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Pharmacogenetic Predictors of Adverse Events and Response to Chemotherapy in Metastatic Colorectal Cancer: Results From North American Gastrointestinal Intergroup Trial N9741

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ABSTRACT

Purpose
With three available chemotherapy drugs for advanced colorectal cancer (CRC), response rate (RR) and survival outcomes have improved with associated morbidity, accentuating the need for tools to select optimal individualized treatment. Pharmacogenetics identifies the likelihood of adverse events or response based on variants in genes involved in drug transport, metabolism, and cellular targets.

Patients and Methods
Germline DNA was extracted from 520 patients on the North American Gastrointestinal Intergroup N9741 study. Three study arms were evaluated: IFL (fluorouracil [FU] + irinotecan [IRN]), FOLFOX (FU + oxaliplatin), and IROX (IRN + oxaliplatin). Information on adverse events, response, and disease-free survival was available. Thirty-four variants in 15 candidate genes for analysis based on previous associations with adverse events or outcome were assessed. Genotyping was performed using pyrosequencing.

Results
All variants were polymorphic. The homozygous *UGT1A1*28* allele observed in 9% of patients was associated with risk of grade 4 neutropenia in patients on IROX (55% *v* 15%; $P = .002$). Deletion in GSTM1 was associated with grade 4 neutropenia after FOLFOX (28% *v* 16%; $P = .02$). Patients with a homozygous variant genotype for *GSTP1* were more likely to discontinue FOLFOX because of neurotoxicity (24% *v* 10%; $P = .01$). The presence of a *CYP3A5* variant was significantly associated with RR on IFL (29% *v* 60%; $P = .0074$). Most previously published genotype-toxicity or -efficacy relationships were not validated in this study.

Conclusion
This study provides a platform to evaluate pharmacogenetic predictors of response or severe adverse events in advanced CRC. Pharmacogenetic studies can be conducted in multicenter trials, and our findings demonstrate that with continued research, clinical application is practical.

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INTRODUCTION

The practice of medicine is in the midst of a transition from the current reliance on treatment based on average outcomes across populations to biologically based patient management. A number of factors drive these efforts, including the challenge of selecting among equivalent therapies, the emerging influence of the patient in health care decisions, and the realization that health care systems cannot afford ineffective, yet expensive, medications. The application of pharmacogenetic techniques provides an opportunity to enrich the proportional benefit to patients in an economical manner. This is especially true in cancer therapy, where initial treatment provides the best opportunity for disease control or palliation. In addition, the adverse drug events from chemotherapy can be severe, debilitating (even lethal), and expensive, making therapies associated with equal efficacy and less adverse events attractive to patients and practitioners alike.[1,2]

Recent clinical studies suggest that genomic findings can translate into improvements in clinical practice.[3-8] Indeed, many studies have suggested an association between genetic variants and either adverse events or efficacy end points. However, these

studies are often small (< 100 patients), from a single center, and do not have comparative treatment arms in which to determine whether a marker is prognostic only for overall outcome or allows prediction for an effect of a specific therapy.

Colorectal cancer (CRC) is a disease for which the management could be optimized using pharmacogenetics. The treatment has changed because of the availability of three cytotoxic drugs with distinct mechanisms of action, in addition to antibody therapy (bevacizumab, cetuximab, and panitumumab).[9-13] These drugs have improved the tumor response, progression-free survival (PFS), and overall survival (OS) of some patients with CRC, but are associated with important drug-specific adverse events. In addition, the cost of these treatments is > $100,000 per year for many patients.[1] The development of multiple active regimens for treatment of this disease is encouraging but, to date, there have been no prospective tools validated that permit selection of the best therapy for an individual patient. The North American Gastrointestinal Intergroup N9741 study (hereafter N9741) of chemotherapy for metastatic CRC offered a unique opportunity for pharmacogenetic research.[14] This trial prospectively included pharmacogenetic sampling in the context of a multicenter study of patients randomly assigned to the three main cytotoxic agents for treating advanced CRC (fluorouracil [FU], irinotecan [IRN], and oxaliplatin). The goal of this work was to assess the impact of published candidate genetic markers for which studies had identified a putative role in the prediction of adverse events or activity in patients receiving combination chemotherapy for advanced CRC.

