4 major haplotypes, i.e., **1A* (wild type), **1G* (IVS10 + 12G > A), **16B* [554C > G (Thr185Ser) and IVS10 + 12G > A], and **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A]. Associations of *CYP3A4* genotypes with irinotecan PK and severe toxicities (grade 3 diarrhea and grade 3 or 4 neutropenia) were investigated.

Results Area under the concentration–time curve ratios of APC/irinotecan, an in vivo parameter for CYP3A4 activity, were significantly higher in females than in males. The male patients with **16B* showed significantly decreased AUC ratios (APC/irinotecan) with 50% of the median value of the *non-*16B* male patients (no **16B*-bearing female patients in this study), whereas no significant alteration in the AUC ratios was observed in the patients with **18B*. A slight trend toward increasing AUC ratios (20%) was detected in both male and female patients bearing **1G*. Multivariate analysis confirmed contributions of *CYP3A4*16B* (coefficient ± SE = −0.18 ± 0.077, $P = 0.021$) and **1G* (0.047 ± 0.021, $P = 0.029$) to the AUC ratio. However, no significant association was observed between the *CYP3A4* genotypes and total clearance of irinotecan or toxicities (severe diarrhea and neutropenia).

Conclusion This study suggested that *CYP3A4*16B* was associated with decreased metabolism of irinotecan to APC. However, the clinical impact of *CYP3A4* genotypes on total clearance and irinotecan toxicities was not significant.

Keywords CYP3A4 · Haplotype · Irinotecan · Pharmacogenetics

Introduction

Human cytochrome P450 3A4 (CYP3A4) is a major CYP enzyme, abundant in the liver and intestine, and is involved in the metabolism of endogenous substances, including steroid hormones, and a variety of exogenous compounds such as environmental chemicals and pharmaceuticals. Large inter-individual differences in liver and intestinal CYP3A4 expression levels are known and thought to be caused by multiple factors including genetic variations, disease status, and modulation by exogenous stimuli, such as smoking, diet, and drugs [5, 18, 31]. The tissue-specific CYP3A4 expression is regulated by constitutive and inducible mechanisms via activation of the nuclear receptors, pregnane X receptor (PXR), constitutive androstane receptor (CAR), and vitamin D receptor (VDR) [5, 18]. Since approximately half of clinical drugs currently in use are metabolized by CYP3A4 [5, 33], it is important to find suitable biomarkers, including genetic polymorphisms, which can reflect in vivo CYP3A4 activity and predict individual responses to CYP3A4-metabolized drugs. Recent progress in pharmacogenetic research has led to the accumulation of knowledge about *CYP3A4* genetic variations responsible for altered expression or function. To date, more than 30 *CYP3A4* variations have been identified (http://www.cypalleles.ki.se/cyp3a4.htm), and large ethnic differences in their frequencies have been recognized. *CYP3A4*1B* (−392A > G), a single nucleotide polymorphism (SNP) in the 5′-flanking region, is found in Caucasians (2–9.6%) and African–Americans (35–67%), but not in Asians [16]. As relatively frequent coding SNPs, **2* [664T > C (Ser222Pro)] (2.7%) and **17* [566T > C (Phe189Ser)] (2%) were detected in Caucasians; **10* [520G > C (Asp174His)] in Caucasians (0.24–2%) and Mexicans (5%); **15* [485G > A (Arg162Gln)] (2–4%) in African–Americans; **16* [554C > G (Thr185Ser)] in East Asians (1.4–5%) and Mexicans (5%); **18* [878T > C (Leu293Pro)] (2.3–10%) in East Asians [2, 4, 17, 24]. We previously identified 25 *CYP3A4* haplotypes in a Japanese population [4]. The haplotypes **6* [including 830_831insA (Glu277fsX8)] (0.1%), **11* [including 1088C > T (Thr363Met)] (0.2%), **16B* [including 554C > G (Thr185Ser)] (1.4%), and **18B* [including 878T > C (Leu293Pro)] (2.8%) were identified, but **1B* (−392A > G) was not found. These findings indicate that ethnic-specific *CYP3A4* haplotypes must be taken into consideration in pharmacogenetic studies.

Irinotecan, an anticancer prodrug, is used for treatment of various cancers including lung and colon, and metabolized by CYP3A4 to produce inactive compounds such as APC (a major CYP3A4-mediated product) and NPC (a minor product) [6, 7]. An active metabolite SN-38 (a topoisomerase I inhibitor) is produced from the parent compound by carboxylesterases (CES) [28] and subsequently glucuronidated by UDP-glucuronosyltransferase 1As (UGT1As) to form inactive compound SN-38G [12] (Fig. 1). The parent compound and its metabolites are mainly excreted into the bile [29], where several ABC transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein 2 (MRP2) are involved in excretion [30]. The dose-limiting toxicities of irinotecan are severe diarrhea and neutropenia, and high plasma concentrations of SN-38 and/or its accumulation in tissues are thought to cause these toxicities [3, 30]. Recent extensive pharmacogenetic studies on irinotecan, mostly focusing on the *UGT1A1* genotypes, have revealed important roles for *UGT1A1*28* and **6* in reduced in vivo UGT activity and enhanced toxicities [1, 8, 9, 11, 13, 22, 26]. On the other hand, CYP3A4 can modulate irinotecan pharmacokinetics (PK). Co-administration of ketoconazole, a CYP3A4 inhibitor and also a potent UGT1A1 inhibitor [34], with irinotecan resulted in a decreased value of the area under the concentration–time curve (AUC) for APC and also increased AUC for SN-38 [14]; and vice versa, co-administration of St. John's Wort,

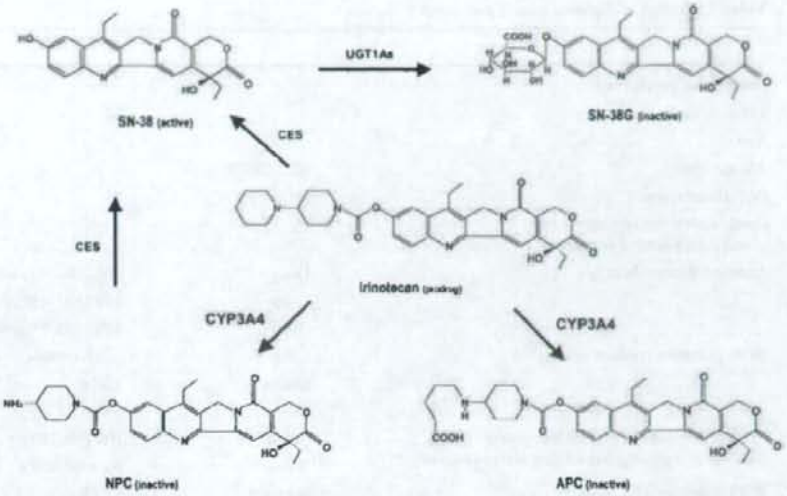


Fig. 1 Irintoecan metabolism in human liver. CYP3A4 mediates oxidation of irinotecan to produce inactive compounds, such as APC (a major CYP3A4-mediated product) and NPC (a minor product)

a CYP3A4 inducer, decreased the AUC of SN-38 [19]. A close association was also reported between in vivo CYP3A4 phenotypes and irinotecan clearance [21]. To date, however, no clinical impact by *CYP3A4* polymorphisms, such as **1B* (−392A > G) and **3* [1334T > C (Met445Thr)], has been demonstrated on irinotecan PK in Caucasians [20]. We previously found that **16* [554C > G (Thr185Ser)] caused decreased in vitro CYP3A4 activities [23]. Furthermore, a significant association of **16B* [harboring 554C > G (Thr185Ser)] was demonstrated with decreased AUC ratios of metabolite/paclitaxel, an in vivo parameter of CYP3A4 activity, in paclitaxel-administered Japanese patients [24].

In this study, to determine the clinical impact of the *CYP3A4* polymorphisms on irinotecan therapy, we identified the *CYP3A4* diplotypes of 177 Japanese cancer patients who received irinotecan and analyzed associations of the *CYP3A4* genotypes with irinotecan PK and toxicities.

Materials and methods

Patients and irinotecan treatment

One hundred seventy-seven patients with cancers who started irinotecan-containing therapy from 2002 to 2004 at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were enrolled for this pharmacogenetic study on irinotecan. 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. No participant received irinotecan previously, and other eligibility criteria included: bilirubin < 2 mg/dl, aspartate aminotransferase (GOT) < 105 IU/l, alanine aminotransferase (GPT) < 120 IU/l, creatinine < 1.5 mg/dl, white blood cell count > 3000/μl, performance status of 0–2, and an interval of at least 4 weeks after the last session of chemotherapy (2 weeks after the last session of radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were applied according to the approved treatment recommendations in Japan: intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly for irinotecan-monotherapy, and 60 mg/m^2 weekly for combination therapy with cisplatin. Profiles of the patients and irinotecan regimens are summarized in Table 1.

Genotyping of *UGT1A1* and *CYP3A4*

DNA was extracted from pretreatment whole-blood samples taken from 177 patients who received irinotecan. Data on *UGT1A1* genetic polymorphisms obtained from the same set of DNA samples have been published elsewhere [22]. The *CYP3A4* genotypes for 88 patients were previously determined [4]. Additional *CYP3A4* genotyping for the remaining 89 patients was conducted using the pyrosequencing method described previously [24], and the *CYP3A4* diplotypes/haplotypes [4] were inferred using an expectation-maximization-based program, LDSUPPORT [15].

