib has been shown to correlate with high *EGFR* gene copy number.[23] In this study, an exploratory analysis of tumor samples for amplification of *EGFR* gene copy number and somatic mutations of the *EGFR* gene revealed no clear relationship between *EGFR* mutation or gene amplification status and clinical outcome in patients receiving vandetanib. The *EGFR* mutation frequency of 4% (1 of 27 patients) is lower than that previously reported,[24,25] and further studies are needed to evaluate *EGFR* mutation status as a possible predictive marker for vandetanib therapy in advanced NSCLC.

In addition to *EGFR* mutation/amplification status, plasma profiling of cytokines and angiogenic factors may be a feasible approach for identifying blood-based prognostic and activity markers for therapies in NSCLC. Preliminary analysis of plasma concentrations of the angiogenesis markers VEGF and VEGFR-2 in the present study revealed that patients with PR or SD were more likely to have low baseline levels of VEGF than those with PD. It has been shown previously that low pretreatment levels of circulating VEGF correlated with a good response to gefitinib treatment in patients with NSCLC.[26] The significance of the relationship between these biomarkers and clinical outcome requires further investigation.

In conclusion, vandetanib monotherapy (100–300 mg/d) demonstrated antitumor activity with an acceptable safety and tolerability profile in Japanese patients with advanced NSCLC. Based only on this study, there is no com-

pelling evidence to identify the optimal dose of vandetanib monotherapy in this population of patients; further investigation of vandetanib doses in the range 100 to 300 mg is warranted in Japanese patients with advanced NSCLC. Other randomized phase II studies of vandetanib in advanced NSCLC have demonstrated improvements in progression-free survival with vandetanib 300 mg as a monotherapy versus gefitinib[12] and with the combination of vandetanib 100 mg and docetaxel.[14] Phase III evaluation of vandetanib in a broad population of patients, both as monotherapy at 300 mg (versus placebo in patients previously treated with anti-EGFR therapy [ZEPHYR]; versus erlotinib [ZEST]) and at 100 mg in combination with docetaxel (ZODIAC) or pemetrexed (ZEAL), has been initiated in global trials.

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SNP Communication

Genetic Variations and Haplotypes of *ABCC2* Encoding MRP2 in a Japanese Population

Kimie SAI[1,2,*], Yoshiro SAITO[1,2], Masaya ITODA[1], Hiromi FUKUSHIMA-UESAKA[1], Tomoko NISHIMAKI-MOGAMI[2], Shogo OZAWA[1,3,a], Keiko MAEKAWA[1,2], Kouichi KUROSE[1,4], Nahoko KANIWA[1,4], Manabu KAWAMOTO[5], Naoyuki KAMATANI[5], Kuniaki SHIRAO[6,b], Tetsuya HAMAGUCHI[6], Noboru YAMAMOTO[6], Hideo KUNITOH[6], Yuichiro OHE[6], Yasuhide YAMADA[6], Tomohide TAMURA[6], Teruhiko YOSHIDA[7], Hironobu MINAMI[8,c], Yasuhiro MATSUMURA[9], Atsushi OHTSU[10], Nagahiro SAIJO[11] and Jun-ichi SAWADA[1,2]

[1]*Project Team for Pharmacogenetics,* [2]*Division of Functional Biochemistry and Genomics,*
[3]*Division of Pharmacology,* [4]*Division of Medicinal Safety Science,*
National Institute of Health Sciences, Tokyo, Japan
[5]*Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan*
[6]*Division of Internal Medicine, National Cancer Center Hospital*
[7]*Genomics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan*
[8]*Division of Oncology/Hematology,*
[9]*Investigative Treatment Division, Research Center for Innovative Oncology,*
[10]*Division of GI Oncology/Digestive Endoscopy,*
[11]*Deputy Director, National Cancer Center Hospital East, Chiba, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: The multidrug resistance-associated protein 2 (MRP2) encoded by the *ABCC2* gene is expressed in the liver, intestine and kidneys and preferentially exports organic anions or conjugates with glucuronide or glutathione. In this study, all 32 exons and the 5′-flanking region of *ABCC2* in 236 Japanese were resequenced, and 61 genetic variations including 5 novel nonsynonymous ones were detected. A total of 64 haplotypes were determined/inferred and classified into five *1 haplotype groups (*1A, *1B, *1C, *1G, and *1H) without nonsynonymous substitutions and *2 to *9 groups with nonsynonymous variations. Frequencies of the major 4 haplotype groups *1A (−1774delG), *1B (no common SNP), *1C (−24C>T and 3972C>T), and *2 [1249G>A (Val417Ile)] were 0.331, 0.292, 0.172, and 0.093, respectively. This study revealed that haplotype *1A, which has lowered activity, is quite common in Japanese, and that the frequency of *1C, another functional haplotype, was comparable to frequencies in Asians and Caucasians. In contrast, the haplotypes harboring 3972C>T but not −24C>T (*1G group), which are reportedly common in Caucasians, were minor in Japanese. Moreover, the allele 1446C>T (Thr482Thr), which has increased activity, was not detected in our Japanese population. These findings imply possible differences in MRP2-mediated drug responses between Asians and Caucasians.

Keywords: ABCC2; MRP2; genetic variation; haplotype; amino acid change

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**To whom correspondence should be addressed:* Kimie SAI, Ph.D., *Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel: +81-3-3700-9478, Fax: +81-3-3707-6950, E-mail: sai@nihs.go.jp*
[a]Present address: Department of Pharmacodynamics and Molecular Genetics, Faculty of Pharmaceutical Sciences, Iwate Medical University, Iwate, Japan.
[b]Present address: Department of Medical Oncology, OITA University Faculty of Medicine, Yufu, Japan
[c]Present address: Medical Oncology, Department of Medicine, Kobe University Hospital and Graduate School of Medicine, Kobe, Japan.

As of October 7, 2007, the novel variations reported here are not found in the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).

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Introduction

The multidrug resistance-associated protein 2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT) is a 190–200 kDa transmembrane glycoprotein comprised of 1545 amino acids and belongs to the superfamily C of ATP-binding cassette (ABC) transporters. This transporter is expressed on hepatic canalicular membranes, intestinal apical membranes, luminal membranes of renal proximal tubules, placental epithelial cells, and the blood brain barrier.[1] MRP2 exports endogenous and exogenous substances, preferentially organic anions or conjugates with glucuronide, glutathione and sulfate.[1-3] This protein originally identified in cisplatin-resistant tumor cells[4] is shown to confer drug resistance to other anti-cancer drugs, such as vincristine and doxorubicin.[5,6]

MRP2 is encoded by the *ABCC2* gene located on chromosome 10q24 and consists of 32 exons (31 coding exons) and spans 69 kb. Several *ABCC2* genetic variations have been detected in patients with Dubin-Johnson syndrome (DJS), an autosomal recessive disease characterized by hyperbilirubinemia with conjugated bilirubin or increased coproporphyrin excretion in urine.[2,7] Recent studies on *ABCC2* have identified common single nucleotide polymorphisms (SNPs) such as −24C>T and −3972C>T (Ile 1324Ile) among several ethnic populations, and several studies have suggested their association with altered MRP2 expression or function.[8-17] In more recent studies on *ABCC2* haplotypes covering an extended 5′-flanking region, close linkages were found among −1549A>G in the 5′-flanking region and two common SNPs −24C>T and −3972C>T (Ile1324Ile).[8] In addition, as possible functional SNPs, −1774delG in Koreans[8] and −1019A>G in Caucasians[10] were reported. However, there is little information on detailed haplotype structures throughout the gene, and comprehensive haplotype analysis in Japanese has not yet been conducted.

We previously analyzed *ABCC2* genetic variations within all 32 exons and the proximal 5′-flanking region (approximately 800 bp upstream of the translation initiation site) using established cell lines derived from Japanese cancer patients to obtain preliminary information on *ABCC2* SNPs in Japanese.[18] In this study, to reveal *ABCC2* haplotype structures in Japanese, we resequenced the *ABCC2* gene including the distal 5′-upstream region (approximately 1.9 kb upstream from the translation initiation site) as well as all 32 exons in 236 Japanese subjects and conducted haplotype analysis using the detected genetic polymorphisms.

Materials and Methods

Human DNA samples: Genomic DNA samples were obtained from blood leukocytes of 177 Japanese cancer patients at two National Cancer Center Hospitals (Tokyo and Chiba, Japan) and Epstein-Barr virus-transformed lymphoblastoid cells prepared from 59 healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP consortium (Tokyo, Japan). Written informed consent was obtained from all subjects. Ethical review boards of all participating organizations approved this study.

PCR conditions for DNA sequencing: We sequenced all 32 exons of the *ABCC2* gene and approximately 800 bp upstream of the translation initiation codon (proximal 5′-flanking region) as described previously and also extended the sequenced region to 1.9 kb upstream of the translation initiation site (distal 5′-flanking region). Briefly, for amplification of the proximal 5′-flanking region and 32 exons, 5 sets of multiplex PCR were performed from 200 ng of genomic DNA using 1.25 units of Z-taq (Takara Bio. Inc., Shiga, Japan) with 0.3 uM each of the mixed primers as shown in **Table 1** [1st PCR]. The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72°C for 190 sec. Next, each exon was amplified separately using the 1st PCR product by Ex-Taq (0.625 units, Takara Bio. Inc.) with appropriate primers (0.3 uM) (**Table 1**) [2nd PCR]. The conditions for the second round PCR were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of the distal 5′-flanking region, multiplex PCR was performed from 25 ng of genomic DNA using 1 unit of Ex-Taq (Takara Bio. Inc.) with 0.4 uM each of the 2 sets of primers as shown in **Table 1** [PCR]. The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min.

