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Population Pharmacokinetics and Adverse Events of Erlotinib in Japanese Patients with Non-small-cell Lung Cancer: Impact of Genetic Polymorphisms in Metabolizing Enzymes and Transporters

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Determinants of interindividual variability in erlotinib pharmacokinetics (PK) and adverse events remain to be elucidated. This study with 50 Japanese non-small-cell lung cancer patients treated with oral erlotinib at a standard dose of 150 mg aimed to investigate whether genetic polymorphisms affect erlotinib PK and adverse events. Single nucleotide polymorphisms (SNPs) in genes encoding metabolizing enzymes (CYP1A1, CYP1A2, CYP2D6, CYP3A4, CYP3A5, UGT1A1, UGT2B7, GSTM1, and GSTT1) or efflux transporters (ABCB1, and ABCG2) were analyzed as covariates in a population PK model. The ABCB1 1236C>T (rs1128503) polymorphism, not ABCB1*2 haplotype (1236TT–2677TT–3455TT, rs1128503 genotype TT–rs2032582 TT–rs1045642 TT), was a significant covariate for the apparent clearance (CL/F), with the TT genotype showing a 29.4% decrease in CL/F as compared with the CC and the CT genotypes. A marginally higher incidence of adverse events (mainly skin rash) was observed in the TT genotype group; however, patients with high plasma erlotinib exposure did not always experience skin rash. None of the other SNPs affected PK or adverse events. The ABCB1 genotype is a potential predictor for erlotinib adverse events. Erlotinib might be used with careful monitoring of adverse events in patients with ABCB1 polymorphic variants.

Key words erlotinib; population pharmacokinetics; genetic polymorphism; adverse event

Erlotinib is an epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitor, for which a daily dose of 150 mg has been shown to be effective for improving overall survival in non-small-cell lung cancer (NSCLC) patients who have failed at least 1 prior chemotherapy regimen.1,2) Furthermore, erlotinib demonstrated significant prolongation of progression-free survival versus standard chemotherapy in EGFR mutation–positive NSCLC patients.2,3) Based on these results, erlotinib monotherapy has received regulatory approval in over 100 countries, including countries in Europe, the United States, and Japan.

Erlotinib comes in tablets of 3 strengths (150, 100, 25 mg), and individual dose reduction following adverse events is recommended.4,5) Skin rash and diarrhea are the most common adverse events, and these lead to discontinuation of therapy or dose reduction in many patients. It is reported in the U.S. package insert that skin rash occurred in 75% of patients receiving erlotinib as second- or third-line treatment for NSCLC,6) and skin rash was observed in 98% of Japanese patients in clinical studies.5,7) The incidence of skin rash is reported to be related to erlotinib exposure6); therefore, a search for a dose regimen that has adequate efficacy while avoiding rash would greatly contribute to the improvement of a patient’s QOL. Diarrhea is a dose limiting toxicity and is probably related to drug-induced EGFR inhibition.8) Hepatotoxicity is also a serious side effect, and precautions regarding hepatotoxicity are mentioned in the package insert in the United States and Japan.

Erlotinib is metabolized in the human liver, primarily by the CYP isoforms 3A4, 3A5, 1A1, and 1A2.11–13) Erlotinib has been identified as a substrate of the ATP-binding cassette (ABC) transporters ABCB1 (P-glycoprotein/MDR1) and ABCG2 (BCRP).14) The concentration of erlotinib in plasma has been shown to display large interindividual variability in clinical studies. Several population pharmacokinetics (PK) analyses have found that total bilirubin, alpha 1-acid glycoprotein, and smoking status affect its apparent clearance (CL/F).9,15) Studies have also focused on polymorphisms of the CYP and ABC genes. Rudin et al.16) and Phelps et al.17) reported that polymorphisms of CYP3A4 and CYP3A5 tend to affect erlotinib CL/F. ABCG2 polymorphism has also been reported to influence erlotinib CL/F.18) Our previous study showed that the ABCB1*2 haplotype (1236TT–2677TT–3455TT, rs1128503 genotype TT–rs2032582 TT–rs1045642 TT), was a significant covariate for the apparent clearance (CL/F), with the TT genotype showing a 29.4% decrease in CL/F as compared with the CC and the CT genotypes. A marginally higher incidence of adverse events (mainly skin rash) was observed in the TT genotype group; however, patients with high plasma erlotinib exposure did not always experience skin rash. None of the other SNPs affected PK or adverse events. The ABCB1 genotype is a potential predictor for erlotinib adverse events. Erlotinib might be used with careful monitoring of adverse events in patients with ABCB1 polymorphic variants.