Pharmacokinetics and toxicities

Pharmacokinetic analysis for irinotecan in 176 patients (data on one patient was unavailable) was performed as

Table 1 Profiles of Japanese cancer patients in this study

			No. of patients
Patients for genotyping			177
(Male/female)			(135/42)
Age			
Mean/range	60.5/26–78		
Performance status	0/1/2		84/89/4
Combination therapy, tumor type and initial dose of irinotecan[a]			
Irinotecan monotherapy	Lung	100 (60–100)/w	21
	Colon	150 (120–150)/2w	28
	Others	100 (100–150)/w	7
With platinum-containing drug[b]	Lung	60 (50–90)/w	58
	Stomach	70/2w	9
	Others	60/w	5
With 5-fluorouracil (5-FU)/leucovorin (LV)[c] or tegafur/gimeracil/oteracil potassium[d]	Colon	100 (90–180)/w or 150/2w	34
	Others	90/w or 100/w	2
With mitomycin C (MMC)[e]	Stomach	150/2w	10
	Colon	150/2w	1
With amrubicin[f]	Lung	60/w	2

[a] The median value and range in the parentheses are shown. "/w" and "/2w" represent weekly and biweekly, respectively

[b] Mostly, cisplatin (60 or 80 mg/m^2) was administered after irinotecan treatment

[c] LV (10 mg/m^2) was administered right after irinotecan treatment and then followed by 5-FU treatment. (500 mg/m^2 injection); or LV (200 mg/m^2) was administered simultaneously with irinotecan and followed by 5-FU treatment (400 mg/m^2 bolus injection and 2.0–2.4 g/m^2 infusion)

[d] Tegafur (80 mg/m^2 per day)/gimeracil/oteracil potassium was administered twice (before irinotecan treatment and on the next day)

[e] MMC (5 mg/m^2) was administered just before irinotecan treatment

[f] Amrubicin (30 or 35 mg/m^2) was administered 24 h after irinotecan treatment

previously described [26]. Briefly, heparinized blood was collected before administration of irinotecan, and 0, 0.3, 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan and APC were determined by HPLC [25], and AUC_{inf} and other PK parameters were calculated using the trapezoidal method of the 202 non-compartmental model for a constant infusion in WinNonlin ver. 4.01 (Pharsight Corporation, Mountain View, CA, USA). As for the co-administered anti-cancer and other drugs which were administered within 1 week before irinotecan-treatment, no drugs significantly affected the PK parameters related to CYP3A4 activity. Information on foods and drinks taken by the patients which might induce or inhibit CYP3A4 activity was not available.

A complete medical history and data on physical examinations were recorded prior to irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Statistical analysis

Statistical analysis on the differences in PK parameters between sexes and among *CYP3A4* genotypes was performed using the Mann–Whitney test or Kruskal–Wallis test, and associations of *CYP3A4* genotypes with the irinotecan toxicities were assessed by the Chi-square test, using Prism version 4.0 (GraphPad Prism Software Inc. San Diego, CA, USA). $P = 0.05$ (two-tailed) was set as a significant level of difference. Multivariate analysis for the log-transformed AUC ratio (APC/irinotecan) was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, co-administered drugs, serum biochemistry parameters at baseline, and genetic factors (including *CYP3A4* haplotypes and the *UGT1A1*6 or *28* haplotype obtained in our previous study [22]) as independent variables. Multivariate analysis on toxicities (grade 3 diarrhea or nadir of absolute neutrophil counts) was conducted for the patients who received irinotecan monotherapy, where the variables included dosing interval and the absolute neutrophil count at baseline, in addition to the other patient background and genetic factors described above. The variables in the final

models for both AUC ratio and toxicities were chosen by the forward and backward stepwise procedure at the significance level of 0.1 using JMP version 6.0.0 software (SAS Institute, Inc., Cary, NC, USA).

Results

Sex difference in PK parameters

Since hepatic CYP3A4 levels were reported to be significantly higher in females than in males [24, 32], we first analyzed the sex differences in the major PK parameters for irinotecan and APC, a major CYP3A4 metabolite (Table 2). As for irinotecan, lower total clearance and MRT, and higher AUC/dose were observed in females, but the differences (3, 5 and 3%, respectively) were not significant. A small but significant increase in C_{max}/dose for irinotecan was observed in females. This is attributable to the smaller distribution volume of females. On the other hand, the median values of AUC/dose and C_{max}/dose for APC of the females were significantly higher than those of the males (1.29- and 1.33-fold, respectively). The AUC ratio (APC/irinotecan), a parameter of in vivo CYP3A4 activity, was significantly higher (1.28-fold) in females than in males. These findings suggest that these differences may reflect the higher CYP3A4 activity in the females.

CYP3A4 genotypes

CYP3A4 diplotypes/haplotypes in 177 Japanese cancer patients were determined according to the previous definition [4]. The *CYP3A4* haplotypes found in this population were **1A* (wild type), **1G* (IVS10 + 12G > A alone), **16B* [554C > G (Thr185Ser) and IVS10 + 12G > A], and **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A]. In the current study, neither **6* [830_831insA (Glu277fsX8)] nor **11* [1088C > T (Thr363Met)] were found. The frequencies of **1G*, **16B*, and **18B* were 0.215, 0.014, and 0.020 (Table 3), and they were comparable to those obtained in previous reports [4, 24]. Note that the haplotypes **16B* and **18B* were detected only in male patients.

Associations of *CYP3A4* genotypes with PK parameters

Considering the significant sex difference in APC levels, associations between the *CYP3A4* genotypes and PK parameters were analyzed for each sex separately. In male patients, no significant differences among the *CYP3A4* genotypes were observed for total clearance and MRT of irinotecan (Fig. 2a, b). In females, a slightly but significantly lower (10%) median value for MRT of irinotecan was observed in patients bearing **1G* compared with those carrying the wild type (**1A*/**1A*) ($P = 0.022$, Mann–Whitney test) (Fig. 2b), whereas no significant **1G*-dependency was observed for total clearance (Fig. 2a). No significant

Table 3 Frequencies of *CYP3A4* haplotypes (A) and diplotypes (B) for Japanese cancer patients in this study

(A) Haplotype group[a]	No. of chromosomes ($N = 354$)	Frequency
**1A*	266	0.751
**1G*	76	0.215
**16B*	5	0.014
**18B*	7	0.020
(B) Diplotype	No. of patients ($N = 177$)	Frequency
1A*/1A*	100	0.565
1G*/1A*	55	0.311
1G*/1G*	10	0.056
16B*/1A*	4	0.023
16B*/1G*	1	0.006
18B*/1A*	7	0.040

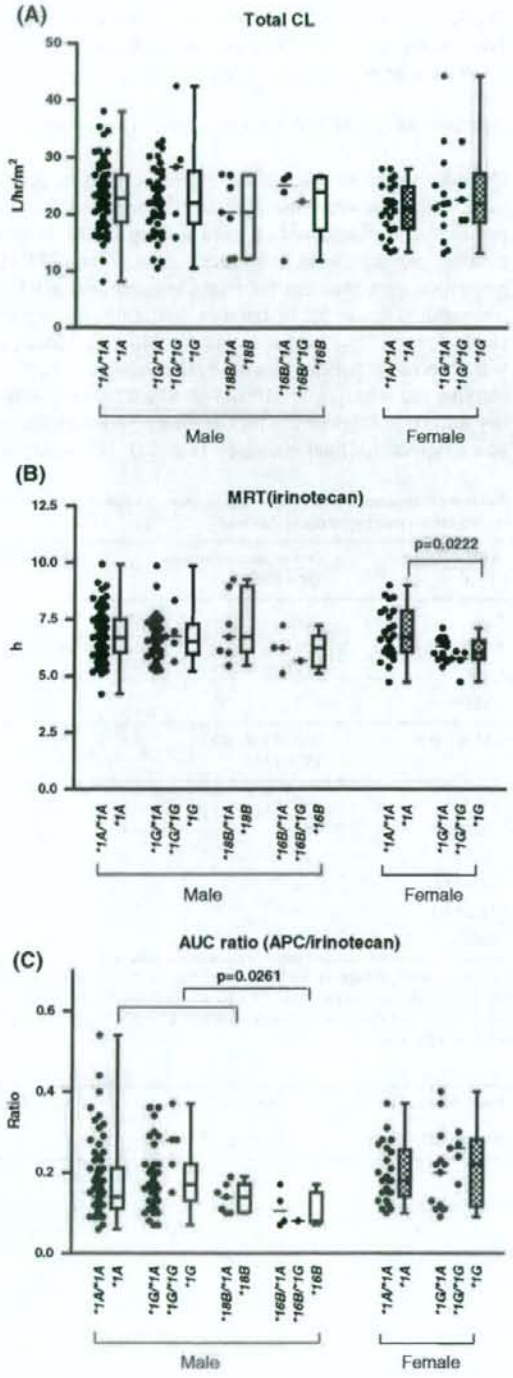
[a] Groups based on tagging SNPs of major haplotypes previously defined [4]; **1A* wild type, **1G* IVS10 + 12G > A; **16B* 554C > G (Thr185Ser) and IVS10 + 12G > A; **18B* 878T > C (Leu293Pro) and IVS10 + 12G > A

Table 2 Pharmacokinetic parameters for irinotecan-administered Japanese patients and sex differences

Parameters	Male ($N = 134$) Median (25–75%ile)	Female ($N = 42$) Median (25–75%ile)	P value[a]
Irinotecan			
Total CL (l/h per m^2)	22.6 (18.5–26.9)	21.8 (17.8–25.1)	0.242
AUC/dose (10^{-3} h m^2 per l)	44.4 (37.3–54.1)	45.8 (39.8–55.8)	0.242
C_{max}/dose (10^{-3} m^2 per l)	10.0 (8.96–11.3)	11.4 (10.4–12.4)	0.0003
MRT (h)	6.61 (6.01–7.40)	6.29 (5.78–7.12)	0.202
APC			
AUC/dose (10 h m^2 per l)	6.72 (5.23–9.49)	8.66 (6.57–13.1)	0.0071
C_{max}/dose (10^{-3} m^2 per l)	0.560 (0.430–0.805)	0.745 (0.610–1.14)	0.0007
AUC ratio (APC/irinotecan)	0.151 (0.114–0.210)	0.194 (0.132–0.266)	0.0179

CL clearance; *MRT* mean residence time

[a] Mann–Whitney test



◀ **Fig. 2** Association of *CYP3A4* genotypes with irinotecan pharmacokinetics in Japanese cancer patients. The values of mean residence time (MRT) of irinotecan in female patients were significantly lower in those with **1G* than those with the wild-type (**1A/*1A*) ($P = 0.0222$, Mann–Whitney test). The levels of the AUC ratio (APC/irinotecan), a parameter of CYP3A4 activity, in male patients were significantly lower in those with **16B* than those without **16B* ($P = 0.0261$, Mann–Whitney test)

differences in C_{max}/dose for irinotecan among the genotypes were observed in both males and females (data not shown). Regarding the AUC ratio (APC/irinotecan) in males, a significantly lower median value (50%) was observed in patients with **16B* than patients without **16B* (i.e., *non-*16B* patients) ($P = 0.0261$, Mann–Whitney test) (Fig. 2c). In contrast, no significant changes in the AUC ratio (APC/irinotecan) were detected in the male **18B* heterozygotes. In both males and females, a higher median AUC ratio (20%), without statistical significance, was observed in **1G*-bearing patients (**1G/*1A* and **1G/*1G*) than wild-type patients (**1A/*1A*). As for C_{max}/dose of APC, similar trends were observed (without statistical significance): 35% decrease in the median value for **16B* compared with *non-*16B*; 10 and 20% increases in males and females, respectively, for **1G* compared with the wild type (data not shown).