Table 1. Descriptive Baseline Information on the Initial 520 Patients With Blood Sampling for Pharmocogenetics Study and 1,174 Patients for Whom No Blood Was Obtained

Variable	No Blood Drawn (n = 1,174) No.	%	Blood Drawn (n = 520) No.	%	*P*
Age, years					
Median	61.0		61.0		.54
Minimum	19.0		26.0		
Maximum	88.0		85.0		
Disease status					.08
Measurable	983	84	453	87	
Evaluable	189	16	67	13	
ECOG PS					.81
0-1	1,115	95	496	95	
2	57	5	24	5	
Sex					.28
Female	450	38	214	41	
Male	724	62	306	59	
Prior adjuvant chemotherapy					.86
Yes	171	15	78	15	
No	1,001	85	442	85	
Race/ethnicity					.21
White	1,013	86	450	87	
Black	92	8	36	7	
Hispanic	42	4	16	3	
Other	16	14	9	2	
Asian	11	1	9	2	
Grade ≥ 3 adverse events					
Nausea	147	13	62	12	.69
Dehydration	60	5	31	6	.49
Grade ≥ 4 neutropenia	190	16	93	18	.42
Vomiting	131	11	54	10	.61
Diarrhea	254	22	93	18	.07
Febrile neutropenia	103	9	42	8	.61
Paresthesia	92	8	44	8	.69
Median time to event outcomes (months)					
Time to progression	7.5		8.2		.04
Overall survival	17.0		18.1		.15

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status.

PATIENTS AND METHODS

Patient Population

The N9741 clinical study has been previously detailed.[14] Patients characteristics are presented in Table 1. Three regimens—IFL, FOLFOX, and IROX—were tested in this study. Initially, IFL was IRN at 125 mg/m^2 and bolus FU at 500 mg/m^2 plus leucovorin at 20 mg/m^2 on days 1, 8, 15, and 22 every 6 weeks. Midway through the trial, due to adverse events considerations, the doses of IRN and FU were reduced to 100 mg/m^2 and 400 mg/m^2, respectively.[15] FOLFOX was oxaliplatin at 85 mg/m^2 on day 1 and bolus FU at 400 mg/m^2 plus leucovorin at 200 mg/m^2 followed by FU at 600 mg/m^2 in 22-hour infusions on days 1 and 2 every 2 weeks. IROX was oxaliplatin at 85 mg/m^2 and IRN at 200 mg/m^2 every 3 weeks. Treatment continued until progression, unmanageable toxic adverse effects, or withdrawal of consent.

Toxic adverse effects (except paresthesia) were graded using National Cancer Institute Common Terminology Criteria for Adverse Events version 2.0. Paresthesias that resulted in functional impairment that interfered with daily activities or caused disability were classified as grade 3 or 4, respectively. Any grade 3 or 4 toxic effect resulted in an approximately 20% dose reduction for subsequent cycles. The specific adverse events tested for association with genetic markers were diarrhea, vomiting, paresthesia, febrile neutropenia (all grade ≥ 3), and neutropenia (grade ≥ 4). Response was evaluated by computed tomography before therapy and every 6 weeks during therapy until an objective response was attained (either complete or partial response) or the patient progressed. Confirmed response was defined as an objective status of complete or partial response maintained for at least 4 weeks.