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genotype TT–rs1045642 genotype TT) impacted on erlotinib exposure in 28 Japanese patients. However, these results are inconsistent and none of those analyses clearly explains the determinants of the large interindividual variability in the plasma concentration of erlotinib.

Here, with the aim of developing an erlotinib dose regimen that would minimize its adverse effects, we analyzed single nucleotide polymorphisms (SNPs) of the PK-related genes CYP1A1, CYP1A2, CYP3A4, CYP3A5, ABCB1, and ABCG2. We also analyzed CYP2D6 since erlotinib is partially metabolized by CYP2D6. UDP glucuronosyltransferase (UGT) 1A1 and UGT2B7 were analyzed since erlotinib inhibits these enzymes thus erlotinib is possibly metabolized by these enzymes. Glutathione-S-transferase mu1 (GSTM1) and theta1 (GSTT1) genes which are reported to play important roles in hepatotoxicity were analyzed since hepatotoxicity is a serious side effect of erlotinib. We selected all known SNPs before study initiation. We then examined the effect of these genetic polymorphisms on the PK of erlotinib by using population PK analysis. Furthermore, the relationships between these genotypes and adverse effects of erlotinib are reported here.

METHODS

Patients and Study Design  Fifty Japanese patients with NSCLC who were treated with oral erlotinib at a standard dose of 150 mg were enrolled in a prospective clinical study. All patients had failed at least 1 prior chemotherapy regimen, and they were treated with erlotinib as a second-line or later treatment. Erlotinib was administered once daily in the morning and the dosage was not modified during the treatment period. Erlotinib treatment was continued until disease progression was observed, unacceptable adverse event became apparent, or the patient refused to continue receiving treatment. Co-medications which were known to affect erlotinib PK were prohibited during study treatment. This study was reviewed and approved by the relevant institutional review boards and signed informed consent was obtained from all patients prior to participation. These procedures were in accordance with the Helsinki Declaration.

Blood samples were collected from 28 patients at 5 to 18 time points (serial sampling at 0, 2, 4, 6, 8, 12, and 24 h after administration on day 1 and 8, and trough sampling at day 15, 29, and later), and trough blood samples at steady state (day 8 or later) were collected from 20 patients at a single time point.

Adverse events were assessed using the Common Terminology Criteria for Adverse Events (CTCAE version 3.0). Since adverse events might be confounded by the number of treatment periods, the grade of erlotinib-induced adverse event was determined during the first 30 days.

Genetic Polymorphism  Genomic DNA samples from 50 patients were obtained from whole blood by using a MagNA Pure LC DNA Isolation Kit I Tissue (Roche Diagnostics, Mannheim, Germany). Genotyping was then carried out using the following methods.

Applied Biosystems TaqMan SNP Genotyping Assay

Detailed explanations of the genotyping methods used in this study have been described in a previous report. The variant genotypes in Supplementary Material 1 were determined by using real-time quantitative PCR (qRT-PCR) on an ABI PRISM 7900HT system (Applied Biosystems, Foster City, CA, U.S.A.).

Direct Nucleotide Sequencing

The genotyping assays for the genes listed in Supplementary Material 2 were performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit with an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Primer sets for each gene are listed in Supplementary Material 2.

PCR-Restriction Fragment Length Polymorphism (RFLP)

The gene alleles listed in Supplementary Material 3 were genotyped. DNA fragments containing the polymorphic sites were amplified by PCR using the appropriate forward and reverse primers (Invitrogen LIFE Technologies Japan, Tokyo, Japan) (Supplementary Material 3). The products were then digested with restriction enzymes. The digested products were analyzed by electrophoresis on a 2% agarose gel in the presence of ethidium bromide. Genotype was determined based on individual fragment lengths.

