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Pharmacogenomic and Pharmacokinetic Determinants of Erlotinib Toxicity

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Abstract

Purpose—To assess the pharmacogenomic and pharmacokinetic determinants of skin rash and diarrhea, the two primary dose-limiting toxicities of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor erlotinib.

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Patients and Methods—A prospective clinical study of 80 patients with non–small-cell lung cancer, head and neck cancer, and ovarian cancer was performed. Detailed pharmacokinetics and toxicity of erlotinib were assessed. Polymorphic loci in *EGFR*, *ABCG2*, *CYP3A4*, and *CYP3A5* were genotyped, and their effects on pharmacokinetics and toxicities were evaluated.

Results—A novel diplotype of two polymorphic loci in the *ABCG2* promoter involving −15622C/T and 1143C/T was identified, with alleles conferring lower ABCG2 levels associated with higher erlotinib pharmacokinetic parameters, including area under the curve ($P = .019$) and maximum concentration ($P = .006$). Variability in skin rash was best explained by a multivariate logistic regression model incorporating the trough erlotinib plasma concentration ($P = .034$) and the *EGFR* intron 1 polymorphism ($P = .044$). Variability in diarrhea was associated with the two linked polymorphisms in the *EGFR* promoter ($P < .01$), but not with erlotinib concentration.

Conclusion—Although exploratory in nature, this combined pharmacogenomic and pharmacokinetic model helps to define and differentiate the primary determinants of skin and gastrointestinal toxicity of erlotinib. The findings may be of use both in designing trials targeting a particular severity of rash and in considering dose and schedule modifications in patients experiencing dose-limiting toxicities of erlotinib or similarly targeted agents. Further studies of the relationship between germline polymorphisms in *EGFR* and the toxicity and efficacy of EGFR inhibitors are warranted.

INTRODUCTION

Erlotinib is the only epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor currently approved for marketing in the United States. The most common adverse effects of erlotinib are skin rash and diarrhea.[1–3] Both of these toxicities can be severe and can lead to discontinuation of therapy. A strong but unexplained association between skin rash and survival has been noted for patients given erlotinib for several epithelial malignancies, including lung cancer, head and neck cancer, and ovarian cancer.[3] Intriguingly, both this toxicity spectrum and the association between rash and clinical benefit have been observed across classes of EGFR inhibitors.

Rash and diarrhea associated with EGFR inhibitor use both demonstrate high interindividual variability. Several potential explanations for this observation have been suggested, including pharmacodynamic and pharmacokinetic (PK) variability.[4,5] Defining determinants of interindividual variability may provide critical insight, guiding the design of future clinical research by defining rational strategies for maximizing clinical benefit and minimizing adverse effects in patients treated with these agents.

Germline polymorphisms can have a major effect on drug pharmacokinetics and pharmacodynamics.[6] *EGFR*, encoding the direct target of erlotinib, is highly polymorphic.[7,8] An intronic microsatellite polymorphism has been associated with *EGFR* expression, with the repeat length of cytosine-adenosine (CA) nucleotides inversely correlating with *EGFR* mRNA and protein level, as well as erlotinib sensitivity in vitro.[9–12] There are marked interethnic differences at this intronic locus.[7]

More recent studies have identified single nucleotide polymorphisms (SNPs) in the 5′-regulatory region of *EGFR*. Two, −216G/T (rs712829) and −191C/A (rs712830), are in the essential promoter region of *EGFR*. The variant −216G/T has been associated with increased *EGFR* promoter activity and gene expression mediated by an altered interaction with Sp1, whereas −191C/A is close to one of major transcription start sites.[8] Recently, −216G/T was reported to be associated with gefitinib response and toxicity in lung cancer patients.[13] Anonsynonymous SNP at codon 497 of *EGFR* (rs11543848), a G to A alteration, results in substitution of the amino acid Arg (R) by Lys (K).[14] This is the only common missense polymorphism of *EGFR* reported to date, and the K allele seems to decrease the activity of EGFR.[15] Whether these polymorphisms are involved in the mechanism underlying side effects and responsiveness to EGFR tyrosine kinase inhibitors (TKIs) in cancer patients remains incompletely understood.

Previous studies of EGFR inhibitors found an association between drug steady-state plasma concentrations and the severity of skin rash and diarrhea.[16,17] Variation in genes involved in the pharmacokinetics of TKIs may contribute to these adverse reactions. Erlotinib is a substrate for both CYP3A4 and CYP3A5.[18] These two genes are highly and polymorphically expressed.[19,20] Polymorphisms in the *CYP3A5* gene can lead to significant interindividual and interracial differences in CYP3A-dependent drug metabolism.[21,22] *CYP3A5*3* is a common A>G transition within intron 3 of *CYP3A5* (rs776746), which creates a cryptic splicing site and leads to a truncated CYP3A5 protein production.[21] G/G homozygotes lack CYP3A5 expression, whereas individuals with at least one wild-type allele (A/A or A/G) express CYP3A5.[21] A common A>G transition in the 5′ regulatory region of CYP3A4 (CYP3A4*1B, rs2740574) has been associated with prostate cancer risk[23–25] and may also moderately increase CYP3A4 activity,[26] though a substantial effect of this SNP on the hepatic expression of CYP3A4 has not been demonstrated.[27–30] These two polymorphisms are linked.[31] It is unknown whether haplotypes of these two SNPs affect the metabolism of erlotinib and influence the interindividual variability in erlotinib toxicity.