Following the PCR, products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx-96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. Genbank NT_030059.12 was used as the reference sequence.

Linkage disequilibrium (LD) and haplotype analyses: Hardy-Weinberg equilibrium and LD analyses were performed using SNPAlyze 3.1 software (Dynacom Co., Yokohama, Japan). Pairwise LDs were shown as rho square (r^2) and $|D'|$ values in **Figure 1**. Diplotype configurations (haplotype combinations) were inferred by LDSUPPORT software, which determined the posterior probability distribution of diplotype configurations for each subject based on estimated haplotype frequencies[19].

Results and Discussion

In this study, sixty-one *ABCC2* genetic variations including 36 novel ones were detected in 236 Japanese subjects

Table 1. Primer sequences used in this study

Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]
PCR (Ex-taq)			
5′-Flanking (for −1.9 k to −1.7 k)	CCACCAGTGCCAAGAGAAGTAT	CACAAGTCATCTGGAAAACACA	20289134–20289443
5′-Flanking (for −1.7 k to −950)	ATGAGGTGGTATCTAACTGTGG	AAATGTTTTCTGTAGGGACGGG	20289392–20290182
1st PCR (Z-taq)			
5′-Flanking (for −1.2 k) to exon 6	ATACTGCATGGGTGGTTATG	AACCTGCCTCCAAATTTTTC	20289942–20303347
Exons 7 to 11	GGAGAATCACTTTGAAGCCG	CTAGCAAGTGTGAGGGGTGT	20304874–20314079
Exons 12 to 19	TCTGTGAATGTGGCAAAACT	GGATCTACCAAGAATTTAGC	20315189–20328004
Exons 20 to 25	GATGAGCATTTTCAATTTAC	TCAGTTCACCCAGCACTTAT	20338211–20344941
Exons 26 to 32	GAGCAAGACCTTGTCTCATA	CCATGGATGAATCTCAGATA	20349821–20360334
2nd PCR (Ex-taq)			
5′-Flanking (for −880 to −130)	GGAAGATCGCTTGAACCCAT	TCATCCCAACCATTTAATCG	20290245–20290994
Exon 1	TTGTTGGCCAGCTCTGTTG	TTCTGGTTCTTGTTGGTGAC	20290810–20291254
Exon 2	GGGTAAGGCTGGATATGGAT	CTGGCTCTACCTGAGACAAT	20292767–20293194
Exon 3	CACCGGAAACCATTCTGTTC	TTTGCCTCACTATGGATCCC	20300442–20300773
Exon 4	GCCAGATTAGTCACGACAGT	CCAAAGGAAGTCTACATGGCC	20301708–20302134
Exon 5	CAGGTAAGGAAAAAAAGAGTGG	CCTTGTCATAAAATGGTCTG	20301966–20302418
Exon 6	TATGCCAGAAAATCTGATTA	AGGTGGAACATGAGCTTGAGT	20302499–20303070
Exon 7	GGTGGAGATAGCCTCTGACC	TGCACTGAGAAGTATGAAGTGC	20305320–20305728
Exon 8	CCTGTACAGAGAAGGCCACG	TGCGGTCTTCATGAACACAA	20307385–20307816
Exon 9	GGCTTTGGACAATTCTGGTC	TCCACCCATTGTCTGTGAAC	20308539–20309038
Exon 10	AGGCAAGAAGTCACAGTGCC	TTGCCCAAACTCCCATTAAG	20312158–20312650
Exon 11	ACAGTCAGGCAAGGGCTATG	GACAGGAGGACATGAAACAA	20313420–20313873
Exon 12	GATTTCTATTCCCCACATTT	GAGCTGGGGGTATGGTACAA	20315554–20315983
Exon 13	GTGACCTTGGAGAAGATATT	CTCTTGAAAGTTTACCAGCA	20316189–20316623
Exon 14	TTGCTCAAGGACTGAAATAG	CCTGCTTATCCTCAGAAGAG	20318223–20318732
Exon 15	GGTCTCATGGTCTCATTCTA	GGGTTTATCCTGCACTAGTA	20319650–20320025
Exon 16	AGAAGCACTTTGGGGTCTTGTA	GCTGAAATGGGAAGGAGAATC	20321144–20321581
Exon 17	GCTGAAAAACGATAGTCCAA	TCAACTAGATTACCCCTGTGT	20325354–20325863
Exons 18 and 19	TCACAGGGTGACAAGCAAC	TTGAATCTCTGGGTAGTTTG	20326820–20327678
Exon 20	GAAACCAGCAAGATCAGAGGA	TCACTCAGCTGGCATCAAAG	20338493–20338929
Exon 21	TGACTGTGACATCTGCTTGC	GGACAGAGGACATATTGCTCC	20338927–20339248
Exons 22 and 23	GCATTGTATTTCAGCATTGT	ACAGTGTTGTCTAGGGGGAC	20339701–20340506
Exon 24	GAACACACAGAATCCAACAGA	TCACTTCAGCTTCAGACAGT	20342562–20343001
Exon 25	TCTCATTGGTCTCCTCCTCG	AATTTCACACCACTAGCCAT	20344186–20344672
Exon 26	GAGGCATTGCCTAAGAGTGC	AAAGATGGAGCCAGGGTTTG	20350122–20350523
Exons 27 and 28	GGCAAGGATTGTCTTTCTTA	CGACAGCTGCGGTAAGTCTG	20351928–20352954
Exon 29	AGAGATGGAGTAGCCAGTCAC	CAGCCACAAATGCATATTACC	20353790–20354262
Exon 30	GAAGCTCAACCACAAACCAG	GCTCGACCAGTTTTCAAGAG	20355106–20355610
Exon 31	GCAAGGTACAGCTAGTTGAA	GCGTGATGTAAAATTTTGGC	20358730–20359248
Exon 32	GCTGTGGCTCATTGATTTTC	AAGGTGATAAAACAGAAATG	20359651–20360213
Sequencing			
5′-Flanking (for −1.7 k)	CCACCAGTGCCAAGAGAAGTAT	CACAAGTCATCTGGAAAACACA[b]	
(for −1.7 k to −1.3 k)	GGTATCTAACTGTGGTTTTG	GAAGGAAAGGAGTCAAAGGAAC	
(for −1.5 k to −950)	TCCCACACTGAATGCTGCCTTT	TAGGGACGGGGTCTCACTAT	
(for −880 to −400)	GGAAGATCGCTTGAACCCAT[b]	ATGTGCAGTTTCGCTTCTG	
(for −570 to −130)	CATATAGGCTCACACTGGAT	TCATCCCAACCATTTAATCG[b]	
Exon 1	TGGTTCCTTTTATGTATGGC	GTTCTTGTTGGTGACCACCC	
Exon 2	AAAGCAGTGGGATGTGCTG	TGTCTCTACTGTGCACCAAGG	
Exon 3	CACCGGAAACCATTCTGTTC[b]	TTTGCCTCACTATGGATCCC[b]	
Exon 4	CCTCCTTTCTTCCCATGTTC	CTCAACTTGATGCCATTTAC	
Exon 5	TGGGGCAACCTCTAACTCATA	TGAGACCCAGACATCTTAAA	
Exon 6	TTAGGGTCTCCAAATAAACA	ACTTTCAGAGGAGTGAGAGAGT	
Exon 7	GGTGGAGATAGCCTCTGACC[b]	TGCACTGAGAAGTATGAAGTGC[b]	
Exon 8	CCTGTACAGAGAAGGCCACG[b]	CACAATGCTGTAAGGTTAAG	
Exon 9	GGCTTTGGACAATTCTGGTC[b]	TCCACCCATTGTCTGTGAAC[b]	
Exon 10	GTGCCTTGGAGAAGCTGTGT	TTGCCCAAACTCCCATTAAG[b]	
Exon 11	TCACTGGGCACCTCAAGTTC	GGAATCCATCACCTCTACCA	
Exon 12	ACATTTTGGGGACTATATCT	ATGCCAGCTAGTCTATCAAA	
Exon 13	GGAGGCTGGATGATCCTTAAG	CTCTTGAAAGTTTACCAGCA[b]	
Exon 14	CATCTGTCTATGGTGGGATA	ATAGGCTCAAGACAAATCTC	
Exon 15	GATTTCATTCACCTCCTGTT	CATTTCCCCATGCATTCTAT	
Exon 16	CCAATCTTGAGGGGAAATCT	TCCAAGACCTCACCTACTAGC	