Genetic Analysis

A 20-mL blood sample was obtained in EDTA lavender-top tubes from each patient after written informed consent. The addition of blood sampling for pharmacogenetic studies was approved by each local institutional review board. Blood was obtained at study registration. Each blood tube was labeled with a unique identifier and contained no clinical or demographic information. Genomic DNA was extracted from EDTA tubes using standard techniques and stored at 4°C before use. Genetic variants in FU, oxaliplatin, or IRN drug pathways were selected for analysis, primarily on the basis of association (in previous literature) with adverse events or outcome with one or more of the study drugs (Table 2).[5,16] Five genes were prespecified in the clinical protocol as of primary interest: *TYMS, DPYD, CYP3A4, UGT1A1,* and *GSTM1.* A total of 34 variants (30 single nucleotide polymorphisms [SNPs], one insertion/deletion, two tandem repeats, and one gene deletion) were assessed using polymerase chain reaction and pyrosequencing technologies as previously described (assay information is available in the Data Supplement, online only).[17]

Statistical Analysis

The primary analyses were specified to compare adverse events and activity measures overall and by treatment arm for each polymorphism. Every variant was evaluated for association with every end point. The total number of statistical tests performed was not quantified, and formal protection for multiple comparisons was not attempted. However, as a general protection against multiple comparisons, since five genes were selected to be of primary interest, a two-sided significance level of 0.01 (as opposed to the usual 0.05) was used to

Table 2. Genotype and Allele Frequencies for the North American Gastrointestinal Intergroup Trial N9741 Patients for Toxicity and Outcome Polymorphisms

Gene	Nucleotide	Amino Acid	n	Genotype Frequency			Allele Frequency	
				wt/wt	wt/var	var/var	p	q
ABCB1	1236 C>T	G411G	526	168	257	101	0.56	0.44
ABCB1	3435 C>T	I1145I	540	157	275	108	0.55	0.45
ABCB1	2677 G>T	A893S	541	162	253	103	0.55	0.43†
ABCC1	IVS18-30 C>G	Intron 18	540	369	157	14	0.83	0.17
ABCC1	4002 G>A	S1334S	547	300	210	37	0.74	0.26
ABCC2	−24 C>T	5UTR	543	363	157	23	0.81	0.19
ABCC2	4544 G>A	C1515Y	545	486	57	2	0.94	0.06
ABCC2	1058 G>A	R353H	548	546	2	0	0.999	0.001
ABCC2	1249 G>A	V417I	542	336	170	36	0.78	0.22
ABCC2	3972 G>A	I1324I	492	198	247	47	0.65	0.35
ABCG2	421 C>A	Q141K	507	401	101	5	0.89	0.11
CYP3A4	−329 A>G (*1B)	Promoter	540	473	49	18	0.92	0.08
CYP3A4	1334 T>C (*3)	M445T	516	510	6	0	0.99	0.01
CYP3A5	6986 A>G (*3C)	Splice variant	76	449	547	22	0.11	0.89
CYP3A5	14690 G>A (*6)	Splice variant	545	533	12	0	0.99	0.01
DPYD	IVS14 + 1 G>A (*2A)	Intron 4	544	540	4	0	0.996	0.004
DPYD	1627 A>G (*5)	I543V	520	342	155	23	0.81	0.19
DPYD	2194 G>A (*6)	V732I	546	498	45	3	0.95	0.05
DPYD	85 T>C (*9A)	C29R	546	319	192	35	0.76	0.24
ERCC1	354 C>T	N118N	521	188	238	95	0.59	0.41
ERCC2	−1989 A>G	Promoter	402	148	164	90	0.57	0.43
ERCC2	2133 C>T	D711D	512	256	188	68	0.68	0.32
ERCC2	2251 A>G	K751Q	547	236	235	76	0.65	0.35
GSTM1	*0	Null	548	N/A	N/A	N/A	53%	47%‡
GSTP1	2293 C>T	A114V	547	474	73	0	0.93	0.07
GSTP1	1578 A>G	I105V	463	194	220	49	0.66	0.34
MTHFR	677 C>T	A222V	541	263	225	53	0.69	0.31
MTHFR	1298 A>C	E429A	537	258	223	56	0.69	0.31
MTHFR	1793 G>A	R549Q	545	490	51	4	0.95	0.05
TYMS	1494del	3UTR	546	257	223	66	0.67	0.33
TYMS	TSER	Enhancer	321	103	132	86	0.53	0.47§
UGT1A1	−3156 G>A (*93)	Enhancer	520	262	217	41	0.71	0.29
UGT1A1	(TA)nTAA (*28)	Promoter	468	224	206	32	0.703	0.291¶
XRCC1	1196 G>A	R399Q	534	231	239	64	0.66	0.34

