BRIEF COMMUNICATIONS

Pharmacogenetics of ABCG2 and Adverse Reactions to Gefitinib

George Cusatis, Vanesa Gregorc, Jing Li, Anna Spreafico, Roxann G. Ingersoll, Jaap Verweij, Vienna Ludovini, Eugenio Villa, Manuel Hidalgo, Alex Sparreboom, Sharyn D. Baker

Gefitinib is an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase with activity in non–small-cell lung cancer. Diarrhea and skin toxicity are prominent gefitinib-related adverse events that potentially limit its use. Gefitinib is a substrate for ABCG2 (ABCP, BCRP, MXR), a polymorphic efflux transporter protein that is highly expressed in the intestines and liver. Here we investigated associations between allelic variants of EGFR, ABCG2, and the transporter protein ABCB1 with diarrhea and skin toxicity in gefitinib-treated patients. One variant, a common functional single-nucleotide polymorphism (SNP) in the ABCG2 gene, was associated with diarrhea in 124 patients treated with oral gefitinib 250 mg once daily; seven (44%) of 16 patients heterozygous for ABCG2 421C>A (Q141K) developed diarrhea, versus only 13 (12%) of 108 patients homozygous for the wild-type sequence ($P = .0046$). However, this SNP was not associated with skin toxicity ($P = .99$). The finding suggests that patients with reduced ABCG2 activity due to a common genetic variant are at increased risk for substrate drug–induced diarrhea, with implications for optimizing treatment with such agents. [J Natl Cancer Inst 2006;98:1739–42]

Gefitinib (ZD1839; Iressa), a small-molecule inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, has activity in patients with locally advanced or metastatic non–small-cell lung cancer, with overall response rates of 10%–18% *(1,2)*. Diarrhea is a prominent adverse effect of gefitinib treatment, with grade 1 or 2 toxicity occurring in 19%–36% of patients receiving a daily dose of 250 mg *(3–5)*. The etiology of gefitinib-related diarrhea is unknown, and it is unclear which patient factors are associated with an elevated risk of this adverse effect. Previously, a strong association was noted between gefitinib steady-state plasma concentrations and the severity of diarrhea *(6)*. Skin toxicity is another common side effect of gefitinib therapy that is typically manifested as a papulopustular rash. At a gefitinib dose of 250 mg daily, grade 1 or 2 skin rash occurs in 31%–42% of patients and grade 3 or 4 rash occurs in 1.4%–2.5% of patients *(3–5)*. The etiology of this rash is unknown, but it may be caused by inhibition of EGFR in the skin *(7)*.

ABCG2 (formerly known as ABCP, BCRP, or MXR) is a polymorphic efflux transporter protein that is expressed in intestinal epithelial cells, in proximal renal tubular cells, and on the biliary surface of hepatocytes and that influences the absorption and disposition of various substrates *(8)*. Recent in vitro data demonstrate that the expression of ABCG2 protects EGFR signaling–dependent A431 tumor cells from death after gefitinib treatment, suggesting that gefitinib may be a substrate for ABCG2 *(9)*. Several studies have also indicated that gefitinib at concentrations of at least 10 μM inhibits this efflux transporter *(10,11)*. In vitro studies using HEK293 human embryonic kidney cells transfected with wild-type and mutant ABCG2 demonstrated that both gefitinib and erlotinib, another small-molecule tyrosine kinase inhibitor, are transported by ABCG2 at clinically achievable concentrations (0.1–1.0 μM) *(12)*. Another efflux transporter protein, ABCB1 (P-glycoprotein), has also been shown to interact with gefitinib, although with much lower reactivity *(13)*.