Table 1. continued

Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]
Exon 17	GTGGAATAACTACAAGCACG	TCAACTAGATTACCCCTGTGT[b]	
Exon 18	GGTGACAAGCAACAAAACTA	CCACCATCTTCCCTGTCTTA	
Exon 19	GATGCTCATGTAGGAAAACA	TTTACCATTCCACCCATGGC	
Exon 20	GGCTTCTCTCTCCTTGTTCA	CAAAGAAACAAAGGAAGAGC	
Exon 21	TGACTGTGACATCTGCTTGC[b]	GGACAGAGGACATATTGCTCC[b]	
Exon 22	GCATTGTATTTCAGCATTGT[b]	GATATTTGATGCATGGACGA	
Exon 23	GAATCTGTCTGGACCCTGTA	GTCTAGGGGGACATAATAAT	
Exon 24	ACACACAGAATCCAACAGAT	TCAACATATGACTAAATGGC	
Exon 25	GGAGCCTCTCATCATTCTGC	TTTCACACCACTAGCCATGC	
Exon 26	CCGATCAAGTCAAACCCTCT	TTTGAACCTCAGTCTTCTTT	
Exon 27	TTTCCTTACTCCCTTGTAGA	AAACTTTAGGGACCCATTAT	
Exon 28	CTGCTACCCTTCTCCTGTTC	CCTTCCCTCTGATACTGTGT	
Exon 29	TACCTCCTGTGACTGTGAAT	CAGCCACAAATGCATATTACC[b]	
Exon 30	GCCAGTCCTATCCACCATCT	AACACGAGGAACACGAGGAG	
Exon 31	GATCTGGAACATGAAAATGG	TTTTGGCCAGATTACTTGAC	
Exon 32	GCTCATTGATTTTCACTGCT	AAGGCAAAGGAATAATTATCG	

[a]The reference sequence is NT_030059.12.
[b]The same primer that was used for the 2nd PCR.

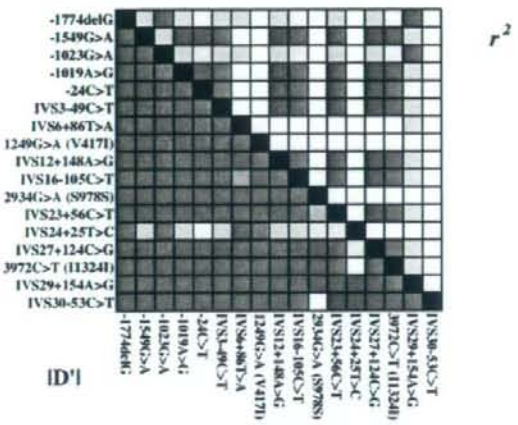

Fig. 1. Linkage disequilibrium (LD) analysis of *ABCC2*
Pairwise LD (r^2 values and |D′|) of polymorphisms detected in no less than 3% of allele frequencies is shown as a 10-graded blue color.

(**Table 2**). All detected variations were in Hardy-Weinberg equilibrium ($p > 0.05$). Novel variations consisted of 5 non-synonymous and 4 synonymous variations in the coding region, 22 in the intronic regions, 3 in the 5′-flanking region, 1 in the 3′- flanking region, and 1 in the 3′-UTR. The novel non-synonymous variations were 1177C>T (Arg393Trp), 1202A>G (Tyr401Cys), 2358C>A (Asp786Glu), 2801G>A (Arg934Gln), and 3320T>G (Leu1107Arg), and their frequencies were 0.002. No statistically significant differences were found in the allele frequencies of all variations between 177 cancer patients and 59 healthy subjects ($P > 0.05$, Fisher's exact test), although a larger number of subjects would be needed to conclude.

The frequency of the known common SNP −24C>T (0.173) was comparable to those reported in Asians (0.17–0.25)[8,12,20] and Caucasians (0.15–0.23)[9,10,14,15,21]. The allele frequency of another common SNP, 3972C>T (Ile1324Ile) (0.216), was also comparable to those in Asians (0.22–0.30)[8,12,20] but lower than those in Caucasians (0.32–0.37)[9,10,14,15,21]. The other major variations in the 5′-flanking region, −1774delG and −1549G>A, were found at frequencies of 0.343 and 0.203, respectively, and these values were similar to those obtained in Koreans (0.34 and 0.21, respectively).[8] However, the relatively frequent SNPs 1446C>G (Thr482Thr) (allele frequency = 0.125), IVS15-28C>A (0.333) and IVS28+16G>A (0.167) in Caucasians[17] were not detected in our study.

The LD profile of the *ABCC* variations (no less than 3% allele frequency) is shown in **Figure 1**. As assessed by r^2 values, close linkages were observed among −1774delG, −1023G>A and IVS29+154A>G, and among −1549G>A, −1019A>G, −24C>T, IVS3-49C>T, IVS12+148A>G, IVS15+169T>C, IVS16-105C>T, IVA23+56C>T, IVS27+124C>G, and 3972C>T (Ile1324Ile). It must be noted that complete linkage was observed between −1549G>A and −1019A>G in our population. In |D′| values, strong LD was also observed almost throughout the region analyzed. Overall, since close associations between the variations were observed throughout the entire *ABCC2* gene, the region sequenced was analyzed as a single LD block for the haplotype inference.

The *ABCC2* haplotype structures were analyzed using 61 detected genetic variations and a total of 64 haplotypes were identified/inferred. **Figure 2** summarizes the haplotypes and their grouping. Our nomenclature system is based on the recommendation of Nebert.[22] Haplotypes without