Abbreviations: wt, wild type; var, variant; p, wild-type allele frequency; q, variant allele frequency.
†*ABCB1* 2677 is a tri-allelic (2677 G>T/A) variant with an A allele (threonine allele) frequency of 2%.
‡*GSTM1* *0 is a deletion polymorphism. Results are stated as percentage of patients with at least one copy of the gene (wt) versus patients with a homozygous gene deletion (var).
§*TYMS* TSER is a tandem repeat polymorphism. Results are stated as three copies of the repeat (wt) or two copies of the repeat (var).
¶*UGT1A1* (TA)nTAA is a repeat polymorphism. Results are stated as six copies of the repeat (wt) or seven copies (var). Alleles containing five or eight copies of the repeat were found at a combined allele frequency of 0.6%.

declare statistical significance for any finding; *P* values from .01 to .05 were considered promising. Given the multitude of statistical tests performed, no positive association should be considered definitive. There were at least 1,088 tests (34 variants × eight outcomes [five toxicity, three efficacy] × four populations [overall, IFL, FOLFOX, IROX]) excluding interaction testing.

Correlation of dichotomous outcomes and genotype status were tested using χ^2 tests; odds ratios and associated 95% CIs were reported as estimates of the association. Univariate logistic regression models were used to explore the association between continuous variables and binary outcomes; multivariate logistic regression was used to adjust for known risk factors (performance status, sex, age). Cox proportional hazards models were used to assess the relationship between polymorphisms and the time to event outcomes, time to progression, and OS. *UGT1A1*28* was tested as a three-level variable (6/6, 6/7, and 7/7 genotypes) using a Cochran-Armitage test for trend for association with categorized outcomes (toxicity and tumor response). Because paresthesias associated with oxaliplatin-based therapy are cumulative and dose-dependent, we also performed analyses for the association between genetic variants and time to the development of neurotoxicity.

RESULTS

Written informed consent for pharmacogenetic studies was obtained from 547 (49%) of the 1,118 patients accrued to N9741 from the time of the amendment to include blood sampling. Usable specimens were obtained from 520 (95%) of the 547 patients: 114 on IFL, 299 on FOLFOX4, and 107 on IROX. A total of 92 (81%) of the 114 patients treated with IFL received the reduced IFL doses, in which IRN was reduced from 125 to 100 mg/m^2/dose and FU was reduced from 500 to 400 mg/m^2/dose. However, because of protocol-specified toxicity-based dose reductions before the protocol amendment, the two IFL cohorts received similar total cumulative doses of IRN. Table 1 compares demographics for patients participating or not in the pharmacogenetic studies. No differences are present in the demographic features, incidence of

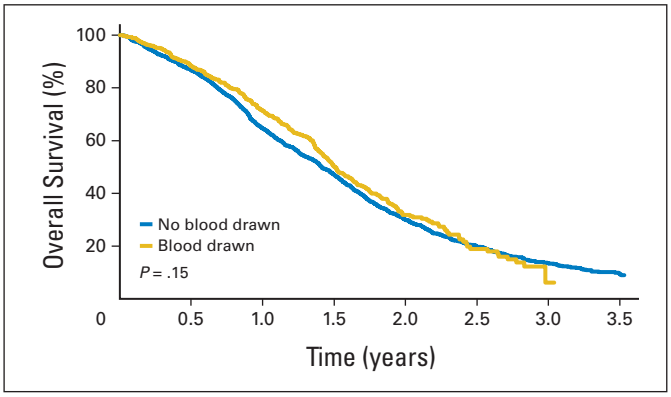

Fig 1. Graphical demonstration that overall survival was not different between patients with blood samples for pharmacogenetic study and patients without blood sampling. Blue line, patients who had no blood drawn; gold line, patients who had blood drawn.

severe systemic toxicity, PFS, or OS of patients with and without blood samples (Table 1, Fig 1).