Multivariate analysis of PK parameters

To further clarify contributions of the *CYP3A4* polymorphisms to APC generation, multivariate analysis was conducted on the AUC ratio (APC/irinotecan) data, where variables included patient backgrounds, irinotecan regimens, and *CYP3A4* (**1G*, **16B* and **18B*) and *UGT1A1* (**6 or *28*) haplotypes. Significant contributions of *CYP3A4*16B* (coefficient ± SE = −0.18 ± 0.077, $P = 0.021$) and **1G* (0.047 ± 0.021, $P = 0.029$) to the AUC ratio (APC/irinotecan) were confirmed, in addition to the contributions of two patient background factors, sex (female) and hepatic function (serum GOT and ALP) (Table 4). No significant associations were observed between the *CYP3A4* polymorphisms and total clearance or MRT of irinotecan (data not shown).

Associations of *CYP3A4* genotypes with toxicities

Severe irinotecan toxicities, grade 3 diarrhea and grade 3 or 4 neutropenia, were monitored in 176 patients during 2 months after starting irinotecan therapy. Since incidences of severe toxicities depended on the irinotecan regimens used and a higher incidence of severe neutropenia with comedication was evident [22], associations of the *CYP3A4*

Table 4 Multivariate analysis of AUC ratio (APC/irinotecan)

Variable	Coefficient	SE	*P* value
Female	0.040	0.016	0.0132
Serum GOT and ALP[a]	0.110	0.021	<0.0001
Serum creatinine[b]	0.132	0.071	0.0651
*CYP3A4*16B*	−0.180	0.077	0.0213
*CYP3A4*1G*	0.047	0.021	0.0291

The values after logarithmic conversion were used

R^2 0.225; Intercept −0.794; *N* 176

[a] Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment

[b] The absolute value (mg/dl) before irinotecan treatment

haplotypes with toxicities were evaluated in patients who received irinotecan monotherapy. Because there was no sex difference in the incidences of severe toxicities, the patients with irinotecan monotherapy were not stratified by sex. Furthermore, significant contributions of *UGT1A1*6* and **28* to neutropenia were previously demonstrated [22]. Therefore, the incidence of severe neutropenia was also evaluated among the wild-type patients without *UGT1A1*6 or *28* (*UGT −/−*). No significant differences in the incidences of severe diarrhea and neutropenia were observed among the *CYP3A4* diplotypes of all or *UGT −/−* patients with irinotecan monotherapy (Table 5). It must be noted that the **16B*-bearing patient ($N = 1$) treated with irinotecan monotherapy did not experience either toxicity. Similarly, for **1G* and **18B*, no statistically significant change in the neutropenia or diarrhea incidence was observed. Multivariate analysis also revealed no significant contribution of the *CYP3A4* polymorphisms to severe diarrhea (logistic model) or absolute neutrophil count nadir (data not shown).

Table 5 Association of *CYP3A4* genotypes with severe toxicities in irinotecan monotherapy

Diplotype	Diarrhea[a]/total (%)	Neutropenia[b]/total (%)	
	All	All	UGT-/-[c]
**1A/*1A*	3/27 (11.1)	5/27 (18.5)	2/11 (18.2)
**1G/*1A*	2/20 (10.0)	5/20 (25.0)	1/9 (11.1)
**1G/*1G*	0/3 (0.0)	2/3 (66.7)	0/0 (–)
**16B/*1A*	0/1 (0.0)	0/1 (0.0)	0/0 (–)
**18B/*1A*	1/4 (25.0)	2/4 (50.0)	0/1 (0.0)
P value[d]	0.8571	0.289	

[a] Grade 3

[b] Grade 3 or 4

[c] Wild type without *UGT1A1 *6 or *28*

[d] Chi-square test

Discussion

In the current study, the higher in vivo CYP3A4 activity in females than in males [24, 32] was suggested from the CYP3A4-mediated APC formation. Since correlations between in vivo CYP3A4 activity and irinotecan PK parameters have been reported [14, 19, 21], clinical impact of *CYP3A4* polymorphisms on irinotecan PK has been presumed. In this study, we demonstrated for the first time a role of *CYP3A4*16B* [554C > G (Thr185Ser) and IVS10 + 12G > A] in reduced APC generation (Fig. 2; Table 4). This finding is concordant with the findings of our previous studies showing a reduced in vitro activity of CYP3A4 by **16* [23] and altered AUC ratios of metabolite/paclitaxel in paclitaxel-administered Japanese patients bearing **16B* [24]. These findings indicate that *CYP3A4*16* could modulate pharmacokinetics of other drugs which are metabolized by CYP3A4. On the contrary, **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A] did not alter the AUC ratios (APC/irinotecan) in irinotecan-administered patients. This also coincides with our previous finding that showed no clinical impact of **18B* on the metabolite/paclitaxel AUC ratio [24].

In the current study, an increasing trend in the AUC ratios (APC/irinotecan) by **1G* (IVS10 + 12G > A) was detected in both males and females, although their increases were small (20% in the median values). In accordance with this tendency, significant reduction in MRT of irinotecan by **1G* was observed in females, whereas this was not significant in males. At present, the reason of this sex-difference in MRT is not clear. Our previous haplotype analysis of the *CYP3A4* and *CYP3A5* regions revealed that *CYP3A4*1G* is mostly linked to *CYP3A5*1* but rarely to *CYP3A5*3* [3] which is a defective allele [10, 16, 17, 33]. Therefore, there is a possibility that *CYP3A5* polymorphisms rather than *CYP3A4*1G* contribute to irinotecan PK. However, this speculation is unlikely because CYP3A5 produces only a very minor metabolite of irinotecan, a de-ethylated product [27]. Since the effect of **1G* was relatively small and was not shown in case of paclitaxel [23], the clinical importance of **1G* should be further evaluated in pharmacogenetic studies on other drugs.

Contrary to the clear reduction in APC production, changes in the PK parameters for the parent compound, i.e., total clearance and Cmax of irinotecan, were not affected by the *CYP3A4* haplotypes. Furthermore, multivariate analysis revealed no associations of the *CYP3A4* haplotypes with the AUC ratio of (SN-38 + SN-38G)/irinotecan, an in vivo parameter for CES activity, and with the AUC ratio of SN-38 (SN-38/irinotecan) (data not shown). We previously observed that the total clearance of irinotecan was affected by other non-genetic factors, such as age, smoking, hepatic and renal functions, and co-administered drugs

(unpublished data), and that the plasma level of SN-38 was largely influenced by *UGT1A1*6* and **28* [22]. Therefore, it is likely that the contribution of CYP3A4 to irinotecan clearance is rather small as compared with other genetic and non-genetic factors.

In accordance with the above observations, no significant associations were observed between the *CYP3A4* haplotypes and severe toxicities (grade 3 diarrhea and grade 3 or 4 neutropenia) in the patients with irinotecan monotherapy (Table 5). Similarly, we observed no significant effect of the *CYP3A4* haplotypes on incidence of the severe toxicities in the patients treated with both irinotecan and cisplatin (data not shown), although the numbers of patients bearing **16B* and **18B* were small. Taken together, the current study indicates that the influence of the *CYP3A4* genotypes on the activation pathway of irinotecan (generation of SN-38) might be small.

In conclusion, the current study suggested that *CYP3A4*16B* was associated with decreased metabolism of irinotecan to APC. However, impact of the *CYP3A4* haplotypes on total clearance of irinotecan and severe toxicities was not significant.

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CASE REPORT

Impaired spermatogenesis by testicular sarcoidosis

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Impaired spermatogenesis by testicular sarcoidosis
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Abstract: Testicular involvement by sarcoidosis is a rare condition. A 23-year-old Japanese man had asymptomatic bilateral testicular lesions, which were detected by gallium scintigram, together with lesions located bilaterally in the uvea, lungs and hilar, and mediastinal lymph nodes and unilateral supraclavicular lymph nodes. Semen analysis demonstrated severely impaired spermatogenesis. Treatment with corticosteroid dramatically improved these lesions and restored spermatogenesis. This case report suggests that testicular sarcoidosis may cause male infertility.

Key words: infertility, male, sarcoidosis, spermatogenesis, testis.

INTRODUCTION

Sarcoidosis is a chronic, non-caseating granulomatous systemic disease of unknown aetiology that most commonly involves the lymph nodes, lungs, skin, uvea and liver. Although sarcoidosis can involve any organ, involvement of the testis has been infrequently reported. The present report describes a patient with sarcoidosis involving the testes with associated impaired spermatogenesis that was restored by steroid therapy.