Several naturally occurring variants in the ABCG2 gene have been described that may affect the expression and/or function of its encoded protein *(14)*. In particular, a functional single-nucleotide polymorphism (SNP) has been identified in exon 5 of the ABCG2 gene, in which a C → A nucleotide transition at position 421 (ABCG2 421C>A) results in a nonsynonymous variant protein in which a glutamine at position 141 is changed to lysine (Q141K) *(15)*. HEK293 cells transfected with this variant demonstrate reduced transport of both gefinib and erlotinib, and the presence of the variant has been associated with greater gefitinib plasma accumulation at steady state in patients receiving gefitinib therapy *(12)*. The most extensively studied ABCB1 variant is a common synonymous C to T transition at nucleotide position 3435 in exon 26 (3435C>T) *(8)*. Although this transition does not change its encoded amino acid, this variant has been associated with reduced mRNA expression *(16)* and tability *(17)* in human hepatic tissue and may have a reduced ability to transport gefitinib. A number of somatic mutations in the EGFR gene have been identified that are associated with increased activity of EGFR tyrosine kinase inhibitors *(18,19)*, and inherited polymorphisms in the EGFR gene have been associated with altered EGFR expression or function *(20–22)*. Two common SNPs in the EGFR promoter have been recently identified: −216G>T, a variant in the Sp1 recognition site in the promoter region, and −191C>A, a variant that affects a region close to a transcriptional start site *(22)*. A haplotype of these two alleles was associated with higher EGFR promoter activity *(22)*. In addition, the length of a tandem repeat $(CA)_n$ in intron 1 of EGFR has been inversely related to EGFR mRNA

Affiliations of authors: The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD (GC, JL, MH, SDB); Division of Medical Oncology, Scientific Institute University Hospital San Raffaele, Milano, Italy (VG, A. Spreafico, EV); Division of Medical Oncology, Policlinico Monteluce Hospital, Perugia, Italy (VL); Institute of Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD (RGI); Department of Medical Oncology, Erasmus MC–Daniel den Hoed Cancer Center, Rotterdam, the Netherlands (JV); National Cancer Institute, Bethesda, MD (A. Sparreboom).

Correspondence to: Sharyn D. Baker, PharmD, PhD, Pharmaceutical Sciences Department, St Jude Children's Research Hospital, 332 North Lauderdale Street, Mail Stop 314, Memphis, TN 38105 (e-mail: sharyn.baker@stjude.org).

See "Notes" following "References."

DOI: 10.1093/jnci/djj469

Table 1. Patient characteristics and genotypes*

Variable	Value
Total enrolled	173
Evaluable for diarrhea/skin toxicity	129 (75%)/134 (77%)
Median age (range), y	67 (25–91)
Sex (male/female)	125 (72%)/48 (28%)
Histological classification	
Adenocarcinoma	80 (46%)
Broncholoalveolar carcinoma	15 (8.6%)
Squamous cell carcinoma	43 (25%)
Large-cell carcinoma	34 (20%)
Adenosquamous carcinoma	1 (0.6%)
TNM classification	
Stage 1B/IIB	2 (1.2%)/2 (1.2%)
Stage IIIA/IIIB	3 (1.7%)/19 (11%)
Stage IV	147 (85%)
Positive smoking history	123 of 146 evaluable (84%)
WHO† performance status (0/1/1)	63 (36%)/74 (43%)/36 (21%)
Median number of days on treatment (range)	92 (5–989+)
ABCB1 3435C>T genotype (CC vs. CT vs. TT)	52 (31%)/79 (47%)/38 (22%)
ABCG2 421C>A genotype (CC vs. CA or AA)	143 (86%)/23 (14%)/1 (0.6%)
EGFR −216G>T genotype (GG vs. GT vs. TT)	56 (35%)/70 (43%)/36 (22%)
EGFR −191C>A genotype (CC vs. CA or AA)	129 (80%)/29 (18%)/4 (2.5%)
EGFR −216G>T/−191C>A haplotype (non-G-C vs. other)	55 (34%)/107 (66%)
EGFR $(CA)_n$ genotype	
n1 + n2 ≤ 35 vs. >35‡	111 (65%)/60 (35%)
n1 and n2 < 18 vs. other	85 (50%)/86 (50%)

*Data are presented as the number of patients with the percentage of the population in parenthesis, unless specified otherwise.

†WHO = World Health Organization.