A total of 34 variants in 15 genes were assessed (18,598 genotypes). All variants were polymorphic in the patient population. All variants were in Hardy-Weinberg equilibrium after correction for ethnicity. The observed allele frequency of all variants was consistent with that in previously published literature. The frequency of a homozygous rare variant genotype ranged from less than 0.1% (*DPYD*2A*) to 47% (*GSTM1* deletion; Table 2). Of note, a *DPYD*2A* heterozygous genotype occurred in 0.74% of patients. Statistically significant ethnic differences between whites and African Americans were present for 10 (29%) of 34 variants.

Adverse Events

A complete set of associations between each SNP and each adverse event considered, overall and by treatment arm, is included in the Data Supplement. *UGT1A1*28* ($P = .003$) and *UGT1A1* −3156 C>T ($P < .001$) were associated with an increased rate of grade ≥ 4 neutropenia, and *UGT1A1* −3156 C>T with an increased rate of grade 3 febrile neutropenia ($P = .005$), pooling across all study arms. Further associations with adverse events were specific to each treatment arm on the study.

Neutropenia. The presence of the homozygous *UGT1A1*28* allele (seven T/A repeats) was observed in 9% of patients and was associated with the risk of grade 4 neutropenia (Fig 2A). In patients receiving the IFL regimen, the impact of *UGT1A1*28* was modest, with a risk of 18% grade 4 neutropenia in the homozygous patients ($P = .25$). The effect was more dramatic in patients receiving the IROX regimen, where 55% of homozygous patients experienced grade 4 neutropenia compared with 10% in six of six patients and 15% in six of seven patients ($P = .002$). *UGT1A1*28* homozygous patients on the FOLFOX regimen also had a trend toward an elevation in grade 4 neutropenia risk ($P = .11$). The *UGT1A1* −3156 G>A putative 5′ promoter enhancer module SNP (*UGT1A1*93*) also displayed similar predictive results, consistent with the high degree of linkage between this variant and *UGT1A1*28*.

*GSTM1*0* was observed in 47% of patients (Table 2). Differential associations with grade 4 neutropenia were seen across the treatment arms. Severe neutropenia in patients on the IFL and IROX arms did not appear to be different based on *GSTM1*0* genotype, whereas