Multiplex PCR

Deletions of GSTM1 and GSTT1 genes were analyzed by using multiplex PCR. The primers used are described in Supplementary Material 4. The PCR products were analyzed by electrophoresis on a 2% agarose gel in the presence of ethidium bromide. PCR products of 273, 480, and 493 base pairs indicated the presence of GSTM1, GSTT1, and β-actin, respectively.

Population Pharmacokinetics Model Development Software

The population pharmacokinetics analysis of erlotinib was performed using a nonlinear mixed-effect model approach with NONMEM software (Version 7.2.0, ICON Development solutions, Dublin, Ireland.) All analyses were performed using the first-order conditional estimation method (FOCE) with interaction. R (version 3.1.1) was used for handling of data and results.

Pharmacokinetics Model

Blood samples were centrifuged and the plasma was frozen at −80°C until analysis. The concentrations of erlotinib in plasma were determined by using HPLC. Determination of the structural model was as described below. Model selection was based on changes in the objective function value (OFV; $p<0.05$). The difference in the OFV obtained by comparing each model is assumed to be asymptotically chi-squared distributed with degrees of freedom equal to the difference in the number of parameters between the 2 models.

Structural models

The following models were tested.

Model 1: One-compartment model with first-order absorption and elimination.

Model 2: Two-compartment model with first-order absorption and elimination.

Model 3: Two-compartment model with first-order and lag-time absorption and elimination.

Model 4: Two-compartment model with zero-order absorption and elimination.

Model 5: Two-compartment model with first-order and zero-order absorption and elimination.

Error model

An exponential model Eq. 1 was evaluated to describe the individual variabilities of pharmacokinetic parameters:

$$\theta_i = \theta \times e^{\eta_i}$$

(1)

where $\theta_i$ represents the parameters of the $i$th subject, $\theta$ repre-
sents the population mean value of the parameter, and \( \eta_i \) is the random effect of the \( i \)th subject on the assumption of a normal distribution with a mean value of 0 and variance of \( \sigma^2 \).

A proportional error model Eq. 2 and a mixed error model Eq. 3 were tested as equations for the residual error:

\[
C_{ij} = PRED_{ij} \times (1 + \epsilon_{ij}) \tag{2}
\]

\[
C_{ij} = PRED_{ij} \times (1 + \epsilon_{ij}) + \epsilon_{ij} \tag{3}
\]

where \( C_{ij} \) represents the \( j \)th observed concentration of the \( i \)th subject, \( PRED_{ij} \) represents the \( j \)th predicted concentration of the \( i \)th subject, and \( \epsilon_{ij} \) is the random effect of the \( j \)th observed concentration of the \( i \)th subject on the assumption of a normal distribution with a mean value of 0 and variance of \( \sigma^2 \).

Covariates of Pharmacokinetic Parameters

The influence of the covariates listed in Tables 1 and 2 was examined. Actual body weight before treatment initiation (listed in Table 1) was used as body weight. EGFR mutation status was excluded since no pharmacokinetic interaction was expected. Smoking status was excluded because most patients did not smoke during the study. In addition, ABCB1 1236C>T (rs1128503) was examined as C/C plus C/T vs. T/T. ABCB1 2677G>T(A) (rs2032582) was examined as T/T vs. the others. The influence of continuous covariates was modeled according to Eq. 4, and that of categorical covariates was modeled according to Eq. 5:

\[
PARCOV = PAR \times (1 + \theta_{cov} \times (\text{Cov} - \text{Med}_{cov})) \tag{4}
\]

\[
PARCOV = \begin{cases} 
PAR (\text{Cov} = 0) \\
PAR \times (1 + \theta_{cov1} \times (\text{Cov} = 1)) \\
PAR \times (1 + \theta_{cov2} \times (\text{Cov} = 2)) 
\end{cases} \tag{5}
\]

where PAR is the population mean value of the parameter, Cov is the value of the covariate, \( \theta_{cov} \) represents the slope of the effect of the covariate, PARCOV represents the individual estimate of the parameter, and \( \text{Med}_{cov} \) represents the median value of the covariate in the study population. Investigation of the covariate effects on the CL/F was undertaken using step-wise covariate modeling.\(^{31}\) The statistical level of significance was set to \( p<0.05 \) for forward inclusion and \( p<0.01 \) for backward deletion, and the final model was thereby determined.