Table 2. Summary of *ABCC2* variations detected in this study

SNP ID			Reference	Location	Position		Nucleotide change	Amino acid change	Frequency (total = 472)
This Study	dbSNP (NCBI)	JSNP			NT_030059.12	From the translational initiation site or from the end of the nearest exon			
MPJ6_AC 2082			8	5′-Flanking	**20289354**	−1774	acttatctgttG/_ttttttttttt		0.343
MPJ6_AC 2078*				5′-Flanking	**20289538**	−1590	tttaatttgttaG/Atgtatgtttgct		0.002
MPJ6_AC 2079			8, 10, 17	5′-Flanking	**20289579**	−1549	tccttatagtatG/Attgtggatatta		0.203
MPJ6_AC 2080			9, 17	5′-Flanking	**20290105**	−1023	tgggaggccaagG/Acagaaggattgt		0.343
MPJ6_AC 2081			10, 17	5′-Flanking	**20290109**	−1019	aggccaaggcagA/Gaggattgttgaa		0.203
MPJ6_AC 2028*				5′-Flanking	**20290395**	−733	acagtttctagcG/Tactgatgccacc		0.004
MPJ6_AC 2029				5′-Flanking	**20290395**	−733	acagtttctagcG/Aactgatgccacc		0.002
MPJ6_AC 2030*				5′-Flanking	**20290715**	−413	ttgcagcagaagC/Tgaaactgcacat		0.002
MPJ6_AC 2003		**ssj0000371**	9, 12, 15–18, 20, 26	Exon 1	**20291104**	−24	tagaagagtcttC/Tgttccagcgca		0.174
MPJ6_AC 2004			18	Exon 1	**20291105**	−23	agaagagtcttcG/Attccagacgcag		0.006
MPJ6_AC 2031		**ssj0000386**	17, 26	Intron 3	**20301785**	IVS3 −49	ctccccctcagtcC/Ttcggttagtggc		0.203
MPJ6_AC 2032*				Intron 6	**20302837**	IVS6 +86	tattttattattT/Attttttgagat		0.076
MPJ6_AC 2033*				Exon 7	**20305479**	732	caagtttgaaacG/Acacatgaagaga	Thr244Thr	0.002
MPJ6_AC 2066*				Intron 7	**20307421**	IVS7 −69	tcacaggctgacC/Gaccctgagctg		0.002
MPJ6_AC 2067*				Intron 7	**20307423**	IVS7 −67	acaggctgaccaC/Accctgagctgct		0.002
MPJ6_AC 2035*				Exon 9	**20308814**	1177	ggtgtaaaagtaC/Tggacagctatca	Arg393Trp	0.002
MPJ6_AC 2068*				Exon 9	**20308839**	1202	tggcttctgtatA/Gtaagaaggtaag	Tyr401Cys	0.002
MPJ6_AC 2036*				Intron 9	**20308859**	IVS9 +13	gtaagcagaataC/Tggcaggtatcac		0.002
MPJ6_AC 2037*				Exon 10	**20312319**	1227	gaccctatccaaC/Tttggccaggaag	Asn409Asn	0.002
MPJ6_AC 2009		**ssj0000388**	17, 18, 20, 23–26	Exon 10	**20312341**	1249	aaggagtacaccG/Attggagaaacag	Val417Ile	0.097
MPJ6_AC 2010			18	Exon 10	**20312549**	1457	ccaagagtaagaC/Tcattcaggtaaa	Thr486Ile	0.019
MPJ6_AC 2069*				Intron 11	**20315600**	IVS11 −67	taaaacatgggtG/Agatcagatacac		0.002
MPJ6_AC 2038		**ssj0000390**	26	Intron 12	**20315952**	IVS12 +148	ccgccccatgccA/Gcttttcctcctt		0.210
MPJ6_AC 2039*				Intron 13	**20318344**	IVS13 −73	tcatggactaacG/Aacaaagtcaaaa		0.002
MPJ6_AC 2070*				Intron 14	**20318515**	IVS14 +14	taaataaatttgG/Taagttgcttccc		0.002
MPJ6_AC 2040*				Intron 14	**20318521**	IVS14 +20	aatttggaagtt(del/ins)†cagcaaactga		0.002
MPJ6_AC 2071*				Intron 14	**20318594**	IVS14 +93	agcaaactgagaG/Tagagtgtggaga		0.002
MPJ6_AC 2041*				Intron 14	**20319757**	IVS14 −62	cggagagagacaC/Tgtgaggcagac		0.002
MPJ6_AC 2042*				Intron 14	**20319758**	IVS14 −61	ggagagagacacG/Atgaggcagaca		0.006
MPJ6_AC 2043		**ssj0000393**	26	Intron 15	**20320054**	IVS15 +169	aaagcaaaggttT/Ctcagccccttcc		0.210
MPJ6_AC 2044*				Intron 15	**20321170**	IVS15 −131	gtcttgtatatcC/Gaaggcaaatttt		0.004
MPJ6_AC 2045*				Intron 16	**20325422**	IVS16 −169	ttgagtccctgagA/Tgtggaataacta		0.004
MPJ6_AC 2046		**ssj0000396**	17	Intron 16	**20325486**	IVS16 −105	tgcacagttattC/Taaatttaagctc		0.214
MPJ6_AC 2072*				Exon 18	**20327159**	2358	tcttctagatgaC/Acccctgtctgca	Asp786Glu	0.002
MPJ6_AC 2012			18, 20, 23	Exon 18	**20327167**	2366	atgaccccctgtC/Ttgcagtggatgc	Ser789Phe	0.008
MPJ6_AC 2073*				Intron 19	**20327555**	IVS19 +3	gaagccacaggtA/Gtgtaagaaggat		0.002
MPJ6_AC 2047*				Intron 19	**20327645**	IVS19 +93	agtatccagtgaA/Tctagatttggaa		0.002
MPJ6_AC 2048				Intron 20	**20338745**	IVS20 +29	gctggcagcccctC/Agtcagctctata		0.002
MPJ6_AC 2049*				Exon 21	**20339052**	2801	ccttgaaaactcG/Agaatgtgaatag	Arg934Gln	0.002
MPJ6_AC 2015		**ssj0000398**	8, 18, 26	Exon 22	**20339944**	2934	aggattgttttcG/Aatattcttcatc	Ser978Ser	0.040
MPJ6_AC 2050*				Exon 22	**20340061**	3051	cgactatccagcA/Gtctcagagggac	Ala1017Ala	0.002
MPJ6_AC 2051*				Exon 23	**20340337**	3181	cacaagcaactgC/Ttgaacaatatcc	Leu1061Leu	0.002
MPJ6_AC 2052		**ssj0000399**	17, 26	Intron 23	**20340470**	IVS23 +56	ggatcttttctgaC/Tagggaggaatta		0.222
MPJ6_AC 2074*				Exon 24	**20342724**	3320	ttacatgcttccT/Gggggataatcag	Leu1107Arg	0.002
MPJ6_AC 2053				Intron 24	**20342843**	IVS24 +25	atggctaagtcaT/Ccctttccttcctc		0.030
MPJ6_AC 2075*				Intron 24	**20342880**	IVS24 +62	agcccagcctctT/Ctcctgagaatct		0.002
MPJ6_AC 2054				Intron 24	**20342926**	IVS24 +108	cactcactcctcC/Tcctcagcagctt		0.023
MPJ6_AC 2055*				Intron 24	**20344318**	IVS24 −56	agaaaggaggaaG/Aatggtggatgcc		0.002
MPJ6_AC 2056*				Intron 26	**20352061**	IVS26 −21	atgatgatttttcA/Ggtcttctggttt		0.002
MPJ6_AC 2057*				Intron 27	**20352227**	IVS27 +44	ggcaaaaacaacA/Gtgcaactccttc		0.008
MPJ6_AC 2058		**ssj0000404**	17, 26	Intron 27	**20352307**	IVS27 +124	aaagttccttttC/Gctctaactcaaa		0.222
MPJ6_AC 2076			26	Exon 28	**20352688**	3927	ccaagtgcggtaC/Tcgacctgagctg	Tyr1309Tyr	0.002
MPJ6_AC 2022		**ssj0000407**	8, 12, 13, 17, 18, 20, 26	Exon 28	**20352733**	3972	cacttgtgacatC/Tggtagcatggag	Ile1324Ile	0.216
MPJ6_AC 2059*				Intron 28	**20352920**	IVS28 +172	agggaaggatagC/Tagccagggatca		0.004
MPJ6_AC 2060*				Intron 29	**20354201**	IVS29 +136	cttgagctagttC/Tcctaggatggac		0.002
MPJ6_AC 2061		**ssj0000408**	26	Intron 29	**20354219**	IVS29 +154	gatggacacgtcA/Gttccagaactt		0.367
MPJ6_AC 2062		**IMS-JST090926**	17	Intron 29	**20355209**	IVS29 −35	cttttctggcatG/Aagccccaacagc		0.015
MPJ6_AC 2063*				Intron 30	**20358793**	IVS30 −92	gggggttttgaA/Gagtctgatctgg		0.008
MPJ6_AC 2064		**IMS-JST185750**		Intron 30	**20358832**	IVS30 −53	ccccctgcctgC/Tgtcttttcctgg		0.051
MPJ6_AC 2077*				3′-UTR	**20359975**	*61*	taatttttatttT/Gtataaaatacag		0.002
MPJ6_AC 2065*				3′-Flanking	**20360190**	*193+83*	ttattcctttgcC/Gtttcatttctgt		0.002a8

*Novel genetic variation

†delGCTTCCCAAACTTATTCGCAGTACTGGTGCCAGAATTTTGATAATACAAGAGCTTAGTAG/insTATTTACCT

‡Numbered from the termination codon.

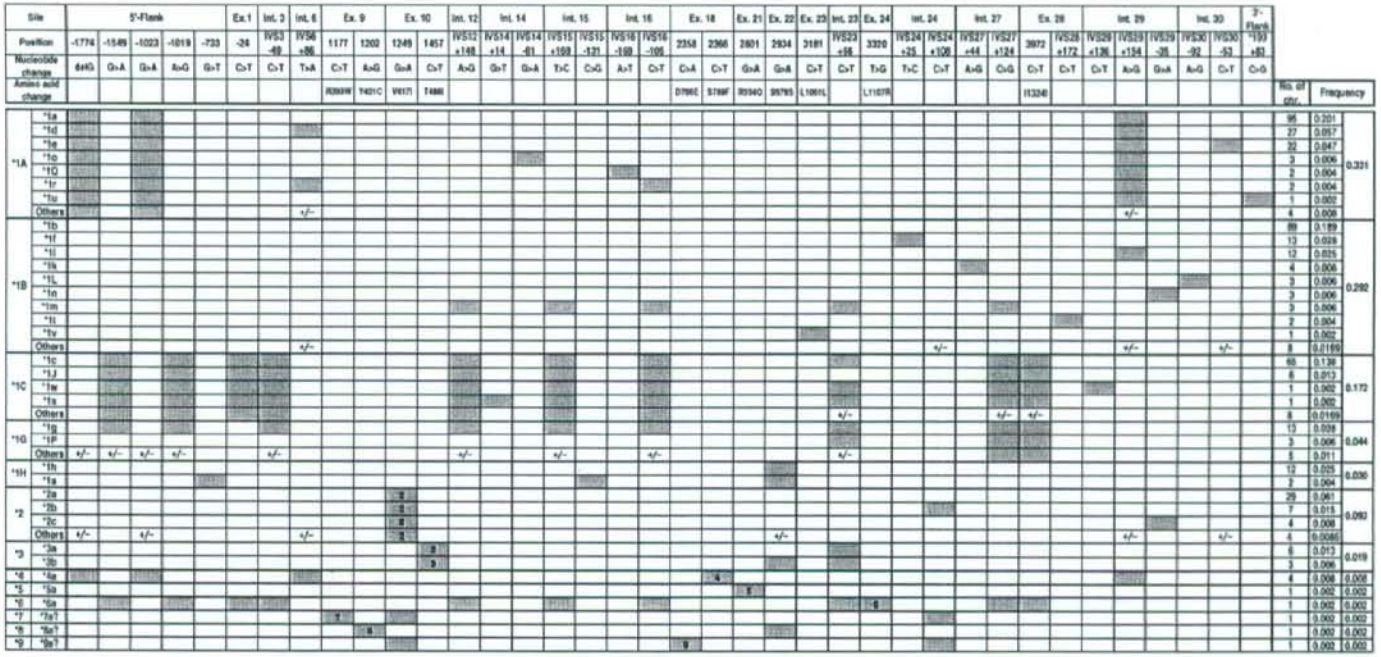

Fig. 2. *ABCC2* **haplotypes in 236 Japanese subjects**
The *1 groups (without nonsynonymous substitutions) were classified into *1A (harboring −1774delG), *1C (harboring −24C>T), *1G [harboring 3972C>T (Ile1324Ile) without −24C>T], *1H [harboring 2934G>A (Ser978Ser)] and *1B [without the common variations]. Marker SNPs for *2 to *9 are indicated by numbers. Rare and ambiguous haplotypes (n = 1) are shown with "?" or grouped into "others".

any amino acid substitution were assigned as the *1 group and named with small alphabetical letters in descending frequency order (*1a to *1x). Haplotypes with nonsynonymous variations were assigned from *2 to *9 groups, and their subtypes were named with small alphabetical letters. The haplotypes (*7a to *9a) were inferred in only one patient and described with "?" due to their ambiguity. Also, ambiguous rare haplotypes in the *1 and *2 groups were classified as "Others" in **Figure 2**. The *1 haplotypes were further classified into the *1A, *1B, *1C, *1G and *1H groups (capital alphabetical letters of the most frequent haplotypes were used) according to the common tagging SNPs, such as −1774delG, −24C>T, 3972C>T (Ile1324Ile), and 2937G>A (Ser978Ser).