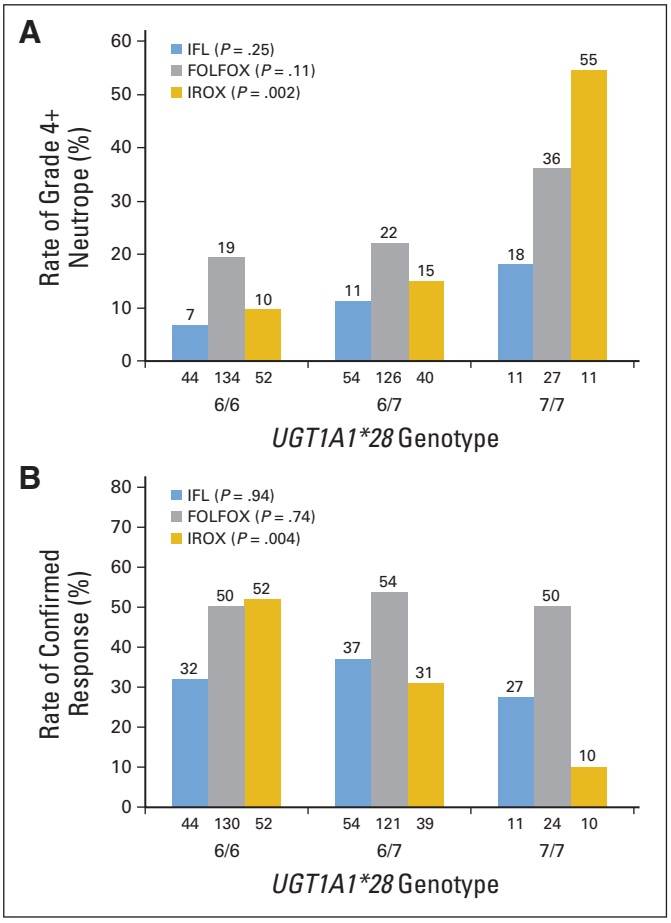

Fig 2. *UGT1A1*28* genotype and severe neutropenia (A) or confirmed response (B). IFL, fluorouracil (FU) + irinotecan (IRN); FOLFOX, FU + oxaliplatin; IROX, IRN + oxaliplatin.

FOLFOX-treated patients with *GSTM1*0* had a 1.7-fold higher risk compared with patients with an intact gene (28% *v* 16%; $P = .016$; Fig 3). The *GSTP1* status (variants at either codon 105 or 114) was related to the risk of grade ≥ 3 febrile neutropenia in patients treated with

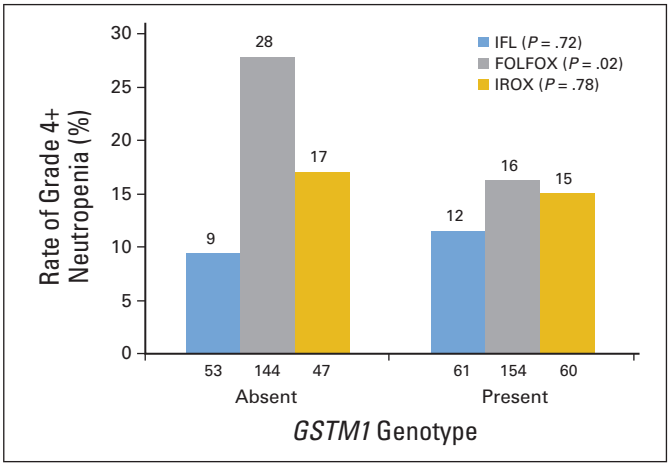

Fig 3. Relationship between *GSTM1*0* and grade 4 neutropenia. IFL, fluorouracil (FU) + irinotecan (IRN); FOLFOX, FU + oxaliplatin; IROX, IRN + oxaliplatin.

FOLFOX, with a rate of 14% in patients with the T/T genotype compared to 3% in the other patients ($P < .001$).

Neurotoxicity. Severe paresthesias were therapy-limiting adverse events in patients receiving oxaliplatin on either the FOLFOX or IROX arms. Indeed, 61% of patients in this cohort came off therapy for reasons other than progressive disease on the FOLFOX arm.[18] Thus, we tested the hypothesis that genetic variation in either excision repair or detoxification of platinum adducts would be associated with risk of oxaliplatin-related peripheral neuropathy. Genetic variants in *GSTM1*, *XRCC1*, *ERCC1*, and *ERCC2* were not associated with neurotoxicity. Patients with a *GSTP1* I105V genotype of T/T were more likely to discontinue FOLFOX because of neurotoxicity (24% *v* 10% for other; $P = .01$). In addition, in patients treated with IROX, only patients with T/T genotype experienced grade 3 neurotoxicity (eight of 43, 18.6% *v* none of 54, 0%; $P = .003$). This trend, however, was not present in FOLFOX-treated patients, where the rate of grade ≥ 3 neurotoxicity was similar in T/T and other patients (11.7% and 11.9% respectively; $P = .70$).

Vomiting. In patients treated with IROX, *UGT1A1* −3156 C>T was associated with the rate of grade 3 vomiting, with rates of 71.4% in *UGT1A1*93* (A/A) patients, as opposed to 22.1% in the comparative group ($P = .004$).