Model Evaluation

Goodness-of-fit plots were evaluated by visual inspection of diagnostic scatter plots. A nonparametric bootstrap resampling technique was adopted to validate the reliability and stability of the final model.\(^{15}\) One thousand bootstrap replicates were generated by random resampling of the original data set with replacement. The stability and performance of the final model were assessed by using a visual predictive check (VPC). The VPC was performed 1000 times by simulating concentrations from the final model with the use of the original data set. The distribution of the observed concentrations was visually compared to the simulated distribution.

Statistical Analysis

The associations between genotypes and adverse event grades were determined by a Fisher’s exact test. An exploratory analysis was performed to assess time to appearance of grade 2 or higher skin rash by Kaplan–Meier methodology.

RESULTS

Population Characteristics and Genotype Frequencies of Selected Genes

PK data were available for a total of 48 of the 50 patients and pharmacogenetic data were available for all of the patients. Patient characteristics are summarized in Table 1. Approximately half of the patients were males (27 males vs. 23 females), and the median age was 68 years. The predominant histological classification was adenocarcinoma (\( \geq 80\% \)). The performance status of most patients (94\%) was less than 2, and only 3 patients (6\% of population) were current smokers (Table 1). The genotype frequencies of the selected genes are summarized in Table 2. All genotypes were in Hardy–Weinberg equilibrium, with the exception of ABCG2 \(-15622C\geq T\), CYP1A1 2455A>G (rs1048943), and UGT2B7 211G>T (rs12233719) (\( p<0.05 \)).

Population PK Model

A two-compartment model with
first-order absorption and elimination described the data well and was selected as the base model. Residual error was best characterized by a proportional error model. Parameter estimates are presented in Table 3. Twenty genotypes and 3 haplotypes, and 12 patient characteristics were individually tested by inclusion in the base model. Only ABCB1 1236C>T was a statically significant covariate for CL/F, with the TT genotype showing a 29.4% decrease in CL/F as compared with

<table>
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<tr>
<th>Gene</th>
<th>rs Number</th>
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<td>8</td>
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Table 2. Genotype Frequencies of Selected Genes Relevant to Erlotinib Disposition
the CC and the CT genotypes (Table 3). The interindividual variability in CL/F decreased by 10.6% (from 41.5 to 37.1%) after inclusion of the TT genotype as a covariate in the model. In final model, the eta-shrinkage for CL/F, K_a, V_{2/F}, and V_{1/F} and the epsilon-shrinkage were 11.3, 44.3, 29.8, 44.2, and 11.7%, respectively.

Goodness-of-fit plots obtained for the final model (Fig. 1) show that the final model fitting is acceptable and no systematic deviation over either time or population prediction value was observed. The medians and 2.5th and 97.5th percentiles for all parameter estimates obtained by the bootstrap method are listed in Table 3. The population PK parameter estimates for all parameter estimates obtained by the bootstrap method were comparable to those generated using the original data set, indicating acceptable accuracy in the bootstrap analyses.

