CYP2C9 and CYP2C19 Polymorphic Forms Are Related to Increased Indisulam Exposure and Higher Risk of Severe Hematologic Toxicity
Anthe S. Zandvliet,1 Alwin D.R. Huitema,1 William Copaalu,3 Yasuhide Yamada,4 Tomohide Tamura,4 Jos H. Beijnen,1,5 and Jan H.M. Schellens2,5

Abstract Purpose: The anticancer agent indisulam is metabolized by the cytochrome P450 of enzymes CYP2C9 and CYP2C19. Polymorphisms of these enzymes may affect the elimination rate of indisulam. Consequently, variant genotypes may be clinically relevant predictors for the risk of developing severe hematologic toxicity. The purposes of this study were to evaluate the effect of genetic variants of CYP2C9 and CYP2C19 on the pharmacokinetics of indisulam and on clinical outcome and to assess the need for pharmacogenetically guided dose adaptation.

Experimental Design: Pharmacogenetic screening of CYP2C polymorphisms was done in 67 patients treated with indisulam. Pharmacokinetic data were analyzed with a population pharmacokinetic model, in which drug elimination was described by a linear and a Michaelis-Menten pathway. The relationships between allelic variants and the elimination pharmacokinetic parameters (CL, V_max, K_m) were tested using nonlinear mixed-effects modeling. Polymorphisms causing a high risk of dose-limiting neutropenia were identified in a simulation study.

Results: The Michaelis-Menten elimination rate (V_max) was decreased by 27% (P < 0.0001) for heterozygous CYP2C9*3 mutants. Heterozygous CYP2C19*2 and CYP2C19*3 mutations reduced the linear elimination rate (CL) by 38% (P < 0.0001). The risk of severe neutropenia was significantly increased by these mutations and dose reductions of 50 to 100 mg/m^2 per mutated allele may be required to normalize this risk.

Conclusions: CYP2C9*3, CYP2C19*2, and CYP2C19*3 polymorphisms resulted in a reduced elimination rate of indisulam. Screening for these CYP2C polymorphisms and subsequent pharmacogenetically guided dose adaptation may assist in the selection of an optimized initial indisulam dosage.

Indisulam is a sulfonylamine anticancer agent that disrupts cell cycle progression in the G1-S transition (1–3). Indisulam was well tolerated, but had only moderate single-agent activity in several phase II studies (3–8). The compound is currently being evaluated as an adjuvant to standard chemotherapy in multiple phase II studies for the treatment of solid tumors. Phase I studies showed that reversible neutropenia and thrombocytopenia were the dose-limiting toxicities of indisulam (9–14). The pharmacokinetic properties of the compound have been extensively studied (9–16). Drug clearance decreased with increasing dose, which was indicative for the saturable elimination of indisulam. A semiphysiologic population pharmacokinetic-pharmacodynamic model was developed, which included two parallel pathways for drug elimination: a saturable pathway with Michaelis-Menten kinetics and a linear pathway (16, 17). The interindividual variability of the maximal rate of Michaelis-Menten elimination (V_max) was 45%. Differences between patients in hepatic metabolic capacity account for this variability. The pharmacokinetic-pharmacodynamic model showed a clear relationship between pharmacokinetics and hematologic toxicity (17). Patients with impaired metabolic capacity may have a relatively high risk of severe myelosuppression due to higher drug exposure.

Results of a clinical mass balance study showed that more than 98% of indisulam is metabolized before it is excreted into the urine or feces (18). No data regarding the activity or toxicity of the metabolites are available. The major metabolite, O-glucuronide indisulam, is formed by glucuronidation of a hydroxyl metabolite of indisulam (18, 19). The hydroxyl metabolite is highly reactive and is immediately conjugated to form O-glucuronide or O-sulfate indisulam. Therefore, the formation of this hydroxyl metabolite may be a rate-limiting process in the metabolism of indisulam.

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Hydroxylation of indisulam is catalyzed by cytochrome P450 enzymes. The contribution of CYP isoenzymes in CYP-dependent metabolism of indisulam was studied previously using human recombinant isozymes. Taking into account the human liver microsome content of each isozyme (20), it was estimated that CYP2C9 had the highest contribution in indisulam metabolism, followed by CYP2C19 (study report, Eisai Co., Ltd., 2002). Concisely, hydroxylation by CYP2C9 and CYP2C19 may be rate limiting for the metabolism of indisulam.

Polymorphisms in the CYP2C genes may affect the rate of elimination of indisulam. Consequently, treatment outcome may be altered. Furthermore, variant genotypes may be clinically relevant predictors for the risk of severe hematologic toxicity. The purpose of the current study was to evaluate the effect of allelic variants of CYP2C9 and CYP2C19 on indisulam pharmacokinetics in cancer patients.