The most frequent *1 group, *1A, harbors the common SNPs −1774delG and −1023G>A in the 5′-flanking region and mostly IVS29+154A>G, and the frequency of *1A (0.331) is almost the same as that in healthy Koreans (0.323) reported by Choi *et al.*[8] They have shown that −1774delG reduced promoter activity both at the basal level and after induction by chenodeoxycolic acid (CDCA), a component of bile acids, and that the haplotype bearing −1774delG is associated with chemical-induced hepatitis (cholestatis and mixed types).[8] Therefore, it is possible that *1A can affect the pharmacokinetics or pharmacodynamics of MRP2-transported drugs.

The *1B group haplotypes (0.292 frequency) harbor no or any intronic or synonymous variations the functions of which are unknown. The functional significance of variations in the *1B group, including the most frequent SNP IVS24+25T>C, needs further confirmation.

The third group *1C (0.172 frequency) harbors the known common SNPs −1549G>A, −1019A>G, −24C>T, IVS3−49C>T, and 3972C>T (Ile1324Ile), except for one rare ambiguous haplotype lacking 3972C>T (Ile1324Ile). The *1C haplotypes also harbor IVS12+148A>G, IVS15+169T>C and IVS16−105C>T. The haplotypes bearing −1549G>A, −24C>T and 3972C>T (Ile1324Ile) are commonly found in Korean populations (frequency 0.14–0.25)[8] and Caucasians (0.14–0.17).[10,14,21] The functional importance of the tagging SNP in the *1C group, −24C>T, has been reported by several researchers; *e.g.*, reduced promoter activity,[8,11] reduced mRNA expression in the kidney,[11] association with chemical-induced hepatitis (hepatocellular type),[8] and influence on irinotecan-pharmacokinetics and pharmacodynamics.[12,16] For other SNPs in the *1C group, functional alterations *in vitro* have not been shown; no change in promoter activity by −1549G>A, no influence of IVS3−49C>T on splicing, and no change induced by 3972C>T (Ile1324Ile) on MRP2 expression or transporter activity.[8] Although −24C>T caused reduced promoter activity in the absence of the bile acid CDCA,[8,11], enhanced promoter activity of −24C>T under induction by CDCA has been demonstrated.[8] Therefore the function of this SNP might depend on cholestatic status.

Our data demonstrated that −1019A>G was closely associated with the other *1C SNPs (complete linkage with −1549G>A). The close linkage between −1019A>G and −1549G>A was also observed in Caucasians, but their linkages with −24C>T and 3972C>T were relatively weak.[14] In contrast, another study on Caucasians reported that −1019A>G was exclusive to −1549G>A, −24C>T and 3972C>T.[10] Although the reasons for these discrepancies are not clear, some ethnic differences might exist in the 5′-flanking region.

The *1G group harbors 3972C>T (Ile1324Ile) but not −24C>T. Caucasians have haplotypes bearing 3972C>T (Ile1324Ile) without −24C>T at frequencies of 0.15–0.20.[10,21] In contrast, the frequency of the corresponding haplotype group in our study (*1G) was much lower (0.044). Although no *in vitro* effect of 3972C>T (Ile1324Ile) was shown,[8] its *in vivo* association with increased area under the concentration-time curve of irinotecan and its metabolites was reported in Caucasians.[13]

The *1H group (*1h and *1s) harbors a synonymous substitution of 2934G>A (Ser978Ser) (0.03 frequency). No influence of 2934G>A(Ser978Ser)on MRP2 expression or transport activity has been shown.[8]

As for haplotypes with nonsynonymous substitutions, eight haplotype groups (*2 to *9) were identified. The *2 [including 1249G>A (Val417Ile)] was the most frequent among them, and its frequency (0.093) was similar to those for Asians (0.10–0.13)[8,12,20] and slightly lower than those for Caucasians (0.13–0.22).[9,10,14,15,21] The haplotype frequencies of *3 [harboring 1457C>T (Thr486Ile)] and *4 [2366C>T (Ser789Phe)] were 0.019 and 0.008. Other rare haplotypes with novel nonsynonymous variation, *5 [2801G>A (Arg934Gln)], *6 [3320T>G (Leu1107Arg)], *7 [1177C>T (Arg393Trp)], *8 [1202A>G (Tyr401Cys)], and *9 [2358C>A (Asp786Glu)] were found each in only one subject as heterozygote at a 0.002 frequency. No functional significance of the marker SNP [1249G>A (Val417Ile)] of *2 has been shown *in vitro*,[8,23] but its *in vivo* associations with lower MRP2 expression in the placenta[24] and chemical-induced renal toxicity[25] have been reported. The variation 2366C>T (Ser789Phe) (*4) has been shown to cause reduced MRP2 expression and alter localization *in vitro*,[23] but clinical data are limited. Functional changes in *3 [1457C>T (Thr486Ile)] and *5 to *9 (novel nonsynonymous variations) are currently unknown. Possible effects of these amino acid substitutions were speculated using PolyPhen analysis (http://genetics.bwh.harvard.edu/pph); its prediction is based on the analysis of substitution site [e.g., a substitution in transmenbrane domain is assessed by the predicted hydrophobic and transmembrane (PHAT) matrix score], likelihood of the substitution assessed by the position-specific independent count (PSIC) profile scores, and protein 3D structures. This analysis predicted a possible functional change of Leu1107Arg (*6) due to substitution in

the transmembrane region (PHAT matrix element difference = −6), and probable functional effects of Arg393Trp (*7) (PSIC score difference = 3.053), Tyr401Cys (*8) (3.382) and Asp786Glu (*9) (2.277), but no functional effects of *3 (1.446) and *5 (0.326).

In conclusion, the current study provided detailed information on *ABCC2* variations and haplotype structures in Japanese and also suggested a large ethnic difference in the frequencies of 3972C>T(Ile1324Ile) and 1446C>G (Thr482Thr) and their related haplotypes between Asians and Caucasians. This information would be useful for studies investigating the clinical significance of *ABCC2* alleles and haplotypes.

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Weekly Epoetin Beta Maintains Haemoglobin Levels and Improves Quality of Life in Patients with Non-Myeloid Malignancies Receiving Chemotherapy

Yasuhiro Suzuki[1], Yutaka Tokuda[1], Yasuhiro Fujiwara[2], Hironobu Minami[3], Yasuo Ohashi[4] and Nagahiro Saijo[5]

[1]Department of Breast and Endocrine Surgery, Tokai University School of Medicine, Kanagawa, [2]Division of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo, [3]Division of Oncology/Hematology, Department of Medicine, National Cancer Center Hospital East, Chiba, [4]Department of Biostatistics/Epidemiology and Preventive Health Sciences, School of Health Sciences and Nursing, University of Tokyo, Tokyo and [5]National Cancer Center Hospital East, Chiba, Japan

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Objective: This study was aimed at investigating the effectiveness and safety of once-weekly epoetin beta for anaemic cancer patients receiving chemotherapy.
Methods: A total of 104 patients with a haemoglobin level of $\leq$11.0 g/dL were enrolled. Patients received a once-weekly subcutaneous dose of 36 000 IU epoetin beta for 12 weeks. If the increase in the haemoglobin level was $<$1.0 g/dL after 6 weeks, or a red blood cell transfusion was required between days 15 and 42, the dose of epoetin beta was increased to 54 000 IU from the subsequent week. The primary endpoint was the percentage of patients who achieved a haemoglobin increase of $\geq$2.0 g/dL; the haemoglobin response rate. Quality of life (QOL) was assessed using the Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire.
Results: The haemoglobin response rate was 66.3% among the 98 patients (breast cancer: $n = 25$; malignant lymphoma: $n = 21$; ovarian cancer: $n = 20$; lung cancer: $n = 15$; other cancers: $n = 17$) assessable for a haemoglobin response. Thirty-nine patients (39.8%) required a dose escalation to 54 000 IU. At the end of the study, QOL assessable patients ($n = 96$) showed a mean improvement in the FACT-An total fatigue subscale score (FSS) of 0.3 points from baseline. Patients with a haemoglobin response had a mean change in the total FSS of +3.2, compared with −3.4 for patients without a haemoglobin response. No serious adverse event of epoetin beta was observed.
Conclusions: Epoetin beta administered at an initial dose of 36 000 IU once-weekly was well tolerated, with increased haemoglobin levels and improved QOL in anaemic cancer patients receiving myelosuppressive chemotherapy.