CASE REPORT

A 23-year-old Japanese man was diagnosed with bilateral uveitis and secondary glaucoma, suggesting ophthalmic sarcoidosis, 2 weeks prior to referral. On admission, physical examination was normal. Blood counts, liver and renal function tests including urinalysis were normal, except for an elevated serum angiotensin-converting enzyme (SACE) activity of 38.5 IU/L (normal range 7.7–29.4 IU/L), slight elevation of CRP of 0.4 mg/mL and elevated Ig (IgG and IgA, 1725 and 441 mg/mL, respectively). Tuberculin skin test was negative. Pulmonary function tests, arterial blood gas analysis and ECG were normal. CXR revealed remarkable bilateral hilar enlargement and widening of the upper mediastinum. Chest CT confirmed bilateral hilar and mediastinal lymphadenopathy, and small infiltrates in the right upper lobe. A 67-gallium-citrate scintigram showed abnormal accumulations in both eyes, the right supraclavicular region, both hila, the mediastinum and both testes, with additional suspected accumulations in both parotid and submandibular glands (Fig. 1, left). Transtracheal fine-needle aspiration cytology of the mediastinal lymph nodes revealed epithelioid cells. A transbronchial lung biopsy specimen contained only bronchial epithelium. BAL fluid (from the right B^5 bronchus with recovery of 146 mL of 200 mL instilled in four fractions), which was macroscopically normal, contained an increased total cell count of 4.9×10^5/mL and an elevated population of lymphocytes (26%) with an elevated ratio (2.9) of CD4- to CD8-positive lymphocytes. Culture of the specimens obtained by transtracheal fine-needle aspiration and BAL were negative for bacteria including acid-fast bacilli. These findings were consistent with a diagnosis of sarcoidosis.

The testes were normal in size and to palpation, but ultrasonographic images demonstrated hypoechoic lesions within the parenchyma (Fig. 2). Serum α-fetoprotein, human chorionic gonadotropin

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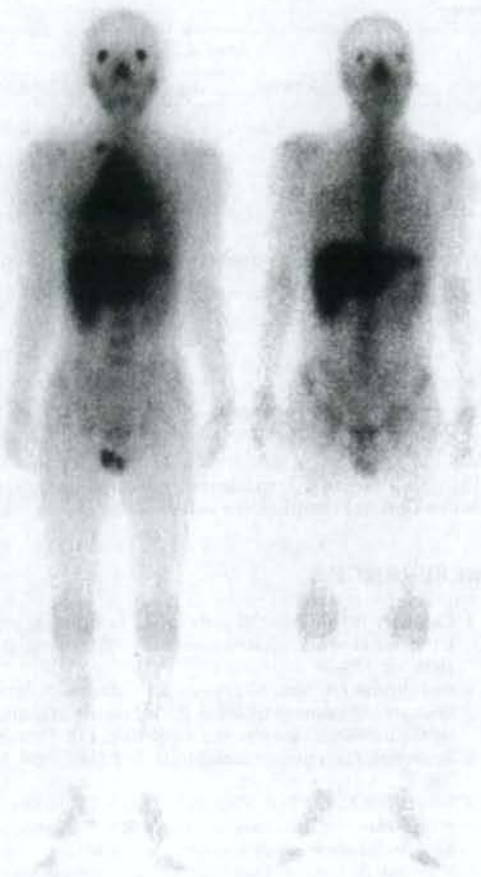

Figure 1 A 67-gallium-citrate scintigram before treatment (left) clearly showed abnormal accumulations in both eyes, both hila, the mediastinum, both testes and the right supraclavicular region. Eight months after the start of prednisolone treatment, accumulations in the mediastinal, hilar and right supraclavicular lymph nodes had disappeared completely, and there was significant decrease in the accumulations in the testes and eyes (right).

β-subunit, luteinizing hormone, follicle-stimulating hormone, prolactin and testosterone were all within the normal range. Semen analysis revealed a decrease in the number and motility of sperm, and an increased population of deformed spermatozoa, suggesting severely impaired spermatogenesis possibly causing male infertility (Table 1).

The ophthalmic symptoms and lesions disappeared dramatically after the topical application of 0.01% betamethasone. Oral prednisolone was started at an initial dose of 60 mg second daily for 3 months, followed by gradual tapering of the dosage. At 2 months, levels of CRP and SACE activity were normal, at 0.1 mg/mL and 15.6 IU/L, respectively. The bilateral hilar and upper mediastinal enlargements on CXR had significantly diminished in size at 3.5 months, and 5.5 months after the start of therapy they had resolved completely. Gallium scintigram 8 months after the start of therapy showed the accumulations in the mediastinal, hilar and right supraclavicular lymph nodes were cleared, and the accumulations in the testes and eyes were significantly decreased (Fig. 1, right). Serial semen analyses also showed gradual recovery of spermatogenesis with therapy (Table 1).

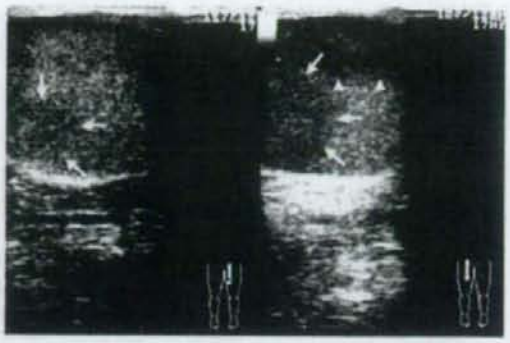

Figure 2 Ultrasonographic views of the right (left) and left (right) testes (sagittal plane) demonstrated hypoechoic lesions in the testicular parenchyma (arrows), suggesting sarcoidosis. Another hypoechoic area (arrowheads) was an artefact.

DISCUSSION

The patient presented with ophthalmic symptoms only, and further investigation supported the diagnosis of sarcoidosis, with testicular involvement. The differential diagnosis for the testicular lesions detected by gallium scintigram and ultrasonography included germ cell tumours, inflammatory diseases and/or epididymides as well as sarcoidosis. Malignant lesions, however, were unlikely because of the bilateral involvement and prompt improvement with steroid therapy, and other inflammatory lesions were also unlikely in the presence of concomitant lesions in the uvea, mediastinal and hilar lymph nodes.

The unique feature of the present case was the involvement of the testes. Intrascrotal sarcoidosis is a very rare condition. Carmody and Sharma, after an extensive review of the English literature, identified 43 patients reported to have this condition. Among these, 24 had testicular involvement, and 12 of these had testicular involvement alone. Of the 24 cases with testicular involvement, 19 patients were black, two were white and the skin colour and/or ethnicity of three was not specified.[1] The typical patient developing testicular sarcoidosis is a middle-aged black

Table 1 Semen analysis before and after the start of steroid therapy

	Before therapy	After starting therapy	
		5 weeks	34 weeks
Dose of oral prednisolone	—	60 mg/2 days	15 mg/2 days
Volume (mL)	4.6	6.6	5.4
Count of spermatozoa ($\times 10^6$/mL)	1.6	24.0	39.0
Deformed spermatozoa (%)	86	34	54
Total motility (%) (a, b, c in %)[†]	20 (0, 7, 13)	43 (7, 22, 14)	48 (8, 17, 23)

[†]Categorized by WHO guideline (1999). Categories a, b and c represent rapid linear, slow/non-linear and non-progressive movement, respectively. Analyses were performed after 4- to 7-day abstinence.
—, not applicable.

man with symptomatic enlarged testes and systemic sarcoidosis including hilar lymphadenopathy. This predominance of testicular lesions in black patients is consistent with the prevalence of sarcoidosis, which is 20 times more common in black people than in white people; the female-to-male ratio is 10.[2] In 1993, Revington[3] reported the first case of sarcoidosis in a patient of Asian extraction, a Bangladeshi, who presented with testicular sarcoidosis, and Toyoshima *et al.*[4] in 2000 reported the first Japanese patient with this rare complication.

The approach to the diagnosis of testicular sarcoidosis is controversial; some authors[5,6] have strongly recommended aggressive diagnostic procedures including scrotal exploration or even orchiectomy because of the rarely reported coincidence of systemic sarcoidosis and testicular malignancy;[7–10] others[2,11] have advocated biopsy by means of inguinal exploration. The present patient's strong wish for offspring in the future, despite his impaired spermatogenesis, prevented the use of invasive diagnostic procedures that might have additional deleterious effects on his fertility. Little is known about the fertility of patients with testicular sarcoidosis. Turk *et al.* stated that it was reasonable to assume that fibrosis and occlusion of the ductus epididymis could cause azoospermia.[11] The present data support this premise. Semen analysis prior to treatment revealed severely impaired spermatogenesis, with dramatic recovery after corticosteroid therapy. Although the mechanism by which spermatogenesis is damaged by testicular sarcoidosis is unclear, the restoration of fertility after only 5 weeks of treatment may indicate impairment at a later stage of spermatogenesis, as the entire process normally requires about 64 days.[12]

A young Japanese man with testicular sarcoidosis accompanied by ophthalmic and thoracic lesions is reported. Gallium scintigram was very useful in detecting the asymptomatic testicular lesions. Testicular sarcoidosis can now be added to the list of causes of male infertility. Chest physicians and infertility specialists need to be aware of this condition.