‡n1 = allele 1; n2 = allele 2; number indicates number of CA tandem repeats.

expression *(21)* and protein levels *(20)*. Moreover, a combined $(CA)_n$ repeat length on both alleles of 35 or less has been associated with greater in vitro sensitivity to erlotinib in head and neck cancer cell lines and a greater incidence of skin toxicity in patients with colorectal cancer *(23)*.

Here we examined the association between common inherited polymorphisms in ABCG2 and EGFR and gefitinib-related toxicity. A cohort of 173 consecutive Caucasian patients with non–small-cell lung cancer (125 males and 48 females; median age = 67 years, range = 25–91 years) at the Scientific Institute University Hospital San Raffaele, Milano, Italy, and Policlinico Monteluce Hospital, Perugia, Italy, received treatment with oral gefitinib at a dose of 250 mg once daily on a compassionate use basis until disease progression, as part of the Iressa Expanded Access Programme. Details related to patient selection criteria and toxicity assessments have been described elsewhere *(24)*. The study protocol was approved by the Institutional Review Boards (Milano and Perugia), and signed informed consent was obtained from all patients. Genomic DNA was isolated from plasma samples, and each patient's genotypes for EGFR at positions −216 and −191, for ABCG2 at position 421, and for ABCB1 at position 3435 were determined by a polymerase chain reaction–based technique with direct nucleotide sequencing (see Supplementary Methods, at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol98/issue23). The association between variant

Table 2. Factors potentially associated with diarrhea and skin toxicity*

	Diarrhea					Skin toxicity				
	No. evaluable					No. evaluable				
Variant genotypes or clinical factors	Total	Individual categories†			*P* value	Total	Individual categories†			*P* value
ABCB1 3435C>T (CC vs. CT vs. TT)	125	43	56	26	.85	130	41	60	29	.67
ABCG2 421C>A (CC vs. CA or AA)	124	108	16	0	.0046	128	109	19	0	.99
EGFR −216G>T (GG vs. GT vs. TT)	119	46	46	27	.23	123	49	47	27	.093
EGFR −191C>A (CC vs. CA or AA)	119	93	26	0	.56	123	95	28	0	.65
EGFR −216G>T/−191C>A (haplotype non-G-C vs. other)	119	40	79		.33	123	41	82		.99
EGFR $(CA)_n$										
n1 + n2 ≤ 35 vs. >35‡	127	80	47		.99	132	84	48		.26
n1 and n2 < 18 vs. other	127	63	64		.59	132	65	67		.15
Baseline patient characteristics										
Sex (male vs. female)	129	94	35		.79	134	99	35		.10
Smoking status (ever vs. never)	109	90	19		.30	111	91	20		.61
Performance status (0 or 1 vs. 2)	129	112	17		.62	134	117	17		.63
Histology (adenocarcinoma vs. other)	129	68	61		.81	134	71	63		.99
Time from diagnosis (≤12 vs. >12 mo)	128	61	67		.24	133	65	68		.48
Stage (I, II, or III vs. IV)	129	23	106		.85	134	23	111		.80
Prior platinum as first-line treatment (yes vs. no)	129	91	38		.60	134	92	42		.99
Prior chemotherapy (0 or 1 vs. ≥2)	129	77	52		.99	134	81	53		.46
Age	129				.85	134				.60

*Statistical associations were evaluated with a Fisher's exact test, except age (Mann–Whitney *U* test), using the software package NCSS version 2005 *(26)*. The total number of evaluable patients differed between groups because toxicity data were not collected completely, patients had early disease progression, and/or complete genotyping data were not available.

†Each individual category is specified by the parenthetical information in the first column. Some comparisons included three categories, whereas others included only two categories, leading to empty cells.

‡n1 = allele 1; n2 = allele 2.

genotypes or patient characteristics and toxicity (e.g., diarrhea versus no diarrhea) was determined by a Fisher's exact test. In view of the relatively low frequencies of the ABCG2 421C>A and EGFR −191C>A alleles (Table 1), the heterozygous and homozygous variant genotypes were pooled in the analysis on the basis of predicted similarity in outcomes.