Materials and Methods

Clinical studies. Indisulam pharmacokinetics have been extensively studied during a phase I program that consisted of seven clinical studies (9–14). Indisulam was administered at various dose levels in 1- or 2-h infusions every 3 weeks (Table 1). Full pharmacokinetic sampling was done during the first treatment cycle, and hematologic parameters were monitored twice weekly. In a pharmacogenetic substudy, which was done in three out of these seven trials, patients were screened for CYP2C allelic variants. Both Caucasian and Japanese patients were included in the substudy because mutant allele frequencies of CYP2C9 were expected to be high in Caucasians and mutant allele frequencies of CYP2C19 were expected to be high in Japanese populations.

Pharmacokinetic and genetic data from this substudy were the primary focus of the present pharmacogenetic analysis. In addition, pharmacokinetic data from the remaining four clinical trials were used to precisely determine the pharmacokinetic characteristics of indisulam. The study protocols were approved by medical ethics committees of the centers in which the studies were carried out. Written informed consent was obtained from each patient.

Bioanalysis. Indisulam plasma concentrations of the Caucasian patients were measured using a validated high-performance liquid chromatography (HPLC) method coupled with electrospary ionization tandem mass spectrometry (21). In the Japanese population, concentrations were measured in plasma, plasma ultrafiltrate, and erythrocytes. After sample pretreatment, a validated HPLC method with UV detection was used for quantification of indisulam (14). Both methods were extensively validated and cross-validated according to Food and Drug Administration guidelines (22). Considering the successful cross-validation of the two methods, we did not discriminate between these methods during data analysis.

Genotyping analysis. Pharmacogenetic screening of the Caucasian patients was done for the *2, *3, *4, and *6 mutations of CYP2C9 and for the *2, *3, *4, *5, and *6 polymorphisms of CYP2C19. DNA was isolated from peripheral lymphocytes using the Nucleon BACC kit (Amersham Life Science) and Qiagen kits. Fluorescent allele specific hybridization was used to determine the genotype for CYP2C9*2 and CYP2C9*3. An amplification refractory mutation system (ARMS) was applied for CYP2C19*3 and CYP2C9*5. The remaining mutations were determined by real-time PCR methods. Japanese patients were genotyped for the *2 and *3 mutations of CYP2C9 and CYP2C19 as described by Yamada et al. (14).

Population pharmacogenetic data analysis. Pharmacokinetic data of indisulam were analyzed with a previously developed population pharmacokinetic model using NONMEM (version V, level 1.1; Globomax LLC). The analysis was done using the first-order estimation method in NONMEM after logarithmic data transformation. The population pharmacokinetic model described the distribution and elimination of indisulam for various dosage levels and administration regimens in both Japanese and Caucasian patients (16). The elimination model comprised two parallel pathways: a linear pathway (described by the linear clearance CL) and a saturable Michaelis-Menten pathway (described by a maximal elimination rate, \( V_{\text{max}} \), and a Michaelis-Menten constant \( K_{m} \).)

In the current study, the elimination model was extended to evaluate the impact of CYP2C polymorphisms on the pharmacokinetics of indisulam. The relationships between mutations of the CYP2C9 and CYP2C19 genes and each of the three elimination pharmacokinetic parameters (CL, \( V_{\text{max}} \), and \( K_{m} \)) were verified.

Allelic variants were incorporated in the population model as covariate relationships according to Eq. A. A pharmacokinetic parameter \( P \) had a typical value of \( P_{\text{pop}} \) in wild-type patients. The typical value of heterozygous patients was equal to \( P_{\text{pop}} \) reduced by \( \times 100\% \). Homozygous mutations were assumed to have twice the impact of heterozygous mutations, and the corresponding typical value of \( P \) was reduced by \( 2 \times 100\% \) as compared with wild-type:

\[
P = P_{\text{pop}} \times (1 - (\theta \times \text{heterozygous} + 2 \times \theta \times \text{homozygous}))
\]

(\( A \))

Polymorphisms that were observed in the study population at a frequency >2% were tested for their effect on the linear elimination of indisulam (CL) and for their impact on the saturable elimination pathway (\( V_{\text{max}} \) and/or \( K_{m} \)). Discrimination between models with and without a pharmacogenetic effect was based on the log-likelihood ratio test. A P value of 0.001 was considered statistically significant. This univariate analysis was followed by a multivariate test. After inclusion of the pharmacogenetic relationships that were statistically significant in the univariate analysis, a backward elimination procedure was done. Only effects of allelic variants that were