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ORIGINAL ARTICLE

Increased incidence of interstitial pneumonia by CHOP combined with rituximab

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Abstract Several authors have reported interstitial pneumonia (IP) during rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy, while others have encountered *Pneumocystis jirovecii* pneumonia during rituximab-combined bi-weekly CHOP. Herein, we report that 13 of 90 (14%) patients developed IP during R-CHOP therapy, compared with none of 105 patients treated with CHOP alone as a historical control. There were no differences in baseline data between patients undergoing the two therapies. Among R-CHOP-treated patients, serum β-D-glucan was increased in 8 of 12 (75%) IP patients compared with none of 30 non-IP patients examined. In five IP patients who underwent sputum evaluation, two were positive for *P. jirovecii* by the polymerase chain reaction and another two were positive for *Candida albicans*. No other organisms were detected as causative pathogens. Treatment with steroids, sulfamethoxazole-trimethoprim (ST), and antifungals was effective. Our results suggest that R-CHOP raises the incidence of IP, possibly through increasing the susceptibility to *P. jirovecii* and fungal infection. The need for prophylactic antifungals and ST during R-CHOP should be evaluated by randomized controlled trials.

Keywords Interstitial pneumonia · Rituximab · R-CHOP · β-D-glucan

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1 Introduction

Rituximab, a chimeric mouse–human monoclonal antibody against CD20, is used for monotherapy, as well as combination chemotherapy, against CD20-positive B-cell non-Hodgkin's lymphoma (NHL), and its efficacy has been demonstrated with a low incidence of adverse effects [1]. There have been several case reports of interstitial pneumonia (IP) in patients treated with rituximab, and allergic mechanisms or increased susceptibilities to infection have been suggested [2–7]. Recently, IP has been shown to be caused by *Pneumocystis jirovecii* (*P. carinii* pneumonia, PCP) during treatment with rituximab plus bi-weekly cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP-14) with or without etoposide [8, 9]. These reports indicated that PCP occurred in the immunosuppressive state induced by rituximab or dose-intense agents. However, IP development in rituximab-treated patients has not been systematically analyzed, and its incidence and etiology in such patients therefore remain unclear.

Here, we compared the incidence of IP in 90 B-cell NHL patients treated with rituximab-combined CHOP (R-CHOP) with that in 105 similar patients treated with CHOP alone as a historical control. IP was diagnosed in 13 of 90 patients treated with R-CHOP compared with none of

the 105 patients receiving CHOP alone, and the incidence was significantly higher in R-CHOP-treated patients. The characteristics of IP patients were analyzed, and the possible etiology is discussed.

2 Patients and methods

A total of 90 patients with CD20-positive B-cell NHL undergoing R-CHOP therapy between April 2005 and October 2006 and 105 patients with B-cell NHL who received CHOP therapy between June 2000 and December 2004 at our institute were compared regarding their characteristics and the incidence of IP by *t*-tests and Fischer's exact test. The study protocol and sampling were approved by the Institutional Review Board of the Cancer Institute Hospital. Due to the retrospective nature of this study, written informed consent was not obtained from any patients.

Histological diagnosis was confirmed by an expert hematopathologist (K.T.), and staging was performed according to the Ann Arbor classification. For patients with stages Ib-IV, CHOP chemotherapy was repeated six times in both treatment groups, and a standard dose of rituximab (375 mg/m^2) was administered on day 1 of each cycle for patients who received R-CHOP. For patients with stage Ia, CHOP or R-CHOP chemotherapy was repeated for three cycles and rituximab was continued as for patients with stages Ib-IV, which was followed by radiotherapy. Antifungals and sulfamethoxazole-trimethoprim (ST) were not administered prophylactically to patients treated with either RCHOP or CHOP therapy, because PCP or fungal infection has not been proven to be aggravated by the addition of rituximab to CHOP.

We performed chest and abdominal computed tomography (CT) to evaluate the response of each lymphoma to treatment. Furthermore, chest X-rays and CT were carried out in patients who developed a high fever and dyspnea during or after treatment. IP was diagnosed on the basis of chest CT revealing bilateral diffuse pulmonary interstitial infiltrates, and hypoxia without hypercapnia on arterial blood gas analysis. In order to confirm the diagnosis, peripheral white blood cells (WBCs), serum lactate dehydrogenase (LDH), C-reactive protein (CRP), and Klebsvonden Lungen-6 (KL-6) were assessed in all patients who developed IP. We also monitored the serum immunoglobulin G (IgG) level in patients. As a test for infection, we measured serum β-D-glucan, antigens for *Candida*, *Aspergillus*, *Klebsiella*, and adenovirus, cytomegalovirus (CMV) antigenemia, and antibodies against *Mycoplasma*. Blood and sputum from some patients were cultured. We further analyzed the serum level of β-D-glucan in 30 patients receiving R-CHOP without any symptoms or signs suggesting IP during the same period as a control.

3 Results

Table 1 shows the characteristics of patients who received R-CHOP and CHOP, and the incidences of IP. There were no significant differences in the baseline characteristics, including the median age, sex, distribution of histology, disease stage, or International Prognostic Index between patients receiving the two treatments. IP was diagnosed in 13 of 90 (14.3%) patients treated with R-CHOP and none of 105 patients receiving CHOP. As other lung complications, two cases of bacterial pneumonia and one case of development of tuberculosis were detected in the R-CHOP group, and five cases of bacterial pneumonia in the CHOP group.

All IP cases during R-CHOP were diagnosed as outpatients. Furthermore, 12 of the cases occurred between 60 and 120 days after treatment initiation of (6, 1, 1, and 4 cases were diagnosed during the third, fourth, fifth, and sixth cycles of treatment, respectively). Chest radiographs showed bilateral diffuse ground-glass opacity in all patients. There was no cystic lesion, fibrotic area with honeycombing, or consolidation in air bronchograms. Patients developed severe hypoxia of 46.2–62.1 (mean 51.2) mmHg of PaO_2 without hypercapnia (mean $PaCO_2$; 35.4 mmHg, range 33.0–40.4), but no patients underwent a respiratory function test. No cases had a history of any other lung disease, disease progression nor lung involvement of the lymphoma at the onset of IP (Table 2).

At the onset of IP, the peripheral WBC counts were within the normal range in seven patients, elevated in two

Table 1 The characteristics of patients treated with both R-CHOP and CHOP

	R-CHOP $n = 90$ n (%)	CHOP $n = 105$ n (%)	P-value
Median age	66.5	68	0.2
Sex			
Male	46 (51)	60 (57)	0.4
Female	44 (49)	45 (43)	
Histology			
DLBCL	73 (81)	86 (82)	0.8
FL, others	17 (19)	19 (18)	
Stage			
I–II	48 (53)	56 (53)	0.9
III–IV	42 (47)	49 (47)	
IPI			
L, L-I	64 (71)	62 (59)	0.8
H, H-I	26 (29)	43 (41)	
IP	13 (14)	0	<0.05

R-CHOP rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *IPI* International Prognosis Index, *L* low, *L-I* low-intermediate, *H* high, *H-I* high-intermediate, *IP* interstitial pneumonia

Table 2 Clinicopathological data in 13 patients developing interstitial pneumonia

Case	Age	Sex	Histology	Stage	Duration from start of therapy (days)	Status at onset	Symptom
1	62	F	DLBCL	I A	83	CR	No
2	75	F	DLBCL	I A	89	CR	No
3	83	F	DLBCL	II A	60	PR	Dyspnea
4	81	F	DLBCL	I B	78	PR	Dyspnea
5	67	F	DLBCL	II A	102	CR	Fever
6	60	F	DLBCL	II A	83	CR	No
7	42	M	DLBCL	IV A	158	CR	Fever
8	75	F	DLBCL	II A	78	CR	Fever
9	71	F	DLBCL	IV A	99	NC	Fever
10	64	M	FL	IV A	62	PR	Fever
11	70	M	DLBCL	I A	71	PR	Fever
12	36	F	DLBCL	I A	96	CR	Fever
13	61	M	DLBCL	I A	60	CR	Dyspnea

M male, *F* female, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *CR* complete response, *PR* partial response, *NC* no change, *ND* not done

patients, and low in four patients. In 5 of 13 patients, IP occurred within 10 days of the completion of granulocyte colony-stimulating factor (G-CSF) therapy. Serum LDH levels were above normal in 12 patients, and the KL-6 level was above normal in 6 of 10 patients examined (Table 3).

Among R-CHOP-treated patients, the serum level of β-D-glucan was above normal (~10 pg/ml; range 20.2–130.8 pg/ml) in 8 of 12 (75%) IP patients examined, and negative in all 30 non-IP patients assessed. All IP patients were negative for *Candida* and *Aspergillus* antigens in peripheral blood. Furthermore, 11 patients tested were negative for CMV antigenemia, adenovirus, and *Klebsiella* antigens, and *Mycoplasma* antibodies. In five patients whose sputum was examined, *P. jirovecii* DNA was detected by the polymerase chain reaction in two patients, and *Candida albicans* was detected by culture in another two patients.

In 12 patients with IP, the serum IgG level was monitored every 2 months. Prior to treatment, the median serum IgG level was 1,193 mg/dl (range 832–1,736) and within the normal range in 11 of 12 patients, and the median serum IgG level at the onset of IP was 794.5 mg/dl (range 572–1,082) and below the normal range (820–1,700 mg/dl) in 10 (83.3%) of 12 patients.

For IP treatment, we used steroids, ST, and antifungals in the majority of patients. All patients responded well to treatment, and recovered within 2–3 weeks. The changes in β-D-glucan, CRP, and LDH are shown in Table 4. After recovery from IP, five patients received R-CHOP therapy and four underwent radiotherapy while receiving antifungal and anti-PCP treatment. No patient experienced recurrence of IP.

4 Discussion

Several studies have reported an association between IP and rituximab-combined chemotherapy. However, there have been no prospective studies on this adverse event, and the etiology remains unclear. Herein, we compared the incidence of IP in patients with B-cell NHL treated by R-CHOP with that in patients treated by CHOP alone. There were no cases of IP among patients treated with CHOP, whereas 13 of 90 (14.3%) patients treated with R-CHOP developed IP, although there were no significant differences in baseline data between the two treatment groups.