Key words: anaemia – erythropoietin – cancer – chemotherapy – quality of life

INTRODUCTION

Anaemia is a common complication of cancer patients undergoing chemotherapy. Symptoms of anaemia, including fatigue, palpitations, dizziness and dyspnea markedly reduce patient activity, resulting in impaired quality of life (QOL). In most cases, however, physicians hesitate to prescribe red blood cell (RBC) transfusions until the haemoglobin level is $<$8.0 g/dL, even if the patient has symptoms related to anaemia, such as fatigue. Although the safety of blood transfusion has improved in recent years, risks still remain, such as viral infections, graft versus host disease and haemolytic reactions.

In Europe and the United States, erythropoietin (EPO) agents have widely been used since the 1990s for the treatment of chemotherapy-induced anaemia. Although a three-times weekly dosing schedule was initially introduced (1–3), this schedule was inconvenient for outpatients. Several studies reported that once-weekly dosing of EPO increased the

For reprints and all correspondence: Yutaka Tokuda, Department of Breast and Endocrine Surgery, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan. E-mail: tokuda@is.icc.u-tokai.ac.jp

haemoglobin level and improved QOL in a manner comparable with those obtained by three-times weekly dosing (4,5).

Since EPO agents have not been approved for the treatment of chemotherapy-induced anaemia in Japan, we previously conducted a dose-finding study of weekly epoetin beta in patients with malignant lymphoma or lung cancer, resulting in a recommended weekly dose of 36 000 IU (6). In this prospective study, we investigated the haemoglobin response, the effects on QOL and the safety of once-weekly epoetin beta in anaemic patients with non-myeloid malignancies. We also investigated the effects of dose escalation to 54 000 IU in patients showing insufficient haemoglobin increase.

PATIENTS AND METHODS

Patient Eligibility

Inclusion criteria were as follows: (a) histological or cytological confirmation of non-myeloid malignancy diagnosis, (b) treatment with cyclic chemotherapy, (c) anaemia (haemoglobin level $\leq$11.0 g/dL) considered to be primarily chemotherapy-induced, (d) life expectancy of at least 4 months, (e) aged between 20 and 79 years, (f) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, (g) eligibility for the QOL questionnaire and (h) adequate hepatic and renal function.

Exclusion criteria included: (a) iron deficiency (mean corpuscular volume $<80\ \mu m^3$ or iron saturation [{Fe/(Fe+unsaturated iron-binding capacity)} $\times$ 100] $<$15.0%); (b) surgery scheduled during the study period; (c) EPO therapy within 4 weeks prior to the study; (d) documented haemorrhagic lesions; (e) pregnancy, breastfeeding or non-use of adequate birth control measures; (f) history of myocardial, pulmonary, cerebral infarction, serious drug allergy, uncontrolled hypertension, hypersensitivity to any EPO agent or any serious complication; and (g) tumor in the central nervous system.

Study Design and Treatment Schedule

This multicentre, open-label study was conducted at 14 sites in Japan.

The protocol was approved by the institutional review board of the respective hospitals, and written informed consent was obtained from all patients who participated in the study.

The initial dose of epoetin beta (Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) was 36 000 IU, and a once-weekly treatment was administered subcutaneously for 12 weeks. If the patient's haemoglobin level did not increase by $\geq$1.0 g/dL from baseline after 6 weeks of treatment, or an RBC transfusion was required between days 15 and 42, the dose of epoetin beta was increased to 54 000 IU weekly from the subsequent week. If the haemoglobin level increased to $\geq$14.0 g/dL, epoetin beta was discontinued until the haemoglobin level decreased to $\leq$12.0 g/dL, and was then restarted at two-thirds (24 000 IU or 36 000 IU) of the previous dose (36 000 IU or 54 000 IU). RBC transfusion was allowed at the discretion of the investigator during the study. An oral daily dose of 100–200 mg elemental iron was recommended if the mean corpuscular volume was $<80\ \mu m^3$ or the iron saturation was $<$15.0%.

QOL was evaluated at baseline and week 12 using the Japanese Functional Assessment of Cancer Therapy-Anaemia (FACT-An) questionnaire (7,8), a well-validated instrument. In this study, the FACT-An total fatigue subscale, which consists of 13 fatigue related questions, was mainly analysed. The FACT-An total fatigue subscale scores (FSS) range from 0 to 52, with higher scores indicating less fatigue.

Evaluation of Efficacy and Safety

The American Society of Clinical Oncology/The American Society of Hematology guidelines (9) stipulate that the criteria for the haemopoietic effect should be an increase in haemoglobin level $\geq$1.0–2.0 g/dL in 6–8 weeks. Furthermore, there are reports (2,6), which showed that QOL is improved in patients with an increase in haemoglobin level of $\geq$2.0 g/dL.

The primary endpoint of the study was the percentage of patients achieving an increase in the haemoglobin level of $\geq$2.0 g/dL from the baseline between weeks 4 and 12, the haemoglobin response rate, excluding the data within 28 days after an RBC transfusion. The secondary endpoint was the change in FSS after 12 weeks of treatment. The percentage of patients receiving RBC transfusions between day 28 and the end of the study was also assessed. It was not expected that treatment with an EPO agent could influence transfusion requirements before day 28.

Adverse events (AEs) were assessed during the 12-week treatment period and during a 1-week observation period after the last dosing. Anti-erythropoietin antibodies were measured by the enzyme-linked immunosorbent assay and radio-immunoprecipitation (RIP) assay, and detection by either was judged as positive.

Statistical Analysis

We expected that 90 patients would need to be enrolled in the study to obtain a haemoglobin response rate of 70 $\pm$ 10% (95% confidence interval [CI]), as the primary endpoint.

Patients who received at least one dose of the study drug comprised the safety population. For efficacy analysis, the full analysis set (FAS) population was defined as eligible patients who received at least one dose of the study drug.

The changes in the haemoglobin level and FACT-An scores were calculated by subtracting each patient's baseline values from the last values. The rates of increase in haemoglobin before and after dose escalation were compared using a linear mixed-effects model. The potential factors influencing the change in FSS were examined by multiple

regression analysis. Pearson correlation coefficients were calculated to assess the association between changes in the haemoglobin level and FACT-An scores.

RESULTS

Demographics and Baseline Characteristics

A total of 104 patients were enrolled in the study between February and November 2004. Five patients discontinued the study before the first dosing for the following reasons: patient eligibility criteria violation, $n = 3$; patient denial, $n = 1$; and disease progression, $n = 1$. Thus, 99 patients were administered epoetin beta. One patient was excluded because of non-compliance with the eligibility criteria, leaving 98 patients as the FAS population. Eighty-seven patients (88.8%) completed all 12 weeks of the study. Eleven patients (11.2%) withdrew from the study. The primary reasons for withdrawal were progressive disease and AEs.

The demographics and baseline characteristics of the FAS population are listed in Table 1. Common types of cancer were breast ($n = 25$), malignant lymphoma ($n = 21$), ovarian ($n = 20$) and lung ($n = 15$). The mean age was 58.4 years (range: 23–78), and the mean body weight was 50.7 kg (range: 31.7–74.0). Most of the patients had an ECOG PS of 0 or 1 and a tumour stage of III or IV. The main chemotherapeutic agents used during the study were platinum for lung and other types of cancer, anthracycline for malignant lymphoma, taxane for breast cancer and platinum plus taxane for ovarian cancer. All patients met the criterion that they should not be iron-deficient at the time of enrollment.

Haemoglobin Response

The mean change in the haemoglobin level from baseline to the end of the study was 2.47 g/dL (standard deviation [SD]: 2.09; range: −2.8 to 6.0), as shown in Fig. 1. Figure 1 shows the mean changes in haemoglobin levels by tumour type. The pattern of changes in haemoglobin level was similar for the different tumour types. The mean increase in the haemoglobin level in patients with and without an initial EPO level of ≥100 mIU/mL were 1.76 g/dL (SD: 2.60) and 2.50 g/dL (SD: 1.85), respectively.

The haemoglobin response rates, defined as the percentage of patients achieving an increase in haemoglobin level of ≥2.0 g/dL from the baseline between weeks 4 and 12, are listed in Table 2. The overall haemoglobin response rate was 66.3% (65 of 98 patients). The median time to the haemoglobin response was 56 days from the first dosing, analysed by the Kaplan–Meier method. The percentage of patients with a haemoglobin level of ≥12.0 g/dL between weeks 4 and 12 was 59.2% (58 of 98 patients).

The percentage of patients who required dose escalation to 54 000 IU was 39.8% (39 of 98 patients). In these patients, the haemoglobin level increased after dose escalation, and the change in the haemoglobin level was 1.23 g/dL (SD: 2.19) at the end of the study. The haemoglobin response rate was 33.3% (13 of 39 patients) in patients who required dose escalation. The rate of haemoglobin increase before and after dose escalation was 0.023 g/dL/week (Weeks 0–6) and 0.266 g/dL/week (Weeks 7–12), respectively ($P = 0.0055$).

For three patients, the drug treatment was discontinued when the haemoglobin level exceeded 14.0 g/dL, and was restarted at a dose of 24 000 IU when the haemoglobin level decreased to ≤12.0 g/dL.