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**Table 1. Patient characteristics and dose levels**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasian</th>
<th>Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>46</td>
<td>21</td>
</tr>
<tr>
<td>Primary tumor type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Pancreas carcinoma</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Gastrointestinal carcinoma</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma of unknown primary site</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Patient characteristics</td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>25/21</td>
<td>15/6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 (43-119)</td>
<td>61 (44-79)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 (153-193)</td>
<td>165 (149-180)</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.88 (1.36-2.23)</td>
<td>1.68 (1.39-1.94)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>59 (19-81)</td>
<td>57 (35-70)</td>
</tr>
<tr>
<td>Dose level (mg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>11 (1-h infusion)</td>
<td>—</td>
</tr>
<tr>
<td>400</td>
<td>14 (1-h infusion)</td>
<td>—</td>
</tr>
<tr>
<td>500</td>
<td>12 (1-h infusion)</td>
<td>3 (1-h infusion)</td>
</tr>
<tr>
<td>700</td>
<td>7 (1-h infusion)</td>
<td>6 (2-h infusion)</td>
</tr>
<tr>
<td>800</td>
<td>2 (1-h infusion)</td>
<td>6 (2-h infusion)</td>
</tr>
<tr>
<td>900</td>
<td>—</td>
<td>3 (2-h infusion)</td>
</tr>
</tbody>
</table>
The recommended indisulam dosage of 700 mg/m². For each patient, the at least 1 week. With this model, patients were simulated to receive the estimated (relative SE <10%). The simulated group guaranteed that the relative risk could be precisely estimated (relative SE <10%).

Covariate relationships between patient characteristics and pharmacokinetic parameters that were previously identified were also included in the extended pharmacogenetic model (16). Hence, the value of V_max was not only dependent on genotype, but also on the body surface area. Moreover, the linear clearance was not only dependent on genotype, but also on race. A multivariate analysis was done to verify whether genotype could replace race to explain a difference in CI between Caucasian and Japanese patients.

Assessment of clinical relevance. After establishment of statistically significant pharmacogenetic relationships, the clinical relevance of the effects of polymorphisms were assessed in the study population. The dose-limiting toxicities of indisulam were hematologic side effects such as neutropenia and thrombocytopenia (13). We evaluated the role of CYP2C genotypes in the occurrence of grade 4 neutropenia in the patients who participated in this study. Significant relationships between CYP2C polymorphisms and observed nadir neutrophil counts were identified by the Kruskal-Wallis test using SPSS for Windows (version 11.0.1, SPSS Inc.).

Dose adaptation. The simulation study as described above was repeated with adapted dosages of indisulam to determine the dose reduction needed to normalize the risk of dose-limiting neutropenia in patients with high-risk CYP mutations. This analysis aimed at the development of a straightforward method for dose adaptation, which is feasible for implementation in clinical practice.

Results
Pharmacokinetic samples were obtained from a total of 213 patients. A subpopulation of 21 Japanese and 46 Caucasian patients from three clinical studies was genotyped for polymorphisms in CYP2C9 and CYP2C19 genes. Patient characteristics and administered doses of this subpopulation are listed in Table 1. The variant alleles CYP2C9*2, CYP2C9*3, CYP2C9*5, CYP2C19*2, CYP2C19*3, and CYP2C19*4 were observed in the study population. Genotype frequencies are listed in Table 2. Observed allele frequencies in the current study were consistent with previously reported data in both the Caucasian and the Japanese subpopulation (25–32).

Effect of genomic variants on indisulam exposure. Drug exposure was generally increased in patients with CYP2C9 and/or CYP2C19 mutations, which indicates that indisulam elimination was reduced by some of the CYP2C polymorphisms. This is shown in Fig. 1 for all patients who received 500 mg/m² indisulam. Plasma concentration versus time curves show a clear discrimination between mutants (both CYP2C9 and CYP2C19) and wild types at this dose level (Fig. 1A). The area under the concentration-versus-time curve (AUC) were higher for mutants than for wild-type patients (Fig. 1B). Similar plots of other dose levels showed a marked effect of the CYP2C19*2 variant. Patients who were heterozygous for this mutation generally had substantially higher AUC values than wild-type patients. Exposure was further increased in homozygous CYP2C19*2 mutants (data not shown).

To our knowledge, for the first time, the CYP2C9*5 polymorphism was observed in a Caucasian individual. The AUC of indisulam in this patient after administration of 600 mg/m² in a 1-h infusion (2.512 mg/L/h) was almost twice higher than the AUC of three wild-type Caucasian patients who had received the same dosage (AUC range 1.035-1.458 mg/L/h).