Among patients treated with R-CHOP examined regarding their serum level of β-D-glucan, it was increased in 8 (cases 4, 5, 6, 7, 9, 10, 12, and 13) of 12 (75%) IP patients compared with none of 30 non-IP patients as a control. Among five IP patients undergoing sputum examination, *P. jirovecii* was positive in two by the polymerase chain reaction and *C. albicans* was positive in another two, one (case 8) of whom was β-D-glucan-negative. G-CSF was suspected to be the cause of IP in case 9 as IP developed during G-CSF administration with a sudden increase in the leukocyte count, as has been reported [10, 11]. In our study, fever and itching were noted during the first rituximab infusion in 6 of 13 patients. Two (cases 1 and 2) of them were negative for β-D-glucan, and, thus, possibly developed rituximab-induced lung injury during the succeeding rituximab infusion, because it has been reported to develop during the second or later administration in patients showing an allergic reaction on the first infusion [4, 7]. In two patients (cases 3 and 11), we did not identify the cause of the IP.

β-D-glucan has emerged as an adjunctive diagnostic measurement for PCP and invasive fungal infection, although an increase in serum β-D-glucan does not directly lead to a diagnosis of PCP or fungal infection [12–14]. In two patients with IP, PCP was diagnosed by detecting *P. jirovecii* DNA in sputum, which is known to be a less sensitive specimen than bronchoalveolar lavage or

Table 3 Clinical, biochemical data in 13 patients developing interstitial pneumonia

Case	Serum beta-D glucan (pg/ml)	Culture of sputum	LDH (U/l)	KL-6 (U/ml)	WBC count at onset (/μl)	Duration from G-CSF therapy	Infusion reaction	Treatment
1	Negative	ND	395	388	1,600	ND	Yes	mPSL 250 mg, FLCZ, ST
2	Negative	ND	445	832	5,500	14 days	Yes	mPSL 250 mg, FLCZ, ST
3	ND	ND	201	ND	1,700	ND	Yes	mPSL 250 mg, FLCZ, ST, CFPM
4	28.7	*C. albicans*	355	755	4,900	3 days	Yes	mPSL 250 mg, MKFG, CFPM
5	20.2	ND	367	390	2,800	4 days	No	mPSL 250 mg, CFPM
6	100.0	ND	329	ND	400	ND	No	PSL 60 mg, FLCZ, ST
7	50.6	Negative	543	335	4,800	4 days	No	MKFG, ST, CFPM, γ-globulin
8	Negative	*C. albicans*	378	ND	7,000	ND	No	VRCZ, CFPM
9	130.8	ND	489	500	12,800	During therapy	No	mPSL 250 mg, FLCZ, ST
10	48.5	PCP	347	421	4,200	ND	Yes	mPSL 250 mg, VRCZ, ST, CFPM
11	Negative	ND	253	686	10,700	ND	No	PSL 30 mg, ST, FLCZ
12	59.2	ND	450	531	6,500	ND	Yes	mPSL 1,000 mg, ST, FLCZ
13	65	PCP	580	748	6,500	4 days	No	mPSL 500 mg, ST, FLCZ

Infusion reaction indicates fever and itching during first rituximab administration

ND not done, *C. albicans Candida albicans*, *PCP Pneumocystis carinii* pneumonia, *WBC* white blood cell (4,000–8,000), *LDH* lactate dehydrogenase (–235), *KL-6* Klebsvonden Lungen-6 (–499), *G-CSF* granulocyte colony-stimulating factor, *PSL* prednisone, *mPSL* methylpredonisolone, *FLCZ* fluconazole, *ST* sulfamethoxazole-trimethoprim, *CFPM* cefepime dihydrochloride, *MKFG* micafungin sodium, *VRCZ* voriconazole

transbronchial lung biopsy. To clearly detect PCP, further investigations are required.

Recently, the incidences of PCP were reported to be 6 and 13% in patients treated with rituximab-combined bi-weekly CHOP with or without etoposide, respectively, while only 2 of 141 (1%) patients developed PCP following the conventional tri-weekly R-CHOP therapy. Although the authors did not clearly demonstrate the severe depletion of blood T lymphocytes, they suggested that dose-intensification and the addition of rituximab to CHOP-like regimens depleted the cellular immune system through the cytostatic effects of dose-intensified steroids and anti-cancer agents on lymphocytes, resulting in the development of PCP [8, 9]. They also showed that the addition of rituximab to CHOP-like regimens, such as CHOP and CHOP-14, with or without etoposide, increased the incidence of PCP [8]. Our results also suggest that the addition of rituximab to CHOP significantly increases the incidence of IP in patients with B-cell NHL, and some cases were possibly caused by *P. jiroveci* and fungal infection.

Table 4 The changes in clinical, biochemical data from onset of interstitial pneumonia to post treatment

Case	β-D-glucan (pg/ml)	CRP (mg/dl)	LDH (U/l)
1	Neg	0.3→0.1	395→230
2	Neg	0.1→0.1	445→213
3	ND	6.1→0.1	201→222
4	28.7→ND	10.3→0.2	355→277
5	20.2→Neg	0.3→0.1	367→233
6	100.0→Neg	1.9→0.1	329→200
7	50.6→Neg	12.4→0.3	543→357
8	Neg	12.9→0.3	378→229
9	130.8→Neg	4.0→1.8	489→211
10	48.5→Neg	10.0→0.1	347→194
11	Neg	1.1→0.1	253→208
12	59.2→Neg	5.6→0.3	261→252
13	65→Neg	0.2→0.1	580→203

Onset→after treatment for interstitial pneumonia

Neg negative, *ND* not done *CRP* C-reactive protein (–0.5), *LDH* lactate dehydrogenase (–235)

The incidence of IP in our study was unexpectedly high. A previous report indicated that lung injury induced by rituximab alone or in combination with chemotherapy account for less than 0.03% among over 300,000 patients worldwide [2]. On the other hand, other authors reported a lung injury rate of 11% during R-CHOP therapy [1], and a study of rituximab plus bleomycin-containing chemotherapy identified an increased incidence of lung injury through adding rituximab to chemotherapy [15]. These studies did not provide details of lung injury and the incidence of IP. Onsets of IP in 12 of 13 cases in our analysis were in an outpatients setting, as for most patients included in other studies of R-CHOP. The reason for this phenomenon was not clear with these limited data, and, therefore, further investigations such as a multi-center study, or epidemiological approach will be necessary.

Another recent study also demonstrated that the rates of fungal infections were increased in patients treated with

R-CHOP compared with patients receiving CHOP alone. Since most of the infected patients were more than 80 years of age, the authors concluded that the addition of rituximab to CHOP increases the risk of fungal infection in a very elderly population [16]. However, the average age of our population was not remarkably higher than those in previous studies on R-CHOP, and the ages of populations in studies on PCP during R-CHOP-14 were similar to that in the current study [8, 9].

In conclusion, 13 of 90 (14%) patients treated with R-CHOP developed IP, compared with none of 105 patients receiving CHOP alone as a historical control. Although the etiology remains unclear, increased susceptibility to *P. jirovecii* and fungal infection is suggested. This suggests the need to administer prophylactic treatment with antifungals and ST during R-CHOP therapy. The possible increase in the incidence of IP should be kept in mind when CHOP is combined with rituximab, since the treatment outcome for IP on the use of steroids, ST, and antifungals was favorable.

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Interstitial Lung Disease in Japanese Patients with Lung Cancer

A Cohort and Nested Case-Control Study

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Rationale: Interstitial lung disease (ILD) occurs in Japanese patients with non–small cell lung cancer (NSCLC) receiving gefitinib.

Objectives: To elucidate risk factors for ILD in Japanese patients with NSCLC during treatment with gefitinib or chemotherapy.

Methods: In a prospective epidemiologic cohort, 3,166 Japanese patients with advanced/recurrent NSCLC were followed for 12 weeks on 250 mg gefitinib (n = 1,872 treatment periods) or chemotherapy (n = 2,551). Patients who developed acute ILD (n = 122) and randomly selected control subjects (n = 574) entered a case-control study. Adjusted incidence rate ratios were estimated from case-control data by odds ratios (ORs) with 95% confidence intervals (CIs) using logistic regression. Crude (observed) incidence rates and risks were calculated from cohort data.

Measurements and Main Results: The observed (unadjusted) incidence rate over 12 weeks was 2.8 (95% CI, 2.3–3.3) per 1,000 person-weeks, 4.5 (3.5–5.4) for gefitinib versus 1.7 (1.2–2.2) for chemotherapy; the corresponding observed naive cumulative incidence rates at the end of 12-week follow-up were 4.0% (3.0–5.1%) and 2.1% (1.5–2.9%), respectively. Adjusted for imbalances in risk factors between treatments, the overall OR for gefitinib versus chemotherapy was 3.2 (1.9–5.4), elevated chiefly during the first 4 weeks (3.8 [1.9–7.7]). Other ILD risk factors in both groups included the following: older age, poor World Health Organization performance status, smoking, recent NSCLC diagnosis, reduced normal lung on computed tomography scan, preexisting chronic ILD, concurrent cardiac disease. ILD-related deaths in patients with ILD were 31.6% (gefitinib) versus 27.9% (chemotherapy); adjusted OR, 1.05 (95% CI, 0.3–3.2).

Conclusions: ILD was relatively common in these Japanese patients with NSCLC during therapy with gefitinib or chemotherapy, being higher in the older, smoking patient with preexisting ILD or poor performance status. The risk of developing ILD was higher with gefitinib than chemotherapy, mainly in the first 4 weeks.

Keywords: non–small cell lung cancer; interstitial lung disease; Japanese patients; gefitinib, chemotherapy

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Acute interstitial lung disease (ILD) occurs in Japanese patients with non–small cell lung cancer (NSCLC) receiving gefitinib. There is, however, limited knowledge about risk factors for ILD and the incidence of ILD in patients with NSCLC receiving other treatments.

What This Study Adds to the Field

Acute ILD was common in Japanese patients with NSCLC receiving chemotherapy or gefitinib, with higher risk for gefitinib. Age, performance status, smoking, and preexisting chronic ILD were also important risk factors, aiding clinicians in treatment selection.