**Effect of SNPs on Adverse Events**

None of the tested polymorphisms of the selected genes (CYP1A1, CYP1A2, CYP2D6, CYP3A4, CYP3A5, UGT1A1, UGT2B7, GSTM1, GSTT1, ABCB1, and ABCG2) showed significant association with adverse events. However, because ABCB1 1236C>T was the only statically significant covariate for CL/F of erlotinib, we examined the association between ABCB1 polymorphisms with the development of adverse events (Table 4). We analyzed the association between ABCB1 polymorphisms and cumulative incidence of adverse events (grade 3 or higher for all adverse events; grade 2 or higher for skin rash and diarrhea). We evaluated each genotype separately to explore the overall trend. The incidence of adverse events tended to be associated with ABCB1 functionality.33) ABCB1 is highly expressed in vitro on the apical membrane of enterocytes in the entire intestine and on the bile canaliculal membrane of hepatocytes, lower activity leads to higher absorption and lower elimination of erlotinib, thereby lowering CL/F. We previously reported that patients who were homozygous for the ABCB1*2 haplotype (1236TT–2677TT–3455TT) displayed higher plasma area under the curve (AUC) of erlotinib.30) ABCB1 functionality is not statistically significant covariate of CL/F. This discrepancy may be due to the different structural model or to the narrow range of total bilirubin values obtained in the present study.

**DISCUSSION**

This study analyzed a greater number of genetic polymorphisms in candidate genes involving in both the PK pathway and erlotinib toxicity than has previously been attempted. The SNPs in CYP1A1 2455A>G, UGT2B7 211G>T (rs12233719), and ABCG2 (–15622C>T) deviated from Hardy–Weinberg equilibrium. However, the allele frequencies were similar to the frequencies reported in HapMap-JPT32) and in previous report.36)

The population PK parameters obtained in this study were generally similar to those reported in other studies.9,15–18) An exception was the PK parameters for total bilirubin, which, in contrast to previous reports,9,15) was not a statistically significant covariate of CL/F. This discrepancy may be due to the different structural model or to the narrow range of total bilirubin values obtained in the present study.

**Table 3. Population Parameter Estimates**

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Final model</th>
<th>Bootstrap median (2.5th percentile, 97.5th percentile)</th>
<th>Base model</th>
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<td>OFV</td>
<td>−217.854</td>
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<td>−210.639</td>
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<tr>
<td>Typical CL/F (L·h(^{-1}))</td>
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<td>3.62 (3.04, 4.29)</td>
<td>3.2</td>
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<tr>
<td>ABCB1 1236C&gt;T on CL/F</td>
<td>−0.294</td>
<td>−0.299 (−0.45, −0.0963)</td>
<td>—</td>
</tr>
<tr>
<td>Typical K_a (h(^{-1}))</td>
<td>0.451</td>
<td>0.482 (0.0958, 0.961)</td>
<td>0.449</td>
</tr>
<tr>
<td>Typical V_{2/F} (L)</td>
<td>67.2</td>
<td>68.1 (8.13, 103)</td>
<td>66.5</td>
</tr>
<tr>
<td>Typical V_{1/F} (L)</td>
<td>41.1</td>
<td>41.5 (18.3, 74.7)</td>
<td>42</td>
</tr>
<tr>
<td>Typical Q (L·h(^{-1}))</td>
<td>8.97</td>
<td>7.86 (4.07, 21.6)</td>
<td>9.08</td>
</tr>
<tr>
<td>IIV CL/F (%)</td>
<td>37.1</td>
<td>36.3 (27.7, 45.5)</td>
<td>41.5</td>
</tr>
<tr>
<td>IIV K_a (%)</td>
<td>80.6</td>
<td>81.2 (21.0, 145)</td>
<td>79.4</td>
</tr>
<tr>
<td>IIV V_{2/F} (%)</td>
<td>71.3</td>
<td>73.4 (43.7, 152)</td>
<td>71.4</td>
</tr>
<tr>
<td>IIV V_{1/F} (%)</td>
<td>85.7</td>
<td>81.1 (37.8, 157)</td>
<td>83.8</td>
</tr>
<tr>
<td>RE_{prop} (%)</td>
<td>0.0563</td>
<td>0.0548 (0.0385, 0.0730)</td>
<td>0.0563</td>
</tr>
</tbody>
</table>

OFV, objective function value; V_c, central volume of distribution; V_p, peripheral volume of distribution; Q, intercompartmental clearance; IIV CL/F, interindividual variability for CL/F; IIV V_c/F, interindividual variability for V_c/F; IIV V_p/F, interindividual variability for V_p/F; RE_{prop} proportional component of residual error.