Efficacy

A complete set of associations between each SNP and the efficacy outcomes of response rate (RR), time to progression, and OS, overall and by treatment arm, is included in the Data Supplement. Pooling all arms, only *TYMS* 1494del had univariate prognostic significance for confirmed tumor response (RR, 51.7% for A/A patients, 39.2% for other; $P = .0051$). This was present in all arms with virtually identical magnitude (IFL, 41% *v* 29%; FOLFOX, 58% *v* 46%; and IROX, 45% *v* 33%). Within treatment arms, the presence of *CYP3A5*3C* was significantly associated with confirmed response on IFL (RR, 29% *v* 60%; $P = .0074$; Fig 4). No SNPs were significant predictors of PFS or OS, overall or in the three treatment arms individually.

The presence of a *UGT1A1*28* allele did not impact the confirmed RR in the IFL or FOLFOX arms ($P = .75$ and $P = .76$, respectively; Fig 2B). *UGT1A1*28/*28* patients on the IROX arm had a trend toward a decreased RR ($P = .02$). Neither the *TYMS* enhancer region 28 base pair tandem repeat (TSER) nor the 3′ UTR 6 base pair deletion (1494del) were associated with tumor RR or other measures of outcome in any of the three treatment arms (data not shown).

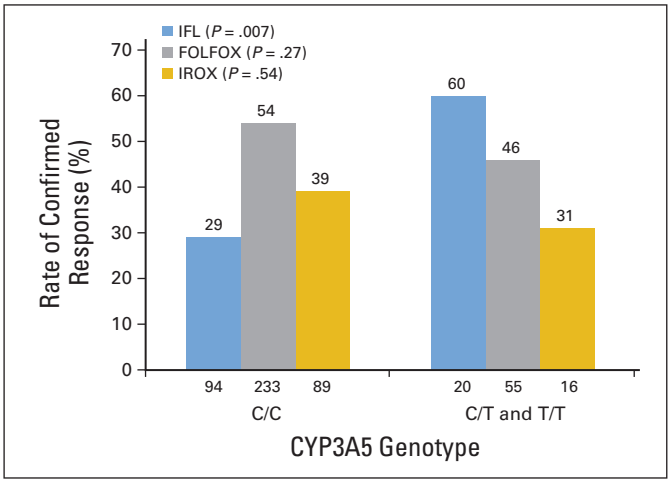

Fig 4. Relationship between *CYP3A5*3C* genotype and confirmed response. IFL, fluorouracil (FU) + irinotecan (IRN); FOLFOX, FU + oxaliplatin; IROX, IRN + oxaliplatin.

DISCUSSION

All biomarkers must be considered in the context of biologic plausibility as we strive to clarify their associations with clinical outcome. In this study, the impact of pharmacogenetic predictors of adverse events or efficacy was largely dependent on the specific therapeutic regimen being used. This should be no surprise, in that factors such as drug dose and concomitant therapy will have an important influence on the predictive power of any genetic variant relevant to drug transport, activation, or metabolism. This was illustrated by our findings relating the *UGT1A1*28* variant and neutropenia. The risk of severe neutropenia was not dramatically influenced by UGT1A1 genotype in the IFL arm, where the dose of IRN in most patients was 100 mg/m^2 weekly for 4 of 6 weeks. However, a clinically significant interaction with *UGT1A1*28* was evident in patients receiving IROX for both neutropenia and vomiting. The administration of IRN in the IROX regimen at twice the dose of the IFL regimen, and with a concomitant marrow toxin (oxaliplatin), was associated with an unacceptable incidence of adverse events in the homozygous *UGT1A1*28* patients.[19]

This matrix effect is particularly important in light of the US Food and Drug Administration changes to the IRN package insert, where *UGT1A1*28* genotype is included in the risk variables. The N9741 data suggest that the IRN package insert requires greater precision about the factors that would make *UGT1A1*28* genotype analysis required for patient management.[19] The need for greater clinical nuance is a theme that is also seen for the genetic prediction of warfarin dose, which is greatly strengthened when applied in the context of demographic and clinical factors, such as patient body size and interacting medications.[20,21] There is a strong need to define a clear pathway for translating genetics into clinical guidance, or we will be buried under an enormous number of potentially useful markers.