Quality of Life

Overall compliance in terms of the percentage of patients who completed the FACT-An was 100% at baseline and 97% (95 of 98 patients) at the end of the study. For three patients who dropped out due to progressive disease and were regarded as missing not at random, the scores at the end of the study were substituted with the minimum scores for all patients. Two patients were excluded from the evaluation of the change in the FSS because the responses to some items were missing.

The mean baseline FSS was 31.8 (SD: 11.4, $n = 98$) points. At the end of the study, the mean change from baseline was 0.3 (SD: 11.8, $n = 96$) points. The mean FSS change in the patients with progressive disease, as judged by each investigator, was −3.8 (SD: 16.7, $n = 15$) points (haemoglobin change: 2.4 g/dL). On the other hand, the mean change in patients without progressive disease was 1.9 (SD: 9.6, $n = 78$) points (haemoglobin change: 2.3 g/dL). These data indicated that progressive disease may be one of the independent variables affecting the change in FSS.

Relationship Between Haemoglobin Response and QOL Score

The results of a multiple regression analysis suggested that the change in the haemoglobin level ($P = 0.014$), the FSS at the initiation of dosing ($P < 0.0001$) and the PS at the end of the study ($P < 0.0001$) largely contributed to the change in the FSS. The correlation coefficient between the change in the FSS and the changes in the haemoglobin level was 0.280, indicating a significant correlation ($P = 0.006$, $n = 96$).

Patients who achieved an increase in the haemoglobin level of ≥2.0 g/dL experienced a 3.2-point mean change in FSS. On the other hand, patients who did not achieve an increase in haemoglobin level of ≥2.0 g/dL experienced a −3.4-point change (Fig. 2). There were no differences in the FSS at the initiation of dosing between patients with and without a change in haemoglobin level of ≥2.0 g/dL (32.0 versus 31.6). These data indicate that the change in FSS is dependent on the change in the haemoglobin level.

Concerning the relationship between the FSS at the initiation of dosing and the change in the FSS, patients with a baseline FSS of ≤36.0 reported greater improvement (mean ± SD: 1.6 ± 13.0) in the FSS at the end of the study (Table 3).

Table 1. Characteristics of the full analysis set population

	Characteristics	Total	Lung	Malignant Lymphoma	Breast	Ovarian	Other types
Sex	Male	27	11	10	0	0	6
	Female	71	4	11	25	20	11
Age (years)	Mean ± SD	58.4 ± 10.8	60.5 ± 10.5	56.5 ± 13.4	58.2 ± 9.0	54.4 ± 11.0	63.8 ± 8.0
	Range	23–78	41–78	23–74	39–77	30–75	40–76
ECOG performance status	0	48	1	9	14	13	11
	1	39	12	9	6	6	6
	2	11	2	3	5	1	0
Tumour stage	I	6	0	1	3	2	0
	II	17	1	4	7	4	1
	III	15	0	3	0	9	3
	IIIA	2	1	0	1	0	0
	IIIB	8	6	0	2	0	0
	IV	50	7	11	12	5	13
Treatment regimen	Platinum based	17	7	2	0	1	7
	Taxane based	28	5	0	19	3	1
	Anthracycline based	28	1	18	6	0	3
	Platinum, Anthracycline based	4	0	1	0	1	2
	Platinum,Taxane based	21	2	0	0	15	4
Weight (kg)	Mean ± SD	50.7 ± 8.2	53.8 ± 8.7	52.7 ± 9.9	47.9 ± 7.2	49.3 ± 6.6	50.9 ± 7.4
	Range	31.7–74.0	38.0–70.7	31.7–74.0	34.0–63.0	34.1–60.0	37.7–65.5
Haemoglobin (g/dL)	Mean ± SD	9.3 ± 1.4	9.6 ± 1.4	9.3 ± 1.4	9.4 ± 1.4	9.2 ± 1.6	9.1 ± 1.4
	Range	5.6–11.9	6.4–11.2	6.5–11.3	5.7–11.9	6.4–11.7	5.6–11.1
MCV (fL)	Mean ± SD	92.3 ± 6.5	89.0 ± 6.4	90.0 ± 5.4	91.9 ± 5.8	94.6 ± 7.5	95.7 ± 5.3
	Range	79.9–107.5	79.9–99.3	80–101	80.3–103.2	81.9–107.5	84–103.4
Reticulocyte (‰)	Mean ± SD	19.7 ± 16.4	20.8 ± 15.1	24.2 ± 24.1	18.0 ± 13.2	21.1 ± 15.6	14.1 ± 10.3
	Range	1–106	2–50	1–106	1–58	1–54	1.1–35.1
Transferrin saturation (%)	Mean ± SD	29.7 ± 22.3	22.4 ± 7.1	41.5 ± 30.6	21.9 ± 16.9	31.1 ± 24.3	30.5 ± 18.0
	Range	4.8–92.9	12.5–35.5	9.9–92.9	4.8–80.6	7.2–90.7	14.0–90.3
Serum endogenous erythropoietin (mIU/mL)	Mean ± SD	119.1 ± 310.5	64.3 ± 69.9	80.7 ± 108.0	88.4 ± 107.1	125.9 ± 144.8	252.0 ± 706.0
	Range	15.7–2970	15.7–254	17.3–399	16.7–472	23.2–578	20.4–2970
Baseline QOL:FACT-An							
Anemia subscale (0–80)	Mean ± SD	50.8 ± 14.5	47.0 ± 15.9	50.6 ± 11.7	47.1 ± 13.7	53.5 ± 11.1	56.7 ± 19.5
	Range	16–80	17–74	26–67	20–71	34–75	16–80
Fatigue subscale (0–52)	Mean ± SD	31.8 ± 11.4	29.6 ± 12.9	30.3 ± 10.6	29.7 ± 10.7	33.9 ± 8.7	36.5 ± 14.1
	Range	4–52	4–52	10–43	7–50	20–48	8–52

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; QOL, quality of life; FACT-An, Functional Assessment of Cancer Therapy-Anemia; MCV, mean corpuscular volume.

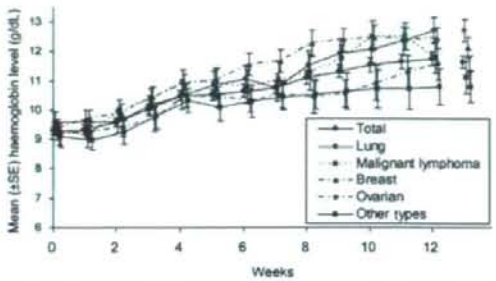

Figure 1. Change in haemoglobin level by tumor type. Mean weekly haemoglobin levels for the FAS population. Haemoglobin values within 28 days after RBC transfusion were excluded. FAS, full analysis set; RBC, red blood cell.

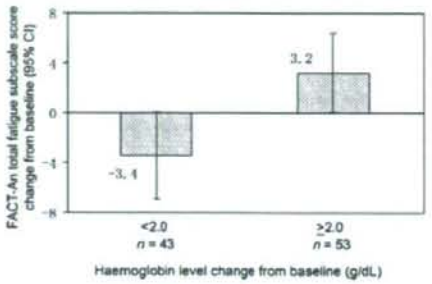

Figure 2. Changes in the FACT-An total fatigue subscale score by change in haemoglobin level. FACT-An, Functional Assessment of Cancer Therapy-Anaemia.

RBC Transfusion Requirement

The percentage of patients who received RBC transfusions between day 28 and the end of the study was only 6.1% (6 of 98 patients). The mean pretransfusion haemoglobin level at the time of the first transfusion was 6.2 g/dL (range: 5.4–7.3 g/dL). The percentage of patients whose haemoglobin level had decreased to <8.0 g/dL or who received an RBC transfusion between day 28 and the end of the study was 20.4% (20 of 98 patients).

Safety

AEs reported by at least 20% of the patients are summarised in Table 4. Death as a result of disease progression was not reported as an AE. Adverse drug reactions reported by at least 5% of patients are listed in Table 5. Among the 133 events in 48 patients (48.5%) that were considered related to the study drug, Grade III events were headache, hypertension, diarrhea, decreased serum potassium, impaired consciousness, anorexia and decreased serum phosphate. Three events (3.0%) of hypertension were reported as possibly related to epoetin beta treatment. An antihypertensive drug was administered after the onset of hypertension in one patient, who had hypertension as a comorbidity before the study. One patient (65-year-old female with malignant lymphoma) experienced a thrombovascular event, a lacunar infarction, at week 6. This event was evaluated as being unrelated to epoetin beta and was attributed to aging.

The incidence and type of AEs in patients who required dose escalation did not differ from those in patients who did not.

In two patients with ovarian and gastric cancer, anti-erythropoietin antibodies were detected only by RIP assay.

Table 2. Haemoglobin response rate by baseline haemoglobin, tumour type and dose escalation

	%	*n*
Response rate*	66.3	65/98
Response rate by baseline haemoglobin, g/dL		
<10.0	68.8	44/64
≥10.0	61.8	21/34
Response rate by tumour type		
Lung	80.0	12/15
Malignant lymphoma	66.7	14/21
Breast	76.0	19/25
Ovarian	65.0	13/20
Other types	41.2	7/17
Response rate by dose escalation		
Yes	33.3	13/39
No	88.1	52/59

*All patients, including those receiving transfusions.

Table 3. Changes in the FACT-An total fatigue subscale score by baseline FSS and final PS

Time period	Baseline		End of treatment		
	n	Mean score (SD)	*n*	Mean score (SD)	Mean change from baseline (SD)
Total	98	31.8 (11.4)	96*	31.8 (13.5)	0.3 (11.8)
Baseline FSS					
≤36.0	62	24.8 (7.9)	62	26.5 (12.0)	1.6 (13.0)
>36.0	36	43.9 (4.0)	34*	41.5 (10.3)	−2.2 (8.8)
Final PS					
0	58	35.5 (11.3)	56*	37.4 (10.3)	2.4 (10.2)
1	28	27.4 (9.1)	28	29.0 (11.5)	1.6 (12.2)
2	4	19.3 (9.4)	4	11.8 (11.4)	−7.5 (7.9)
3	3	29.7 (15.9)	3	21.0 (7.2)	−8.7 (13.8)
4	5	25.7 (7.3)	5	6.4 (7.1)	−19.3 (6.4)

*Two patients missing FSS. Collected but could not be calculated.
FSS, FACT-An total fatigue subscale score; PS, performance status.

Table 4. Frequencies of adverse events ($n = 99$)

Event	n	%	Grade*				
			I	II	III	IV	V
Neutropenia	83	83.8	3	11	24	45	0
Leukopenia	78	78.8	2	16	41	19	0
Nausea	57	57.6	38	11	8	0	0
Thrombocytopenia	55	55.6	21	9	23	2	0
Lymphopenia	52	52.5	0	18	34	0	0
Anorexia	46	46.5	22	13	10	1	0
Fatigue	39	39.4	22	14	3	0	0
Vomiting	36	36.4	18	16	2	0	0
Diarrhea	33	33.3	23	6	4	0	0
Increased lactate dehydrogenase	32	32.3	25	6	1	0	0
Peripheral neuropathy	26	26.3	21	5	0	0	0
Fever	26	26.3	17	7	2	0	0
Constipation	24	24.2	3	13	7	1	0
Increased alanine aminotransferase	24	24.2	15	6	3	0	0
Alopecia	22	22.2	7	15	0	0	0

*National cancer institutes common toxicity criteria, version 2.0.

Table 5. Frequencies of adverse drug reactions ($n = 99$)

Event	n	%	Grade*				
			I	II	III	IV	V
Increased lactate dehydrogenase	10	10.1	9	1	0	0	0
Headache	7	7.1	6	0	1	0	0
Nausea	7	7.1	5	2	0	0	0
Rash	5	5.1	3	2	0	0	0
Back pain	5	5.1	5	0	0	0	0

*National cancer institutes common toxicity criteria, version 2.0.

Neutralisation of EPO activity was detected in neither patient, and the haemoglobin level was elevated after dosing with the study drug. The investigators judged that the antibody did not cause pure red cell aplasia.

When re-examined six months after the last observation, one of these patients (ovarian cancer) was antibody negative, whereas the other (gastric cancer) could not be re-examined, having died of the underlying disease.

DISCUSSION

Several studies have been conducted to assess the effects of EPO agents in anaemic cancer patients, and increased haemoglobin levels and improvement in QOL that correlated with the increased haemoglobin level were reported (1,10).

The objectives of our study were to investigate the effects of an initial once-weekly 36 000 IU dose of epoetin beta on haemoglobin levels and QOL in patients with non-myeloid malignancy undergoing chemotherapy. The criterion for a haemoglobin response, an increase in the haemoglobin level of $\geq$2.0 g/dL, was based on a report that symptoms of anaemia assessed by the FACT-An are improved in patients with a change in the haemoglobin level of $\geq$2.0 g/dL (2,6). According to this index, the haemoglobin response rate in the present study was 66.3% (65 of 98 patients). The increases in haemoglobin levels that were observed were independent of the tumour type or the baseline haemoglobin level. None of the investigators performed a randomised comparison of a dose increase versus an unchanged dose in EPO low responders. In the present study, there was an increase in the rate of haemoglobin increase after dose escalation to 54 000 IU, and the haemoglobin response rate for patients who required a dose escalation was 33.3% (13 of 39 patients).

The secondary endpoint, the change in the FSS, showed an increase of 0.3 points; however, in patients who showed an increase in the haemoglobin level of $\geq$2.0 g/dL, the FSS was increased by 3.2 points, which was significantly higher than the $-$3.4-point change in patients whose haemoglobin level increased by $<$2.0 g/dL. A 3.2-point increase is comparable with the 3 points considered to be a clinically significant change in FSS (11). In addition, the mean change in FSS for patients with progressive diseases (PD) was $-$3.8 points (median: $-$6.5 points, range: $-$37 to 35 points) even though correction of anaemia was observed. In total, excluding PD cases, a 1.9- point improvement was observed.

Investigating the relationship between the FSS at the initiation of dosing and the change in the FSS showed that greater improvements in FSS were observed in patients with lower FSS. The FSS before treatment with epoetin beta was 31.8 $\pm$ 11.4 points, which is higher than the scores (FSS: 22.1–29.7 points, change in FSS: 1.6–5.2 points) in cancer patients with anaemia reported in several randomised trials (1,10,12–14). Nevertheless, the mean initial haemoglobin level (9.3 g/dL) in the present study was equal to the levels in the other trials (9.2–10.1 g/dL). Since it has been reported that the FSS after treatment with an EPO agent is aggravated in patients with an FSS exceeding 36.0 at the initiation of dosing (15), the scores were analysed after stratification at 36.0. This resulted in improved scores (1.6 $\pm$ 13.0 points) for those patients with a baseline score of $\leq$36.0, when compared with patients with a score $>$36.0 ($-$2.2 $\pm$ 8.8 points). The results of a multiple regression analysis of the change in the FSS demonstrated that the change in the haemoglobin level, the FSS at the initiation of dosing and the PS at the end of the study were factors that largely contributed to the change in the FSS. A positive and significant association was observed between

the degree of increase in the haemoglobin level and the degree of improvement in the FSS ($r = 0.280$, $P = 0.006$). It was comparable with the results ($r = 0.2879$, $P = 0.0002$; $r = 0.35$, $P = 0.001$ and $r = 0.2893$, $P < 0.0001$) of three other studies (1,10,16).

The RBC transfusion rate was only 6.1% (6 of 98 patients) between day 28 and the end of the study. As reported for once-weekly epoetin alfa administered to patients with various types of cancer (14), the transfusion rates between week 5 and the end of treatment were 14.5% (24 of 166 patients) for epoetin alfa and 29.3% (48 of 164 patients) for placebo. Furthermore, the mean pretransfusion haemoglobin levels for the first transfusion reported in the previous trial in the United States (7.9 and 7.8 g/dL, respectively) were higher than those (6.2 g/dL) in the present study in Japan. To evaluate the effect of EPO agents, the percentage of patients whose haemoglobin level had decreased to <8.0 g/dL or who received an RBC transfusion was considered to be a more objective index than the RBC transfusion rate in Japan, because RBC transfusion itself is prescribed at the discretion of the investigator and when the haemoglobin level is low.

Epoetin beta was well tolerated in the present study. Most of the AEs were consistent with the underlying disease or with the chemotherapy. Hypertension, which was judged to be related to epoetin beta was observed in three patients. It was alleviated either by no treatment or the administration of hypotensive agents. Lacunar infarction was also observed in one patient. A relationship to epoetin beta was ruled out, however, and this event was judged to be due to aging. Two recently published studies (17,18) targeting higher haemoglobin levels, in which survival was a primary endpoint, have raised concerns that EPO agents may have a negative impact on survival in cancer patients. A meta-analysis of 57 studies, including these two recent studies revealed an overall survival hazard ratio of 1.08 (95%CI: 0.99–1.18) and that uncertainties remain as to whether EPO agents affected survival (19). The FDA has provided new safety information on erythropoiesis-stimulating agents (ESAs), in which the target haemoglobin level is not to exceed 12 g/dL, because analyses of other studies in patients with cancer found a higher chance of serious and life-threatening adverse drug reactions or deaths with the use of ESAs (20). Although, in the present studies, there was no problem with safety when the haemoglobin level at which dosing was withheld was set at 14 g/dL, in consideration of FDA ALERTs, etc., we intend to investigate the use of lower values for target haemoglobin level and haemoglobin level at which dosing should be withheld.

In conclusion, once-weekly epoetin beta treatment increased the haemoglobin level and correspondingly improved the QOL in anaemic patients with non-myeloid malignancies receiving chemotherapy. Additionally, haemoglobin levels could be improved and controlled by once-weekly treatments at an initial dose of 36 000 IU followed by dose adjustment in the range of 24 000–54 000 IU.

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Conflict of interest statement

One of the authors, Hironobu Minami, receives honoraria from Chugai Pharmaceutical Co., Ltd. and Kirin Pharma Co., Ltd.

One of the authors, Yasuo Ohashi, consults on design and data analysis of clinical trials for Chugai Pharmaceutical Co., Ltd.

One of the authors, Nagahiro Saijo, holds stock option for Takeda Pharmaceutical Co., Ltd.

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