Kaplan–Meier methodology. Time to first appearance of grade 2 or higher skin rash is shown in Supplementary Material 5. The median time to onset of grade 2 or higher skin rash appeared to be shorter in ABCB1 TT genotype groups for all ABCB1 SNPs than in the other genotype groups.
These differences between the two studies may have led to the different results obtained. The polymorphism data on ABCB1 is controversial and importance of ABCB1*2 haplotype over SNPs individually (1236C>T, 2677G>A, and 3435C>T) is debatable. However, evidence is accumulating.\(^{{34-36}}\) Both the ABCB1*2 and 1236C>T have significant effect on oxaliplatin\(^{{37}}\) susceptibility and sunitinib toxicities and survival.\(^{{38}}\) Megías-Vericat et al. reported the influence of each SNP on the effectiveness of cytarabine plus anthracyclines\(^{{39}}\) while He et al. emphasized on the impact of the haplotype on the effectiveness of the same therapy.\(^{{40}}\) The impact of the ABCB1 1236C>T SNP rather than the ABCB1*2 haplotype in this study is consistent with a previously published clinical study of methadone\(^{{41}}\) and paclitaxel.\(^{{42}}\)

ABCG2 421C>A (rs2231142) did not result in a significant decrease in CL/F, although a previous study has mentioned the importance of this SNP in a Japanese population.\(^{{18}}\) It should be mentioned that the bioavailability of erlotinib increases in Bcrp1\(^{-/-}\)/Mdr1a/1b\(^{-/-}\) (ABCG2 and ABCB1 knockout) mice.\(^{{14}}\) but that the PK of erlotinib is not substantially affected in ABCG2 knockout mice.\(^{{43}}\) Based on our results and the results of those mouse studies, ABCB1 could play a more important role than ABCG2 in erlotinib absorption.

ABCB1 polymorphisms were marginally associated with the incidence of adverse events. A marginally higher incidence of adverse events and earlier onset of grade 2 or higher skin rash were observed in the TT genotype group (Table 4, Supplementary Material 5). However, the incidence of adverse events

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**Fig. 1. Goodness-of-Fit Plots for the Final Model**

(a) Population predicted concentrations (PRED) vs. observed concentrations (DV). (b) Individual predicted concentrations (IPRED) vs. observed concentrations (DV). (c) Time after first administration (Time) vs. conditional weighted residuals (CWRES). (d) Population predicted concentrations (PRED) vs. conditional weighted residuals (CWRES).
events in the TT genotype group of the ABCB1 1236C>T was not shown to be significantly different due to the lack of sufficient power for statistical analysis because patients were not enrolled based on their genotypes. In a previous report, a higher incidence of skin rash was considered to be related to an increase in the plasma exposure of erlotinib. \(^9\) Although the present population PK analysis showed that the CL/F of erlotinib in the TT genotype group was lower than the CL/F of erlotinib in the other groups, patients with high plasma erlotinib exposure did not always experience skin rash. Therefore, the erlotinib \(AUC\) might not directly cause the skin rash.

ABCB1 is expressed in sweat ducts, blood vessels, nerve sheaths, and muscles of human skin. \(^{44}\) The tissue-to-plasma concentration ratio in the skin of \(mdr1a/1b^{-/-}\) mice is reported to be significantly higher than that in wild-type mice. \(^{45}\) Since the TT genotype of ABCB1 has less activity, the erlotinib concentration in the skin of the TT genotype patients is presumably much higher than that in patients of the other genotypes, and thereby causes skin rash. Furthermore, the higher frequency of TT genotype in Asian population can reasonably explain higher incidence of skin rash in Japanese compared with the other populations. \(^{5, 9}\) whereas the incidence of grade 2 or higher skin rash were not reported to differ significantly between Asians and non-Asians. \(^{46}\) Our result is also consistent with the recent report which shows that ABCB1 1236C>T TT genotype had greater risk of skin rash after the treatment of other EGFR tyrosine-kinase inhibitor, gefitinib. \(^{47}\) Therefore, polymorphism analyses of ABCB1 1236C>T might be beneficial to predict the incidence of skin rash in erlotinib treatment. Nevertheless, erlotinib can be safely used for TT genotype patients provided there is careful monitoring of adverse events.