Detoxification by glutathione has been shown to influence platinum DNA adduct levels in preclinical models and response in small clinical cohorts. Our data demonstrated no clear relationship between genetic variants in either *GSTP1* or *GSTM1* and tumor response. However, the *GSTP1* T/T genotype appears to influence toxicity risk in the oxaliplatin-containing regimens. The *GSTP1* T/T genotype was associated with a heightened risk of both neutropenia and need to discontinue therapy due to neurotoxicity in patients on FOLFOX therapy. Patients with the *GSTP1* T/T genotype on IROX also had a higher incidence of neurotoxicity. Because oxaliplatin-associated neurotoxicity is a major reason for discontinuing active chemotherapy for CRC, tools that prospectively identify patients at high risk for toxicity, if validated, would be clinically useful.

In our analysis, *CYP3A5* genetic variants appear to identify patient groups with differential likelihood of tumor response from IFL treatment. Co-administration of drugs or herbs that inhibit CYP3A enzymes alter IRN pharmacokinetics and are associated with toxicity. However, to the best of our knowledge, this is the first study to evaluate the impact of *CYP3A5* genetic variants in a large patient cohort. The association with *CYP3A5* and response to IFL, but not FOLFOX or IROX, also gives pharmacologic plausibility to this association. The

differences in RR (29% *v* 60%), if validated, would provide valuable information for clinical discussions with patients but not to the point of denying patients access to IRN therapy.

An important outcome of this study is that it clearly establishes that pharmacogenetic studies can be successfully conducted in large, multicenter, international research studies. The study was successfully performed at small community practice sites, as well as larger community clinical oncology programs and comprehensive cancer centers. Indeed, most large clinical trials in the National Cancer Institute cooperative group system now include sampling of peripheral blood for pharmacogenetics.[22] The data from this study are an initial assessment of the utility and potential for large collections of germline DNA to enhance our understanding of disease and patient management.

This study assessed genetic variation only in the germline DNA from blood. While this approach has utility for both toxicity and efficacy end points, there will be important sources of genetic variation in drug effect that are missed. Specifically, somatic mutations in tumor tissue (or cancer cells shed into blood or stool) were not assessed because of the lack of availability of tumor tissue for most patients enrolled. The recently detailed impact of *KRAS* mutations on the activity of epidermal growth factor receptor antagonists clearly demonstrates the importance of including tissue banking as part of large clinical trials to provide an avenue to both discovery and validation of clinically important markers.[23]

This study focused on gene variants from published associations with adverse events or antitumor activity.[5-7] Most of these previous reports were small (< 100 patients), single-institution studies, with no randomized comparisons to give context to the results. Not surprisingly, most of the previous findings were not replicated in this study. In some cases, the explanation is likely to be one of logistics, such as the differences in IRN dosing[7] or FU schedule.[24,25] However, many of the previous findings may be false positives, possibly because of issues of multiple comparisons, nonrepresentative patient samples, the fact that multicenter studies such as N9741 also incorporate the subtle variations in patient management that are masked when only a limited number of centers are included, or the absence of a validation cohort.[26] These include *ABCB1* 3435 C>T, *CYP3A4*1B*, and *MTHFR* 677 C>T.

As we consider the critical need to discover and validate predictive markers, there will also be additional genes of pharmacologic importance that emerge from current scientific investigations, including drug transporters. Putative predictive markers must not be considered in isolation, but rather the analysis must include other important factors, including pathologic findings and patient demographics. The use of robust data sets will allow greater clarity in our quest for predictors of patient therapy.

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