In addition to drug metabolizing enzymes, drug transporters may also be involved in the pharmacokinetics of erlotinib. Recent studies suggest that gefitinib and erlotinib are substrates of ABCG2.[32–35] Two nonsynonymous *ABCG2* SNPs, 421 C>A (Q141K, rs2231142) and 34G>A (V12M, rs2231137), are common.[36–39] The 141K polymorphism has been associated with lower expression and activity of ABCG2 and with higher accumulation of both gefitinib and erlotinib.[35,36,40] A recent clinical study showed an association between 141K and diarrhea in patients treated with gefitinib.[41] We have recently identified four functional polymorphisms in the 5′-regulatory region of *ABCG2* (Poonkuzhali et al, manuscript submitted for publication). The −15994G>A (rs7699188) promoter rSNP (predicted to result in the gain of an HNF4 site) was significantly associated with higher *ABCG2* expression in multiple tissues. Carriers of the −15622C>T (novel) rSNP showed lower *ABCG2* expression in multiple tissues. An intron 1 SNP 16702G>A (rs2046134) was associated with high expression in liver and was predicted to result in the gain of a GATA4 site. Finally, 1143C>T (rs2622604) was associated with low expression in intestine. Whether these polymorphisms affect the pharmacokinetics of erlotinib and other TKIs has not been reported.

We hypothesized that germline polymorphisms in *EGFR* and other candidate genes influence erlotinib toxicity. We conducted a prospective study of 80 patients with lung, head and neck, and ovarian cancer receiving standard dose (150 mg daily) erlotinib to evaluate the impact of the genetic polymorphisms mentioned above on skin rash and diarrhea, the two major adverse reactions.

PATIENTS AND METHODS

Patients

This was a two-institution study conducted at the University of Chicago and the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins in Baltimore, MD. The study was reviewed and approved by the institutional review boards of both institutions, and signed informed consent was obtained from all patients. Patients with lung (n = 43), head and neck (n = 9), and ovarian cancer (n = 28) were treated with 150 mg of oral erlotinib once daily.

Genetic Polymorphisms

Four polymorphisms (−216G/T, −191C/A, intron 1 $(CA)_n$, and 497G/A) in the *EGFR* gene, *CYP3A4*1B*, *CYP3A5*3*, and six polymorphisms (421C/A, 34G/A, −15994G/A, −15622C>T, 16702G/A, and 1143C/T) in the *ABCG2* gene were genotyped in the blood DNA (n = 80). Methods for genotyping and haplotype estimation are included in the Appendix (online only).

Erlotinib Pharmacokinetic Analysis

Plasma samples were collected and erlotinib concentration was measured using high-performance liquid chromatography. Details of the assay are included in the Appendix.

Statistics and Data Analysis

Methods for PK data analyses are provided in the Appendix—Logistic regression was used to examine the association between PK parameters and toxicity. *t* tests and analysis of variance were performed to evaluate the association between the various polymorphisms and PK parameters. Fisher's exact tests were used to analyze the association between genetic polymorphisms and toxicity. Multiple analyses were performed to test for associations under dominant, recessive, and additive genetic models. Multivariable logistic regression models[42] were fit to examine the effects of genetic polymorphisms on toxicity while controlling for PK. Only statistically significant ($P < .05$, boldfaced and italicized) or marginally significant ($.05 \leq P \leq .10$, boldfaced) *P* values are shown in the tables. Further details regarding the statistical methods are provided in the Appendix.

RESULTS

Population PK Modeling: Correlation Between PK Data and Toxicities

Patient characteristics are listed in Appendix Table A1 (online only). Table 1 presents the population parameter estimates. No patient characteristics were significantly associated with any pharmacokinetic parameters.

Because toxicities could be confounded by the number of treatment cycles, only cycle 1 toxicity data were used as a phenotype in this analysis. Thirty-three patients (41%) developed grade 1 skin rash and 25 patients (31%) developed grade ≥ 2 skin rash. Thirty-one patients (39%) had grade 1 diarrhea and nine patients (11%) developed grade ≥ 2 diarrhea. Correlations between toxicity and PK are listed in Table 2. The erlotinib area under the curve (AUC) was marginally associated with grade ≥ 2 rash ($P = .082$). The odds of high-grade toxicity increased by a factor of 1.18 per 10 mg/L × hour increase in the AUC. Steady-state trough level (C_{trough}, mg/L) was significantly associated with rash ($P = .040$), with the odds of grade ≥ 2 rash increasing 1.75-fold per 1 mg/L increase in C_{trough}. No significant or marginally significant associations were detected between any PK parameter and the occurrence of diarrhea.

Correlation Between Genetic Polymorphisms and PK Data

Associations between genetic polymorphisms and AUC, maximum concentration (C_{max}), and C_{trough} are listed in Table 3. *CYP3A4*1B* was marginally associated with AUC and trough levels of erlotinib in a dominant model of the A allele (possibly lower CYP3A4 expression). Patients homozygous for *CYP3A4*1B* (A/A) had 33% higher levels of C_{trough} than patients with A/G genotype and 24% higher levels than patients with G/G genotype ($P = .066$). Homozygotes for *CYP3A5*3* G/G (CYP3A5 nonexpressors) showed a trend toward higher C_{trough} levels relative to A/A or A/G genotypes ($P = .076$ [recessive model]) with similar results for AUC. Because the two polymorphisms are in linkage disequilibrium ($r^2 = 0.44$), haplotypes between them were predicted and diplotypes were assigned to each individual. Patients homozygous for haplotype 1 (*CYP3A4*1B* A-*CYP3A5*3* G, or A-G, 3A5 nonexpressor and possibly lower 3A4 expressor) had a 21% higher AUC ($P = .090$) and 26% higher C_{trough} ($P = .079$) than those with other diplotypes.

The *ABCG2* 1143 C/T or T/T (lower expression) genotype was associated with higher erlotinib AUC and C_{max} ($P = .072$ and $P = .047$, respectively). Patients with −15,622 C/T or T/T (lower expression) genotype had greater C_{max} than those with a C/C genotype ($P = .065$). Moderate linkage disequilibrium ($r^2 = 0.56$) between the two SNPs has been observed, and haplotypes between them were predicted. The 1/4 (C-C/T-T) or 4/4 (T-T/T-T) diplotype was associated with significantly higher AUC and C_{max} ($P = .019$ and $P = .006$, respectively) and marginally higher C_{trough} ($P = .064$).

It should be noted that the number of patients with certain polymorphisms was small (for example, there were only four patients with *EGFR*497 A/A, seven patients with *CYP3A4*1B* G/G, five patients with *ABCG2*16,702 A/A, and nine patients with *ABCG2*-15994 A/A). In the case of *EGFR*497 A/A, the mean AUC, C_{max}, and C_{trough} were noticeably higher than for the other genotypes. Consequently, lack of statistical significance could be due to low statistical power for comparisons involving small sample sizes.

Correlation Between Genetic Polymorphisms and Toxicity

Polymorphisms in the *EGFR* promoter were associated with both skin rash and diarrhea (Table 4). The two promoter polymorphisms, −216 G/T and −191 C/A, were associated with grade ≥ 2 diarrhea ($P = .009$ and $P = .008$, respectively, under a dominant model). Similar

associations were found when comparing with the −216/−191 diplotypes or haplotypes. Only one of 43 patients with either the 1/1 diplotype (T-C/T-C) or the 1/2,3 diplotype (T-C and either G-C or G-A) had grade ≥ 2 diarrhea, compared with eight (23%) of 35 patients in the 2,3/2,3 category (ie, no T-C combination; $P = .027$ for 2 degrees of freedom test; $P = .007$ under a recessive model). The relative frequency of haplotype 1 (T-C) was lower in patients with grade ≥ 2 diarrhea as compared with patients with grade 0 to 1 diarrhea ($P = .003$; data not shown).

The number of patients with skin rash (any grade) in the s/s, s/L, and l/L CA repeat categories were 10 (76.9%) of 13 patients, 33 (80.5%) of 41 patients, and 13 (54.2%) of 24 patients, respectively. Both Fisher's exact test and the test for a linear trend in proportions were marginally significant ($P = .081$ and $P = .067$, respectively). However, as discussed in the Appendix, a Bonferroni correction would require $P < .05/5 = .01$ for statistical significance. The percentage of patients with grade ≥ 2 skin rash in the s/s, s/L, and l/L groups was 7.7%, 41.5%, and 25.0%, respectively. Fisher's exact test was marginally significant ($P = .057$), but the trend test was not significant ($P = .73$). For any grade diarrhea, the toxicity rates in the s/s, s/L, and l/L categories were seven (53.8%) of 13 patients, 23 (56.1%) of 41 patients, and eight (33.3%) of 24 patients, respectively. These differences did not reach statistical significance. There was also no significant association between CA repeat length and grade ≥ 2 diarrhea.

CYP3A4 polymorphisms were marginally associated with skin rash. Individuals with lower CYP3A4 expression (A/A) were more likely to develop rash (46 [78%] of 59 patients) than those with higher CYP3A4 levels (eight [62%] of 13 of A/Gs and three [43%] of seven of G/G homozygotes; $P = .077$). Similarly, the *CYP3A5*3* G polymorphism was also marginally associated with grade ≥ 2 rash ($P = .094$, dominant model) and any grade diarrhea ($P = .062$, recessive model). Finally, patients in the 2,3,4/2,3,4 diplotype category had a lower rate of grade ≥ 2 skin rash than those in the 1/1 or 1/2,3,4 groups ($P = .095$, recessive model). The relative frequency of haplotype 1 (A-G; lower CYP3A expression) was marginally higher in the patients with rash than in those without rash ($P = .089$, data not shown) and significantly higher in those with any grade diarrhea as compared with those without diarrhea ($P = .029$; data not shown).

A marginally significant association was found between *ABCG2* 16,702 G/A polymorphism and any grade skin toxicity ($P = .089$). G/G and A/A patients were more likely to develop toxicity (77% and 80%, respectively) compared with G/A (50%). A more consistent relationship is seen for grade ≥ 2 skin rash, with the G/G genotype exhibiting a higher rate of toxicity as compared with the G/A or A/A polymorphisms under a dominance model ($P = .027$). A marginally significant association was also detected between −15,622 C/T and any grade diarrhea ($P = .066$): 20 (41%) of 49 C/C patients developed diarrhea, compared with 20 of 31 (65%) C/T or T/T patients.

Multivariate Analysis

Logistic regression analyses were performed to evaluate the effects of genetic polymorphisms on toxicity controlling for PK (and vice versa). Because C_{trough} was significantly associated with grade ≥ 2 rash (Table 2) and C_{trough} levels captured the

majority of associations between polymorphisms and PK (Table 3), this variable was chosen as the PK parameter for multivariate analysis. Results are presented in Table 5 for skin rash and in Table 6 for diarrhea. Because of small numbers, multivariable analysis of grade ≥ 2 diarrhea was not performed.

Higher C_{trough} levels were associated with a greater risk of grade ≥ 2 skin rash, with the odds of rash increasing approximately 1.8-fold per 1 mg/dL increase in the trough level. A significant effect was detected for *EGFR* intron 1 $(CA)_n$ repeat (grade ≥ 2 rash) in which the s/L allele length was associated with a higher risk of toxicity relative to s/s ($P = .044$). There was also a marginal association ($P = .070$) between *CYP3A4* and skin rash with lower risk for G/G relative to A/A. The ABCG2 1143 C/T polymorphism was marginally associated with a lower risk of any grade skin rash ($P = .086$), and the 16,702 G/A polymorphism also conferred a lower risk of any grade ($P = .048$) or high-grade ($P = .050$) rash. There were no statistically significant associations between C_{trough} and diarrhea, only a marginal association ($P = .10$) controlling for EGFR 497 G/A. Patients having the G/G *CYP3A5* genotype were at increased risk of any grade diarrhea ($P = .070$), as were patients with the −15,622 C/T polymorphism ($P = .057$).

DISCUSSION

We undertook this prospective study to evaluate the clinical impact on skin rash and diarrhea of the large number of genetic polymorphisms we and others have identified in genes encoding the target for erlotinib, as well as genes associated with its membrane transport and metabolism.[7,8,13,17,18,33,41] Our results indicate that determinants of skin toxicity may include trough erlotinib plasma concentration and variability in the EGFR intron 1 polymorphism ($P = .034$ and $P = .044$, respectively, under a multivariable model). In contrast, diarrhea was correlated with the two linked polymorphisms in the EGFR promoter ($P < .01$), but not with erlotinib concentration. We emphasize that a large number of candidate polymorphic loci were evaluated and multiple analyses of each genetic polymorphism were performed. The multiple testing could lead to the detection of spurious associations, and therefore our findings are in need of further confirmation in independent data sets.

Erlotinib is a metabolic substrate for the phase II enzymes CYP3A4 and CYP3A5.[18] However, *CYP3A4* and *CYP3A5* polymorphisms determining enzyme expression and activity levels demonstrated only marginal associations with either erlotinib pharmacokinetic parameters or observed toxicity.

We report associations between newly discovered polymorphisms in the multidrug transporter gene ABCG2 and erlotinib PK, with lower expressing *ABCG2* alleles correlating with increased erlotinib concentrations, and diplotypes of two linked polymorphisms resulting in strong correlations with erlotinib AUC and C_{max}. Marginal and significant associations were also seen with ABCG2 polymorphic loci and toxicity. We were unable to confirm correlations between previously reported *ABCG2* polymorphisms and drug accumulation or diarrhea in patients treated with the related EGFR inhibitor gefitinib.[35,41] It is unclear whether this represents a pharmacologic difference between gefitinib and

erlotinib. Both agents are known substrates for ABCG2 transport, and both inhibit ABCG2 activity at high concentration.[35]

Previous series have reported separately on gene polymorphisms and on pharmacokinetic variability as correlates of toxicity.[13,35,41] An important aspect of the current report is the integrated analysis of genotypic and pharmacokinetic variability. Multivariate analysis to evaluate the potential interactions between putative determinants of toxicity suggests that interindividual pharmacokinetic variability may be a dominant determinant of erlotinib skin toxicity. Pharmacokinetic variability remains a statistically significant determinant of erlotinib toxicity across all polymorphic loci analyzed (*P* values for C_{trough} ranging from .026 to .052).

Multivariate analysis further suggests that erlotinib associated-diarrhea may be mechanistically distinct from rash. Rash, but not diarrhea, seemed to be correlated with erlotinib pharmacokinetics. This may reflect that gastrointestinal toxicity from erlotinib is primarily luminal and thus may be relatively independent of erlotinib blood levels. EGFR is highly expressed in intestinal lumen. The observed correlations with *EGFR* promoter polymorphisms suggest that EGFR expression may be a more important determinant of erlotinib-associated diarrhea than previously recognized.

Taken together, these data indicate that the toxicities experienced by patients taking erlotinib are multifactorial and determined by distinct parameters in different tissues. The interindividual variability in erlotinib pharmacokinetics, a primary correlate of skin toxicity, has not been adequately explained. That skin toxicity in this study was primarily correlated with erlotinib exposure levels, together with the observed association between skin toxicity and survival, supports exploration of therapeutic strategies based on dose escalation of erlotinib to development of clinically significant (grade ≥ 2) rash. Clinical outcome of patients enrolled in such studies has not been reported. Alternative determinants of interindividual susceptibility to rash and diarrhea, not evaluated in this study, are likely and remain to be identified. A clear understanding of the basis of variability in toxicity to EGFR-directed therapy may ultimately guide use of the currently available agents at optimal doses and in patients most likely to benefit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Table 1

Population Pharmacokinetic Parameter Estimates (± SE) From the Final Model

	Estimate		IIV (CV %)	
Parameter	**Estimate**	**SE**	**Estimate**	**SE**
t_{lag}, hours	0.34		23	9
Ka, 1/h	1.86	0.33	150	66
CL/F, L/h	3.29	0.25	58	25
V/F, L	131	10	61	30
σ_{exp}, CV %	32	12		

Abbreviations: IIV, interindividual variability (variability in individual-specific parameter estimates expressed as coefficient of variation); t_{lag}, lag-time in absorption; ka, absorption rate constant; CL, clearance; V, volume of distribution; F, bioavailability; σ_{exp}, residual variability for exponential error model; CV, coefficient of variation.

Table 2

Association Between PK Parameters (AUC, C_{max}, and C_{trough}) and Toxicity

	Grade 0 *v* 1 +			Grade 0 to 1 *v* 2+		
PK parameter	**OR***	**95% CI**	***P***	**OR***	**95% CI**	***P***
Rash						
AUC	1.08	0.88 to 1.33	NS	1.18	0.98 to 1.43	**.082**
C_{max}	1.19	0.77 to 1.84	NS	1.32	0.89 to 1.96	NS
C_{trough}	1.24	0.70 to 2.17	NS	1.75	1.03 to 2.97	***.040***
Diarrhea						
AUC	1.11	0.93 to 1.33	NS	0.91	0.67 to 1.23	NS
C_{max}	1.21	0.83 to 1.77	NS	0.70	0.35 to 1.39	NS
C_{trough}	1.38	0.84 to 2.27	NS	0.94	0.44 to 2.03	NS

NOTE. *P* values between .05 and .10 were regarded as marginally suggestive of an association (boldfaced), whereas $P < .05$ was considered statistically significant (boldfaced and italicized).

Abbreviations: PK, pharmacokinetic; AUC, area under the curve; C_{max}, maximum concentration; C_{trough}, trough level; OR, odds ratio; NS, not significant.

*For AUC, ORs are per 10 mg/L×h increase; for C_{max} and C_{trough}, ORs are per 1 mg/L increase.

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Table 3

Association Between Polymorphisms and Pharmacokinetic Data

				AUC (mg/L×h)		C_{max} (mg/L)		C_{trough} (mg/L)	
Gene	**Polymorphism**	**Genotype/Diplotype**	**No.**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
EGFR	−216G/T	G/G	35	52.38	26.40	2.71	1.23	1.69	0.99
		G/T	35	50.67	24.80	2.69	1.23	1.56	0.89
		T/T	10	56.94	24.10	2.88	1.13	1.83	0.91
		P			NS		NS		NS
	−191C/A	C/C	64	50.72	25.90	2.65	1.23	1.60	0.96
		C/A+A/A	14	58.32	21.53	3.00	1.10	1.89	0.76
		P			NS		NS		NS
	−216/−191 Diplotype *	1/1	8	56.94	24.10	2.88	1.13	1.83	0.91
		1/2,3	35	50.67	24.80	2.69	1.23	1.56	0.89
		2,3/2,3	35	52.38	26.41	2.71	1.23	1.69	0.99
		P			NS		NS		NS
	Intron 1 $(CA)_n$	s/s	13	53.79	26.32	2.76	1.30	1.71	0.91
		s/L	41	51.97	27.05	2.80	1.25	1.73	1.03
		l/L	24	47.93	21.62	2.56	1.11	1.47	0.74
		P			NS		NS		NS
	497G/A	G/G	35	51.12	23.27	2.64	1.15	1.62	0.80
		G/A	39	51.28	27.47	2.71	1.28	1.62	1.05
		A/A	4	68.35	15.58	3.48	0.79	2.21	0.59
		P			NS		NS		NS
CYP3A4†	*1B A/G	A/A	59	54.36	26.04	2.81	1.25	1.74	0.96
		A/G	13	43.06	23.99	2.32	1.13	1.31	0.82
		G/G	7	46.12	16.75	2.50	0.90	1.41	0.69
		P			**.088**†		NS		**.066**†
CYP3A5‡	*3 A/G	A/A	12	44.74	23.21	2.41	1.13	1.37	0.86
		A/G	17	46.38	24.22	2.47	1.18	1.43	0.86

Gene	Polymorphism	Genotype/Diplotype	No.	AUC (mg/L×h) Mean	AUC (mg/L×h) SD	C_{max} (mg/L) Mean	C_{max} (mg/L) SD	C_{trough} (mg/L) Mean	C_{trough} (mg/L) SD
		G/G	50	55.29	25.76	2.85	1.23	1.78	0.95
		P		**.096**‡		NS		**.076**‡/**.099**§	
CYP3A4-CYP3A5	Diplotype//	1/1	49	55.44	26.01	2.86	1.24	1.78	0.96
		½,3,4	17	46.67	24.21	2.46	1.18	1.46	0.86
		2,3,4/2,3,4	13	44.63	22.23	2.41	1.08	1.36	0.82
		P		**.090**†		NS		**.079**†	
ABCG2	421C/A	C/C	66	50.16	22.90	2.65	1.13	1.57	0.81
		C/A	13	59.92	34.67	2.98	1.55	1.99	1.35
		P		NS		NS		NS	
	34G/A	G/G	69	52.56	25.67	2.73	1.24	1.67	0.94
		G/A	10	46.31	22.11	2.48	1.00	1.42	0.82
		P		NS		NS		NS	
	1143C/T	C/C	51	47.68	23.33	2.49	1.07	1.51	0.89
		C/T+T/T	29	58.20	27.22	3.04	1.35	1.84	0.97
		P		**.072**†		*.047*†		NS	
	16702G/A	G/G	61	51.30	25.60	2.66	1.17	1.64	0.97
		G/A	14	55.05	25.34	2.99	1.41	1.64	0.79
		A/A	5	43.91	21.72	2.23	1.02	1.43	0.76
		P		NS		NS		NS	
	−15994G/A	G/G	45	51.62	27.54	2.68	1.26	1.65	1.04
		G/A	26	48.92	20.79	2.62	1.07	1.50	0.72
		A/A	9	58.33	25.61	2.97	1.35	1.91	0.88
		P		NS		NS		NS	
	−15622C/T	C/C	49	47.99	24.10	2.50	1.12	1.53	0.91
		C/T+T/T	31	57.03	26.20	3.00	1.28	1.79	0.95
		P		NS		**.065**†		NS	
	1143/−15,622 diplotype¶	1/1,2,3	56	47.21	23.06	2.45	1.07	1.50	0.87
		1,4/4	24	61.50	27.46	3.25	1.33	1.92	1.00

Gene	Polymorphism	Genotype/Diplotype	No.	AUC (mg/L×h) Mean	AUC (mg/L×h) SD	C_{max} (mg/L) Mean	C_{max} (mg/L) SD	C_{trough} (mg/L) Mean	C_{trough} (mg/L) SD
		P		*.019*[#]		*.006*[#]		**.064**[#]	

NOTE. *P* values between .05 and .10 were regarded as marginally suggestive of an association (boldfaced), whereas *P* < .05 was considered statistically significant (boldfaced and italicized). NS indicates no significant or marginally significant differences for any test (all *P* values > .10).

Abbreviation: SD, standard deviation.

[*] 1 = (T-C), 2 = (G-C), 3 = (G-A)

[†] Dominant model (*t* test).

[‡] Recessive model (*t* test).

[§] Additive model (linear regression).

[//] 1 = (A-G), 2 = (A-A), 3 = (G-A), 4 = (G-G).

[¶] 1 = (C-C), 2 = (T-C), 3 = (C-T), 4 = (T-T).

[#] *t* test.

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Table 4

Association Between Genetic Polymorphisms and Toxicity

			Toxicity (cycle 1; No. of patients)							
			Skin Rash (grade)				Diarrhea (grade)			
Gene	**Polymorphism**	**Genotype/Diplotype**	**0**	**1**	**2+**	***P****	**0**	**1**	**2+**	***P****
EGFR	−216 G/T	G/G	11	13	11	NS	17	10	8	NS
		G/T	10	14	11	NS	20	14	1	*.027*†/*.009*‡
		T/T	1	5	2		3	5	0	
	−191C/A	C/C	18	29	17	NS	34	26	4	NS
		C/A	3	3	7	NS	5	3	5	*.008*†/*.008*‡
		A/A	1	0	0		1	0	0	
	−216/−191 Diplotypes§	1/1	1	5	2		3	5	0	
		1/2,3	10	14	11	NS	20	14	1	NS
		2,3/2,3	11	13	11	NS	17	10	8	*.027*†/*.007*‡
	Intron 1 $(CA)_n$	s/s	3	9	1	**.081**†/**.067**¶	6	7	0	NS
		s/L	8	16	17	**.057**†	18	17	6	NS
		l/L	11	7	6		16	5	3	
	497 G/A	G/G	9	16	10	NS	17	12	6	NS
		G/A	12	14	13	NS	20	16	3	NS
		A/A	1	2	1		3	1	0	
CYP3A4	*1B A/G	A/A	13	25	21	**.077**†/**.081**‡	27	25	7	NS
		A/G	5	6	2	**.099**‡	9	2	2	NS
		G/G	4	2	1		4	3	0	
CYP3A5	*3 A/G	A/A	5	6	1	NS	9	3	0	**.080**†/**.062**//
		A/G	4	8	5	**.094**‡	10	6	1	NS
		G/G	13	19	18		21	21	8	
CYP3A4-CYP3A5	Diplotypes#	1/1	13	18	18	NS	21	21	7	NS

Gene	Polymorphism	Genotype/Diplotype	Toxicity (cycle 1; No. of patients)							
			Skin Rash (grade)				Diarrhea (grade)			
			0	1	2+	*P**	0	1	2+	*P**
		1/2,3,4	3	9	5	**.095**‖	10	5	2	NS
		2,3,4/2,3,4	6	6	1		9	4	0	
ABCG2	421 C/A	C/C	18	30	18	NS	33	25	8	NS
		C/A	4	3	6	NS	7	5	1	NS
	34 G/A	G/G	20	28	21	NS	36	25	8	NS
		G/A	2	5	3	NS	4	5	1	NS
	1143C/T	C/C	11	26	14	NS	27	17	7	NS
		C/T	10	7	11	NS	13	13	2	NS
		T/T	1	0	0		0	1	0	
	16702G/A	G/G	14	24	23	**.089**†	29	24	8	NS
		G/A	7	6	1	**.065**†/*.027*‡	9	5	0	NS
		A/A	1	3	1		2	2	1	
	−15994G/A	G/G	13	15	17	NS	21	19	5	NS
		G/A	5	15	6	NS	14	8	4	NS
		A/A	4	3	2		5	4	0	
	−15622C/T	C/C	13	23	13	NS	29	14	6	**.066**‡
		C/T	8	10	12	NS	11	16	3	NS
		T/T	1	0	0		0	1	0	
	1143/−15,622 diplotype**	1/1,2,3	14	26	16	NS	31	18	7	NS
		1,4/4	8	7	9	NS	9	13	2	NS

NOTE. *P* values between .05 and .10 were regarded as marginally suggestive of an association (boldfaced), whereas $P < .05$ was considered statistically significant (boldfaced and italicized). NS indicates no significant or marginally significant differences for any test (all *P* values > .10).

* First row, grade 0 versus 1+; second row, grade 0–1 versus 2+.

† Fisher's exact test for 3×2 table.

‡ Dominant model (Fisher's exact test).

§ 1 = (T-C), 2 = (G-C), 3 = (G-A).

¶ Armitage trend test.

‖ Recessive model (Fisher's exact test).

1 = (A-G), 2 = (A-A), 3 = (G-A), 4 = (G-G).

** 1 = (C-C), 2 = (T-C), 3 = (C-T), 4 = (T-T).

Table 5

Multivariable Analysis: Skin Rash (cycle 1)

Gene	Polymorphism	Genotype/Diplotype	OR*	Grade 0 versus 1 + 95% CI	*P*	OR*	Grade 0–1 versus 2 + 95% CI	*P*
EGFR	−216 G/T	G/G	1.0			1.0		
		G/T	1.18	0.42 to 3.31	NS	1.09	0.38 to 3.13	NS
		T/T	3.12	0.34 to 28.7	NS	0.66	0.11 to 4.02	NS
		C_{trough}	1.27	0.72 to 2.26	NS	1.87	1.07 to 3.25	*.027*
			C/C	1.0			1.0	
		−191C/A	C/A+A/A	0.90	0.24 to 0.30	NS	2.47	0.73 to 8.33	NS
			C_{trough}	1.30	0.73 to 2.32	NS	1.77	1.03 to 3.06	*.039*
			1/1	1.0			1.0	
		−216/−191 Diplotypes	1/2,3	0.38	0.04 to 3.52	NS	1.67	0.27 to 10.3	NS
			2,3/2,3	0.32	0.04 to 2.95	NS	1.52	0.25 to 9.320	NS
			C_{trough}	1.27	0.72 to 2.26	NS	1.87	1.07 to 3.25	*.027*
		Intron 1 (CA)n	s/s	1.0			1.0		
			s/L	1.24	0.27 to 5.58	NS	9.47	1.07 to 83.9	*.044*
			l/L	0.37	0.08 to 1.70	NS	5.09	0.51 to 50.5	NS
			C_{trough}	1.21	0.66 to 2.21	NS	1.90	1.05 to 3.44	*.034*
		497 G/A	G/G	1.0			1.0		
			G/A	0.78	0.28 to 2.18	NS	1.25	0.45 to 3.50	NS
			A/A	0.89	0.08 to 10.0	NS	0.59	0.05 to 6.66	NS
			C_{trough}	1.28	0.72 to 2.28	NS	1.88	1.08 to 3.27	*.026*
CYP3A4	*1B A/G	A/A	1.0			1.0		
		A/G	0.48	0.13 to 1.76	NS	0.40	0.08 to 2.06	NS
		G/G	0.22	0.04 to 1.13	**.070**	0.36	0.04 to 3.28	NS
		C_{trough}	1.16	0.64 to 2.10	NS	1.76	1.01 to 3.07	*.047*
CYP3A5	*3 A/G	A/A	1.0			1.0		
		A/G	2.30	0.46 to 11.5	NS	4.66	0.45 to 47.8	NS