In this study, 1 patient developed grade 4 hepatotoxicity. This hepatotoxicity was not expected to be related to either the plasma exposure of erlotinib or to GST genotypes. The plasma ophthalmic acid concentrations of 26 patients at baseline (before erlotinib treatment) were determined by liquid chromatography tandem mass spectrometry as described in a previous report. \(^{48}\) Interestingly, the patient who developed grade 4 hepatotoxicity had a high level of ophthalmic acid (36.9 ng/mL), while the other 25 patients, who did not develop hepatotoxicity, had undetectable (\(<2\) ng/mL) ophthalmic acid. Ophthalmic acid is an analog of glutathione, which acts as an enzyme-catalyzed antioxidant when cells and organisms experience electrophilic stress that arises from metabolic processes. \(^{49}\) This acid is a sensitive indicator of glutathione levels in a mouse model, with a high level of this acid indicating glutathione depletion. \(^{50}\) Furthermore, ophthalmic acid in the serum of paracetamol-induced acute liver failure patients has been reported to be detected more frequently in non-survivors. \(^{51}\) Therefore, the above-described case in our study indicates the possibility that a patient with higher oxidative stress prior to treatment might have a higher risk of developing severe erlotinib-induced hepatotoxicity.

The present study has several limitations. The sample size of this study is smaller than that of a previous report \(^{18}\) \((n=50 vs. n=88)\), and the study was of an observational case-control nature, therefore effect of SNPs on adverse events could not be analyzed by Cox proportional hazard model. The effect of smoking status was not tested because all former smokers

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**Table 4. ABCB1 SNPs and Adverse Events**

<table>
<thead>
<tr>
<th>ABCB1 SNPs</th>
<th>(n)</th>
<th>All toxicity (%)</th>
<th>Skin rash (%)</th>
<th>Diarrhea (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1236C&gt;T; rs1128503</td>
<td>C/C</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>28</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>18</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2677G&gt;T(A): rs2032582</td>
<td>G/A</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>G/T</td>
<td>21</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>T/A</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>6</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3435C&gt;T; rs1045642</td>
<td>C/C</td>
<td>14</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>28</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>8</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

**Fig. 2. Visual Predictive Check**

Open circles: observations; dashed lines: 97.5th percentile, median and 2.5th percentile of observed concentrations; solid lines: 97.5th percentile, median and 2.5th percentile of predicted concentrations.
had quit smoking more than 2 weeks before the initiation of study treatment and therefore the effect of smoking on inducing CYP1A2 activity was negligible. The EGFR polymorphisms which were reported to be associated with erlotinib or gefitinib toxicities were not evaluated, since the EGFR mutation test was not mandatory for second-line or later treatment of erlotinib. However, our study employed concentration mutation test was not mandatory for second-line or later treatment of erlotinib. Further, randomized controlled trials with genotyped patients are needed to clarify the relationship between ABCB1 genotype, tissue erlotinib concentration, and adverse effects.

In conclusion, only ABCB1 1236C>T influenced exposure to erlotinib. This SNP was suggested to be related to the risk of adverse events. Erlotinib might be used with careful monitoring of adverse events for patients with ABCB1 polymorphic variants. The effects of SNPs in other genes (CYP1A1, CYP1A2, CYP2D6, CYP3A4, CYP3A5, UGT1A1, UGT2B7, GSTM1, GSTT1, and ABCG2) were denied. Further clinical studies are needed to confirm the ABCB1 impact and to investigate the mechanism underlying how ABCB1 affects the incidence of adverse events.

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Conflict of Interest This study was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (AH: number, KAKENHI 21590167). C.E. is an employee of Chugai Pharmaceutical Co., Ltd. A.H. has received research funding from Chugai Pharmaceutical Co., Ltd. J.S., S.S., N.I., M.I., S.U., H. Kishi, S.F., H. Kohrogi declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES