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors are a well-established therapy for the treatment of non–small cell lung cancer (NSCLC) in many countries. They are generally well tolerated and not typically associated with the cytotoxic side effects commonly seen with chemotherapy.

The EGFR tyrosine kinase inhibitor gefitinib (IRESSA; AstraZeneca, London, U.K.) was first approved for the treatment of advanced NSCLC in Japan in July 2002. In clinical trials and in preapproval compassionate clinical use, some reports of interstitial lung disease (ILD)–type events had been observed. As the drug was made more widely available in Japan after approval, however, an increasing number of spontaneous reports for ILD appeared.

ILD is a disease that affects the parenchyma or alveolar region of the lungs (1). When associated with drug use, it can present precipitously with acute diffuse alveolar damage, which is fatal in some patients (2). Chest imaging shows ground-glass density and patients present with severe breathlessness. There is no specific treatment, but supportive therapy including oxygen, corticosteroids, or assisted ventilation is indicated. Acute exacerbations of ILD have previously been considered relatively rare in many settings, with Japan as a notable exception (3), but recent studies of patients with idiopathic pulmonary fibrosis (IPF) have challenged this and underlined this important risk (4).

ILD, especially IPF, is a known comorbidity in patients with NSCLC and has also been associated with many other lung cancer therapies (5). Rates of acute ILD events up to and exceeding 10% have been reported in patients receiving chemotherapy and radiotherapy (6–11). It is recognized that ILD is more common in Japan than elsewhere (5, 6, 12, 13).

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When safety reports of acute ILD-type events in gefitinib-treated patients appeared in Japan, there was limited knowledge about ILD in patients with NSCLC. There was a need to better understand baseline incidence on different treatments, risk factors for developing ILD, and whether gefitinib might be associated with increased risk of ILD, or if patient selection or other aspects were involved. A pharmacoepidemiologic study was designed and conducted by an independent academic team together with scientists from AstraZeneca to define the risk and increase understanding of ILD in Japanese patients with NSCLC. Some of the results of this study have been previously reported in the form of conference abstracts (14, 15).

METHODS

See also the online supplement for further details on methods.

Overall Study Design

A nonrandomized cohort study with a nested case-control study component was conducted between November 12, 2003, and February 22, 2006, in 50 centers across Japan. Patients with advanced or recurring NSCLC who had received at least one chemotherapy regimen were eligible for cohort entry. Patients and their physicians selected the most appropriate treatment (gefitinib 250 mg or chemotherapy) and the patients were followed for up to 12 weeks after treatment initiation. Basic data were collected at the start of follow-up and included sex, age, World Health Organization (WHO) performance status (PS), and tumor histology. If a patient switched to a new treatment, he or she could be re-enrolled for a new treatment period of 12 weeks, provided he or she was still eligible.

Patients who developed acute ILD events during the follow-up were registered to the case-control study nested within the cohort as clinically diagnosed potential cases. For each potential case, four patients who had not yet developed ILD were randomly selected as appropriate control subjects from patients registered to the cohort at that time, and extensive clinical and demographic risk factor data were collected on cases and control subjects (*see* Figure E1 in the online supplement).

The study followed Good Clinical Practice procedures. An independent external epidemiology advisory board provided advice on design, conduct, and analysis of the study.

Diagnosis of ILD

To ensure an accurate diagnosis of ILD, several study design components were implemented: (*1*) an information card to all cohort patients, alerting them to the symptoms of ILD; (*2*) internationally agreed criteria for the diagnosis of ILD and a diagnostic algorithm (*see* Figure E2) developed from the American Thoracic Society/European Respiratory Society consensus statement (1); and (*3*) a blinded diagnostic review of all clinically diagnosed potential ILD cases registered to the study by an independent case review board (CRB) of radiologists and clinicians.

Evaluation of Preexisting Lung Conditions

The CRB also blindly evaluated pretreatment computed tomography (CT) scans for the presence of a number of pulmonary conditions: preexisting (chronic) ILD (mainly IPF), drug-induced lung disease, pulmonary emphysema, radiation pneumonitis, lymphangitis carcinomatosis, and healed tuberculosis, and evaluated the extent of normal lung, as well as the extent of areas adherent to pleura.

Detailed Data Collection

For cases and control subjects, detailed data on NSCLC treatment, demography, cancer histology, clinical stage and the presence of metastases, WHO PS, smoking, previous cancer treatments, past and current medical history, surgical history, and concomitant medication and therapy were collected. Data on serious adverse events (SAEs) and hence all-cause mortality were collected for the gefitinib-treated patients in the cohort only; thus, information on mortality from causes other than ILD in chemotherapy-treated patients is not available from this study.

Statistical Analysis

From cohort data, we estimated observed person-time incidence rates as well as two measures of the observed "risk" of acute ILD to a patient: a naive estimate of observed cumulative incidence (incidence proportion, "frequency"), and risk up to 84 days by the Kaplan-Meier method.

Control subjects for the nested case-control study were sampled using incidence density sampling, and consequently the odds ratio (OR) obtained from the case-control analysis estimates the study incidence rate ratio (and approximately estimates the risk ratio) (16).

For the case-control statistical analysis, it was initially verified that the convenience matching for calendar time implicit in the risk set control sampling could be disregarded. In tabular analyses, we then identified potential confounders and risk factors, using as selection criteria a 10% change in the OR estimate for gefitinib versus chemotherapy treatment when stratifying for each factor separately, and a risk factor crude OR of less than 0.5 or more than 2.0, respectively. We also identified potential interactions between treatment and other risk factors, or between two potential risk factors. Modeling using logistic regression then proceeded in the corresponding four steps. Few previous data were available on risk factors for ILD in patients with NSCLC and so a hypothesis-free stepwise process with loose P value criteria ($P < 0.20$) for selection was used throughout to avoid bias.

Two sensitivity analyses were performed. First, to investigate the potential influence of the modeling approach used, a propensity score analysis was performed (17). This analysis provides an alternative way of adjusting for potential confounding bias by stratifying for a compound score based on predictors of treatment (*see* online supplement for details). Second, we estimated the possible bias due to misclassification of disease under reasonable assumptions of diagnostic error.

ILD-related mortality among the patients who developed acute ILD on gefitinib or chemotherapy treatment was obtained. Modeling of risk factors for ILD-related mortality followed a similar process to the ILD risk factor modeling. For gefitinib-treated patients, two additional data items were available: total all-cause mortality, which was analyzed by the Kaplan-Meier method, and SAEs, for which frequencies and possible consequences in terms of treatment discontinuation and death were calculated.

RESULTS

Cohort Subjects and Treatments

Cohort participation rates were high. In 10 sampled study centers, 89.6% of eligible patients were enrolled to the cohort. The number of treatment periods and subjects are summarized in Table 1. In total, 4,423 treatment periods in 3,159 subjects were available for analysis. In the cohort, 70.8% of patients had only one treatment period, 21.5% had two periods, and the remaining 7.8% of patients had three or more treatment periods registered (Table 1). Chemotherapy included a wide range of treatments, the most common being taxane monotherapy, followed by taxane+platinum and gemcitabine+vinorelbine combinations.

Cases and Control Subjects

In the overall cohort data of all treatment periods, clinicians reported 155 suspected cases of acute ILD during the follow-up, of which 122 were confirmed by the CRB after blinded review of CT and clinical data—79 of 103 gefitinib-treated (76.7%) and 43 of 52 chemotherapy-treated (82.7%) subjects. A total of 574 eligible control subjects were sampled from the person-time of the cohort. Almost all ILD cases and selected control subjects consented to participate in the nested case-control study, with final participation rates of 98.1 and 92.0%, respectively. Valid data from the CRB review of CT scans were available for 115 cases and 520 control subjects.

Descriptive Data

On data items available for the full cohort (sex, age, WHO PS, and tumor histology), the control subjects were quite represen-

TABLE 1. NUMBER OF TREATMENT PERIODS AND SUBJECTS IN THE COHORT AND NUMBER OF CASES AND CONTROLS IN THE NESTED CASE-CONTROL STUDY

	Gefitinib (*n*)	Chemotherapy (*n*)	Total (*n*)
Treatment periods registered to cohort	1,901	2,572	4,473
No treatment administered	9	15	24
Ineligible subjects	6	6	12
Protocol deviations	14	0	14
Per-protocol study cohort (treatment periods)	1,872	2,551	4,423
Subjects in cohort (first treatment periods)*	1,489	1,677	3,166
No. of subjects and order of treatment periods registered to the cohort			
1 treatment period: G			1,199
1 treatment period: C			1,036
2 treatment periods: GC			194
2 treatment periods: CG			248
2 treatment periods: CC			228
2 treatment periods: GG			9
3–8 treatment periods†: initial G			81
3–9 treatment periods‡: initial C			166
First gefitinib treatment periods total§	1,849		
Confirmed cases‖	79	43	122
Rejected cases‖	24	9	33
Control subjects	252	322	574

Definition of abbreviations: C = chemotherapy; G = gefitinib.

* Counts the first registered treatment period for each subject.

† 70% of these with three periods.

‡ 78% of these with three periods.

§ Counts the first gefitinib treatment period for all subjects with one or more gefitinib treatment registrations to the cohort; also when their very first registration was for chemotherapy.

‖ Cases registered by clinical investigators to the case-control study and subsequently confirmed or rejected by the case review board (blinded review of case diagnostic data).

tative of the overall cohort (details not shown). Comparisons of the gefitinib- and chemotherapy-treated control groups as representative of the cohort indicated that the former included more women, never-smokers, adenocarcinoma tumors, and poorer PS, as well as less preexisting ILD and pulmonary emphysema on CT scan (Tables 2 and 3). ILD cases, regardless of treatment, were more likely than cohort control subjects to be older, male, smokers, with squamous cell carcinoma histology, and have poor PS (Tables 2 and 3). The frequency of preexisting ILD and pulmonary emphysema was higher in cases, reflected also in a lower extent of normal lung on CT scan.