Clinical characteristics and genotypes for the 173 patients are shown in Table 1. A total of 129 and 134 patients were evaluable for diarrhea and skin toxicity, respectively, after one cycle of treatment (2 months). Grade 1 or 2 diarrhea occurred in 20 (16%) of the 129 patients; one additional patient experienced grade 3 diarrhea. Grade 1 or 2 skin toxicity occurred in 84 (63%) of 134 patients (frequency, 62.7%); one additional patient experienced grade 3 skin toxicity. The associations between studied genotypes as well as baseline characteristics and toxicity are listed in Table 2. Of the 129 patients with evaluable data on diarrhea toxicity, genotype data on the ABCG2 421C>A polymorphism were available for 124 patients. This polymorphism was statistically significantly associated with the occurrence of diarrhea; seven (44%) of the 16 patients with at least one variant ABCG2 421C>A allele developed grade 1 or 2 diarrhea, whereas only 13 (12%) of 108 patients carrying the wild-type sequence for both alleles did ($P = .0046$). The one patient with the homozygous variant genotype had no noticeable toxicity. No other genotypes and patient characteristics were associated with diarrhea, and no studied variables were associated with the development of skin rash.

To our knowledge, this study provides the first evidence that adverse events related to the treatment of ABCG2 substrate drugs are linked to variations in expression and/or function of the protein brought on by a common functional polymorphism in the ABCG2 gene. The mechanism underlying the functional impact of the ABCG2 Q141K amino acid change is not entirely known, but it is most likely associated with reduced protein levels and altered ATPase activity and not with a change in localization of the protein *(14)*. The association we observed between ABCG2 421C>A genotype status and the observed clinical outcome, diarrhea, may reflect a role of this transporter in the oral absorption and/or elimination pathways of gefitinib. In a recent pharmacokinetic study, higher steady-state gefitinib plasma concentrations were observed in patients harboring a variant ABCG2 421C>A allele *(12)*, and higher steady-state plasma concentrations were associated with the occurrence of diarrhea *(6)*. This study was limited by the small sample size and the relatively low frequency of the ABCG2 421C>A variant. Nevertheless, we expect that the results presented here for gefitinib are representative for many other orally ingested drugs that are transported by ABCG2 [e.g., erlotinib and imatinib *(25)*] and that continued investigation in this area will likely have an impact on attempts to further optimize and individualize treatment regimens involving such agents.

References

(1) Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. J Clin Oncol 2003;21:2237–46.

(2) Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 2003;290:2149–58.

(3) Mohamed MK, Ramalingam S, Lin Y, Gooding W, Belani CP. Skin rash and good performance status predict improved survival with gefitinib in patients with advanced non-small cell lung cancer. Ann Oncol 2005;16:780–5.

(4) Razis E, Skarlos D, Briasoulis E, Dimopoulos M, Fountzilas G, Lambropoulos S, et al. Treatment of non-small cell lung cancer with gefitinib ('Iressa', ZD1839): the Greek experience with a compassionate-use program. Anticancer Drugs 2005;16:191–8.

(5) Schutte W, Nagel S, Schaedlich S, Brust D, Blankenburg T. Clinical benefit in NSCLC: advanced-stage patients require symptom-improving palliation. Experiences from the 'Iressa' expanded access program. Onkologie 2005;28:195–8.

(6) Li J, Karlsson MO, Brahmer J, Cusatis G, Hidalgo M, Baker SD. Population pharmacokinetic (PK)-pharmacodynamic (PD) modelfor gefitinib in cancer patients. Proc Am Soc Clin Oncol 2005;41:Abstract 3083.

(7) Perez-Soler R, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? J Clin Oncol 2005; 23:5235–46.

(8) Lepper ER, Nooter K, Verweij J, Acharya MR, Figg WD, Sparreboom A. Mechanisms of resistance to anticancer drugs: the role of the polymorphic ABC transporters ABCB1 and ABCG2. Pharmacogenomics 2005;6: 115–38.

(9) Elkind NB, Szentpetery Z, Apati A, Ozvegy-Laczka C, Varady G, Ujhelly O, et al. Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor Iressa (ZD1839, Gefitinib). Cancer Res 2005;65:1770–7.

(10) Stewart CF, Leggas M, Schuetz JD, Panetta JC, Cheshire PJ, Peterson J, et al. Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. Cancer Res 2004;64:7491–9.

(11) Nakamura Y, Oka M, Soda H, Shiozawa K, Yoshikawa M, Itoh A, et al. Gefitinib ("Iressa", ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, reverses breast cancer resistance protein/ABCG2-mediated drug resistance. Cancer Res 2005;65:1541–6.

(12) Li J, Sparreboom A, Zhao M, Robey RW, Bates SE, Baker SD. Gefitinib and erlotinib, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, are substrates for the breast cancer resistance protein (BCRP)/ABCG2 transporter. Clin Cancer Res 2005;11S:120 (Abstract A251).

(13) Ozvegy-Laczka C, Hegedus T, Varady G, Ujhelly O, Schuetz JD, Varadi A, et al. High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. Mol Pharmacol 2004;65:1485–95.

(14) Gardner ER, Figg WD, Sparreboom A. Pharmacogenomics of the human ATP-binding cassette transporter ABCG2. Curr Pharmacogenomics. 2006;4:in press.

(15) Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, et al. C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther 2002;1:611–6.

(16) Song P, Lamba JK, Zhang L, Schuetz E, Shukla N, Meibohm B, et al. G2677T and C3435T genotype and haplotype are associated with hepatic ABCB1 (MDR1) expression. J Clin Pharmacol 2006;46:373–9.

(17) Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics 2005;15:693–704.

(18) Johnson BE, Janne PA. Selecting patients for epidermal growth factor receptor inhibitor treatment: a FISH story or a tale of mutations? J Clin Oncol 2005;23:6813–6.

(19) Janne PA, Engelman JA, Johnson BE. Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology. J Clin Oncol 2005;23:3227–34.

(20) Buerger H, Gebhardt F, Schmidt H, Beckmann A, Hutmacher K, Simon R, et al. Length and loss of heterozygosity of an intron 1 polymorphic sequence of egfr is related to cytogenetic alterations and epithelial growth factor receptor expression. Cancer Res 2000;60:854–7.

(21) Gebhardt F, Zanker KS, Brandt B. Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. J Biol Chem 1999;274:13176–80.

(22) Liu W, Innocenti F, Wu MH, Desai AA, Dolan ME, Cook EH Jr, et al. A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter. Cancer Res 2005; 65:46–53.
(23) Amador ML, Oppenheimer D, Perea S, Maitra A, Cusati G, Iacobuzio-Donahue C, et al. An epidermal growth factor receptor intron 1 polymorphism mediates response to epidermal growth factor receptor inhibitors. Cancer Res 2004;64:9139–43.
(24) Gregorc V, Ceresoli GL, Floriani I, Spreafico A, Bencardino KB, Ludovini V, et al. Effects of gefitinib on serum epidermal growth factor receptor and HER2 in patients with advanced non-small cell lung cancer. Clin Cancer Res 2004;10(Pt 1):6006–12.
(25) Burger H, van Tol H, Boersma AW, Brok M, Wiemer EA, Stoter G, et al. Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. Blood 2004;104: 2940–2.
(26) Hintze J. NCSS and PASS. Number Cruncher Statistical Systems. Kaysville (UT); 2004. Available at: http://www.ncss.com. [Last accessed: October 27, 2006.]

NOTES

This study was funded in part by the Commonwealth Foundation for Cancer Research. This funding source had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and/or in the decision to submit the paper for publication.

This work was presented previously, in part, at the 41st Annual Meeting of the American Society of Clinical Oncology, held on May 13–17, 2005, in Orlando, FL.

Manuscript received August 1, 2005; revised August 31, 2006; accepted September 28, 2006.