Gene	Polymorphism	Genotype/Diplotype	OR*	Grade 0 versus 1 + 95% CI	P	OR*	Grade 0–1 versus 2 + 95% CI	P
		G/G	1.88	0.50 to 7.09	NS	5.23	0.61 to 45.1	NS
		C_{trough}	1.24	0.69 to 2.22	NS	1.80	1.02 to 3.17	*.043*
CYP3A4-CYP3A5	Diplotypes	1/1	1.0			1.0		
		1/2,3,4	1.81	0.44 to 7.44	NS	0.85	0.25 to 2.90	NS
		2,3,4/2,3,4	0.46	0.13 to 1.65	NS	0.17	0.02 to 1.46	NS
		C_{trough}	1.24	0.69 to 2.23	NS	1.78	1.01 to 3.13	*.047*
ABCG2	421 C/A	C/C	1.0			1.0		
		C/A	0.77	0.20 to 2.88	NS	1.89	0.52 to 6.90	NS
		C_{trough}	1.28	0.73 to 2.27	NS	1.81	1.03 to 3.17	*.039*
	34 G/A	G/G	1.0			1.0		
		G/A	1.74	0.34 to 9.01	NS	1.16	0.26 to 5.18	NS
		C_{trough}	1.29	0.73 to 2.28	NS	1.88	1.08 to 2.35	*.025*
	1143C/T	C/C	1.0			1.0		
		C/T+T/T	0.40	0.14 to 1.14	**.086**	1.39	0.51 to 3.79	NS
		C_{trough}	1.36	0.76 to 2.46	NS	1.70	1.00 to 2.91	**.052**
	16702G/A	G/G	1.0			1.0		
		G/A	0.29	0.09 to 0.99	*.048*	0.12	0.01 to 0.99	*.050*
		A/A	1.25	0.13 to 12.2	NS	0.46	0.05 to 4.49	NS
		C_{trough}	1.27	0.70 to 2.30	NS	1.79	1.02 to 3.12	*.041*
	−15944G/A	G/G	1.0			1.0		
		G/A	1.77	0.55 to 5.73	NS	0.53	0.17 to 1.62	NS
		A/A	0.46	0.10 to 2.06	NS	0.39	0.07 to 2.20	NS
		C_{trough}	1.32	0.74 to 2.36	NS	1.77	1.02 to 3.07	*.042*
	−15622C/T	C/C	1.0			1.0		
		C/T+T/T	0.83	0.30 to 2.30	NS	1.57	0.58 to 4.23	NS
		C_{trough}	1.26	0.71 to 2.22	NS	1.70	1.00 to 2.91	**.052**
	1143/−15,622 diplotype	1/1,2,3	1.0			1.0		
		1,4/4	0.59	0.20 to 1.74	NS	1.22	0.42 to 3.50	NS

Gene	Polymorphism	Genotype/Diplotype	OR*	Grade 0 versus 1 + 95% CI	P	OR*	Grade 0–1 versus 2 + 95% CI	P
		C_{trough}	1.31	0.73 to 2.35	NS	1.71	1.00 to 2.94	**.051**

NOTE. *P* values between .05 and .10 were regarded as marginally suggestive of an association (boldfaced), whereas $P < .05$ was considered statistically significant (boldfaced and italicized). NS indicates no significant or marginally significant differences for any test (all *P* values > .10).

*Odds ratios (OR) for C_{trough} are per 1 mg/dL increase.

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Table 6

Multivariable Analysis: Diarrhea (cycle 1)

Gene	Polymorphism	Genotype/ Diplotype	OR*	Grade 0 versus 1 + 95% CI	*P*
EGFR	−216 G/T	G/G	1.0		
		G/T	0.74	0.28 to 1.91	NS
		T/T	1.51	0.30 to 7.44	NS
		C_{trough}	1.45	0.87 to 2.42	NS
	−191C/A	C/C	1.0		
		C/A+A/A	1.36	0.42 to 4.46	NS
		C_{trough}	1.45	0.87 to 2.42	NS
	−216/−191	1/1	1.0		
	Diplotypes	1/(2–3)	0.49	0.10 to 2.42	NS
		(2–3)/(2–3)	0.66	0.13 to 3.28	NS
		C_{trough}	1.45	0.87 to 2.42	NS
	Intron 1 (CA)n	s/s	1.0		
		s/L	1.09	0.31 to 3.87	NS
		l/L	0.46	0.11 to 1.85	NS
		C_{trough}	1.42	0.84 to 2.39	NS
	497 G/A	G/G	1.0		
		G/A	0.90	0.36 to 2.28	NS
		A/A	0.24	0.02 to 2.63	NS
		C_{trough}	1.55	0.92 to 2.62	**.10**
CYP3A4	*1B A/G	A/A	1.0		
		A/G	0.42	0.11 to 1.55	NS
		G/G	0.70	0.14 to 3.44	NS
		C_{trough}	1.36	0.81 to 2.27	NS
CYP3A5	*3 A/G	A/A	1.0		
		A/G	2.08	0.41 to 10.7	NS

Gene	Polymorphism	Genotype/ Diplotype	OR*	Grade 0 versus 1 + 95% CI	*P*
		C_{trough}	1.30	0.78 to 2.17	NS

NOTE. *P* values between .05 and .10 were regarded as marginally suggestive of an association (boldfaced). NS indicates no significant or marginally significant differences for any test (all *P* values > .10).

*Odds ratios (OR) for C_{trough} are per 1 mg/dL increase.