Cohort Analysis of ILD Occurrence

The observed incidence rate of acute ILD over the entire 12-week follow-up in the overall cohort was 2.8 per 1,000 person-weeks—4.5 in the gefitinib-treated and 1.7 in the chemotherapy subcohort (Table 4). The observed incidence in the gefitinib-treated subcohort was highest in the first 4 weeks after starting treatment, greater than in the chemotherapy-treated subcohort. In the following two 4-week periods, the incidence was lower with no clear difference (Table 4, Figure 1A). The naive cumulative incidence of ILD at 84 days (i.e., observed frequency or proportion of the original cohort that developed ILD in the study) for patients in their first study treatment period was 4.0 and 2.1% for gefitinib- and chemotherapy-treated patients, respectively (Table 4), whereas the estimated theoretical 12-week risk of ILD (i.e., taking competing causes of death and loss to follow-up into consideration; Kaplan-Meier method) was 4.5 and 2.4%, respectively (Table 4, Figure 1B). Thus, the observed cohort rates and risks suggested an association of increased ILD occurrence with gefitinib treatment mainly in the first 4 weeks after treatment initiation. All cohort estimates are unadjusted for imbalances between treatments in other risk factors. Detailed comparisons between the treatments therefore used the adjusted case-control OR (as an estimate of the adjusted incidence rate ratio) to achieve comparability.

Case-Control Analysis of ILD Occurrence and Risk Factors

Major results. The OR of developing acute ILD with gefitinib treatment versus chemotherapy, adjusted for the full predictor model of major confounders together with additional identified important risk factors and interactions, was 3.2 (95% confidence interval [CI], 1.9–5.4) (Table 5). Several risk factors aside from treatment also had strong effects, including WHO PS, as well as smoking status and preexisting ILD together with the extent of normal lung on CT scan, which interacted in a complex way in the model (Table 5, Figure 2). Preexisting ILD was confirmed as a strong risk factor, with OR point estimates ranging from 4.8 to 25.3 depending on the extent of remaining normal lung on CT scan, in comparison with patients without preexisting ILD and high extent of normal lung on CT scan (Table 5). The full set of ILD risk factors in both groups from the final model thus included older age (≥55 yr), WHO PS (≥2), smoking, short duration since NSCLC diagnosis (<6 mo), reduced extent of normal lung on CT scan (<50%), preexisting ILD, and concurrent cardiac disease. Although some potential significant interactions were seen in the initial tabular analyses (Table E1), no significant interactions with treatment (i.e., treatment-specific risk factors, or variation in treatment-related effect in subgroups defined by another risk factor) were identified in the modeling after adjustment for the relevant risk factors.

When the case-control analyses focused on the first 4 weeks after treatment initiation (because the unadjusted cohort analyses above indicated that the bulk of the association with gefitinib appeared to be for this time interval) the estimated OR adjusted for a full predictor model developed on this period's data was 3.8 (95% CI, 1.9–7.7). The same model produced an OR for Weeks 5–8 of 1.6 (95% CI, 0.5–4.8), whereas the final 4-week period had too few cases for an adequate estimate. The estimate for Weeks 5–12 combined, using this same model, was 2.5 (95% CI, 1.1–5.8). The important covariates and predictors were the same in this model as in the model for the full 12-week data, with the exception of age, preexisting cardiac disease, and preexisting pulmonary emphysema, which were not included. Due to sparse data beyond 4 weeks, independent models for Weeks 5–8, 9–12, and 5–12 could not be developed.

Confounding and sensitivity analysis. In the overall 12-week basic analysis, moderately strong confounding by other risk factors was found. The crude OR of developing ILD with gefitinib treatment versus chemotherapy was 2.3 (95% CI, 1.5–3.6). When adjusted for some of the most important potential confounders one at a time, the adjusted OR point estimate for the association of treatment with ILD occurrence ranged from 2.1 to 3.1 (*see* Table E1 for details). The most important confounder was severity of preexisting ILD with strong negative confounding, and the only one that resulted in a lower adjusted OR than 2.3 (positive confounding) was WHO PS.

The propensity score analysis approach identified the following variables as the most important predictors of selecting gefitinib treatment in this study: female sex; nonsmoking status; non–squamous tumor histology; poor PS; preexisting lymphangitis carcinomatosis; no previous gefitinib treatment; and no preexisting ILD, emphysema, or radiation pneumonitis. The

TABLE 2. CHARACTERISTICS OF CONFIRMED CASES AND CONTROL SUBJECTS (AS A RANDOM SAMPLE OF THE STUDY COHORT)

	Cases ($n = 122$)	Controls ($n = 574$)	Gefitinib Control Sample ($n = 252$)	Chemotherapy Control Sample ($n = 322$)
Sex				
Male	92 (75.4)	360 (62.7)	126 (50.0)	234 (72.7)
Female	30 (24.6)	214 (37.3)	126 (50.0)	88 (27.3)
Age				
<55 yr	11 (9.0)	95 (16.6)	43 (17.1)	52 (16.1)
≥55 yr	111 (91.0)	479 (83.4)	209 (82.9)	270 (83.9)
WHO performance status				
0	18 (14.8)	154 (26.8)	68 (27.0)	86 (26.7)
1	69 (56.6)	358 (62.4)	148 (58.7)	210 (65.2)
2–3	35 (28.7)	62 (10.8)	36 (14.3)	26 (8.1)
Histologic type				
Squamous cell carcinoma	29 (23.8)	103 (17.9)	27 (10.7)	76 (23.6)
Adenocarcinoma	80 (65.6)	414 (72.1)	207 (82.1)	207 (64.3)
Others	13 (10.7)	57 (9.9)	18 (7.1)	39 (12.1)
Smoking history				
No	21 (17.2)	192 (33.4)	113 (44.8)	79 (24.5)
Yes	100 (82.0)	382 (66.6)	139 (55.2)	243 (75.5)
Unknown	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
Time since diagnosis of NSCLC				
<0.5 yr	49 (40.2)	153 (26.7)	65 (25.8)	88 (27.3)
0.5 to <1 yr	36 (29.5)	154 (26.8)	67 (26.6)	87 (27.0)
≥1 yr	37 (30.3)	267 (46.5)	120 (47.6)	147 (45.7)
Previous gefitinib treatment				
No	113 (92.6)	465 (81.0)	241 (95.6)	224 (69.6)
Yes	9 (7.4)	109 (19.0)	11 (4.4)	98 (30.4)
Concurrent cardiac disease				
No	111 (91.0)	556 (96.7)	244 (96.4)	312 (96.9)
Yes	11 (9.0)	19 (3.3)	9 (3.6)	10 (3.1)

Definition of abbreviations: NSCLC = non–small cell lung cancer; WHO = World Health Organization.
Values shown are numbers (%).

estimated OR of developing ILD for gefitinib treatment when stratifying by the propensity score was 3.3 (95% CI, 1.9–5.5), very similar to the primary result, suggesting that the primary regression modeling approach well captured the confounding in the data.

If some misclassification of ILD diagnosis remains despite the design features aimed to minimize it, the adjusted OR point estimate of 3.2 may apart from random variation be subject to systematic bias. A sensitivity analysis to evaluate the possible magnitude of such bias due to misclassification of ILD diagnosis suggested that the true study point estimate for the adjusted OR would be expected to lie between 2.6 and 4.8, assuming diagnostic sensitivity of more than 80% for both gefitinib- and chemotherapy-treated patients, and specificity of more than 99.0% for gefitinib and more than 99.5% for chemotherapy. Lower values for sensitivity/specificity were considered very unlikely for this serious condition in a cancer patient population, in this study setting.

Analysis of ILD Mortality

Mortality due to ILD among gefitinib- or chemotherapy-treated patients. The mortality due to ILD for the patients who developed acute ILD was 31.6% (95% CI, 21.6–43.1) among gefitinib-treated patients and 27.9% (95% CI, 15.3–43.7) among those with other treatments; the OR was 1.05 (95% CI, 0.3–3.2) for gefitinib versus chemotherapy, adjusted for relevant risk factors. Several other factors were strong predictors of a fatal outcome for patients with ILD, including age of 65 years or older, smoking history, preexisting ILD, CT scan evidence of reduced normal lung (≤50%), and/or extensive areas adherent to pleura (≥50%), with ORs ranging from 2.4 to 11.7 (*see* Table E2).

Overall mortality among gefitinib-treated patients. In the gefitinib-treated cohort in whom such data were available, an analysis of mortality from all causes by the Kaplan-Meier method showed that cumulative mortality at 12 weeks among the patients who did develop ILD was 58.7%, compared with 14.6% (95% CI, 12.8–16.3) among the large majority who did not develop ILD (Figure 3). For the entire gefitinib cohort, including the subjects who developed ILD, the observed cumulative mortality was 16.0% (95% CI, 14.3–17.8), so that the increased mortality in ILD cases impacted the total survival rate at 12 weeks in the overall gefitinib-treated cohort only to a limited extent, reducing survival from 85.4 to 84.0%.

SAEs among Gefitinib-treated Patients

SAEs were only collected for gefitinib-treated patients in the cohort, and a total of 198 patient registrations reported SAEs (10.5%), of which 38 (2.0%) reported SAEs resulting in a fatal outcome. Within this group, there were 142 patient registrations with drug-related (as reported by the physicians) SAEs (7.5%), of which 30 (1.6%) resulted in a fatal outcome. The majority of these (25 out of 30) were due to ILD-type events. There were 122 patient registrations where study treatment was discontinued due to the reported SAEs (6.5%). SAEs seen in the gefitinib-treated patients were generally consistent with the known safety profile of gefitinib and/or the patient's underlying disease and comorbidities.

DISCUSSION

This study provides important information on ILD in an advanced/recurrent NSCLC setting in Japanese patients in Japan, and it is the largest prospective study of this condition