Table 1. Observed genotype and allele frequencies of the polymorphisms under study

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Nucleotide change (cDNA)</th>
<th>Effect</th>
<th>Caucasian population</th>
<th>Japanese population</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>C420T</td>
<td>R144C</td>
<td>35 10 1 13</td>
<td>21 0 0 0</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>A1075C</td>
<td>I359L</td>
<td>36 9 1 12</td>
<td>19 2 0 4.8</td>
</tr>
<tr>
<td>CYP2C9*5</td>
<td>C1080G</td>
<td>D360E</td>
<td>45 1 0 1.1</td>
<td>21 0 0 0</td>
</tr>
<tr>
<td>CYP2C9*6</td>
<td>818 Del A</td>
<td>Frame shift</td>
<td>46 0 0 0 0</td>
<td>21 0 0 0</td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>G681A</td>
<td>Splicing defect</td>
<td>37 9 0 9.8</td>
<td>10 7 4 36</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>G636A</td>
<td>W212X</td>
<td>46 0 0 0</td>
<td>18 3 0 7.1</td>
</tr>
<tr>
<td>CYP2C19*4</td>
<td>A1G</td>
<td>Initiation</td>
<td>45 1 0 1.1</td>
<td>21 0 0 0</td>
</tr>
<tr>
<td>CYP2C19*5</td>
<td>C1297T</td>
<td>R433W</td>
<td>46 0 0 0</td>
<td>21 0 0 0</td>
</tr>
<tr>
<td>CYP2C19*6</td>
<td>G395A</td>
<td>R132Q</td>
<td>46 0 0 0</td>
<td>21 0 0 0</td>
</tr>
</tbody>
</table>

Abbreviations: Wt, wild type; Het, heterozygous mutant; Hom, homozygous mutant.

significant in the multivariate analysis (P < 0.001) were included in the final pharmacokinetic model.

The CYP2C19*2 and CYP2C19*3 mutations were both known to result in nonfunctional protein (23, 24). Therefore, it would not be plausible to expect different effects of these mutations on indisulam metabolism, and consequently, we did not discriminate between these mutations in the statistical analysis.

Covariate relationships between patient characteristics and pharmacokinetic parameters that were previously identified were also included in the extended pharmacogenetic model (16). Hence, the value of V_max was not only dependent on genotype, but also on the body surface area. Moreover, the linear clearance was not only dependent on genotype, but also on race. A multivariate analysis was done to verify whether genotype could replace race to explain a difference in CI between Caucasian and Japanese patients.

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To our knowledge, for the first time, the CYP2C9*5 polymorphism was observed in a Caucasian individual. The AUC of indisulam in this patient after administration of 600 mg/m² in a 1-h infusion (2.512 mg/L/h) was almost twice higher than the AUC of three wild-type Caucasian patients who had received the same dosage (AUC range 1.035-1.458 mg/L/h).
Population pharmacogenetic data analysis. The CYP2C9*2, CYP2C9*3, CYP2C19*2, and CYP2C19*3 mutations occurred at a frequency >2% and were therefore included in the statistical analysis to evaluate their effect on indisulam pharmacokinetics. In the univariate analysis, the relationships between the CYP2C9*3, CYP2C19*2, and CYP2C19*3 mutations and the Michaelis-Menten elimination rate ($V_{\text{max}}$) were statistically significant. The CYP2C19*2 and CYP2C19*3 polymorphisms also significantly reduced the linear clearance (CL).

The Michaelis-Menten constant ($K_m$) was not significantly affected by any of the polymorphisms.

Upon multivariate evaluation of the univariately selected pharmacogenetic effects, the racial difference in CL was not significantly different from 1 and was therefore excluded from the model. The CYP2C9*3 allelic variant was shown to significantly impair the saturable metabolism of indisulam by a typical 27% reduction of $V_{\text{max}}$ in heterozygous mutants ($P < 0.0001$). The relationships between the CYP2C19 mutations and $V_{\text{max}}$ were not significant in the multivariate analysis and were excluded during the backward elimination procedure. The CYP2C19*2 and CYP2C19*3 polymorphisms resulted in significant reductions of linear elimination of indisulam ($P < 0.0001$), and the typical value of CL was decreased by 38% in heterozygous patients. The final model included two pharmacogenetic effects: CYP2C9*3 ($\sim V_{\text{max}}$) and CYP2C19*2/CYP2C19*3 ($\sim \text{CL}$; Table 3).

Clinical relevance of pharmacogenetic effects. Indisulam clearance was typically reduced in patients with one or more of the genomic variants CYP2C9*3, CYP2C19*2, and CYP2C19*3. Consequently, these patients showed a higher exposure to indisulam. The CYP2C9*3, CYP2C19*2, and CYP2C19*3 polymorphisms were thus expected to cause a higher risk of grade 4 neutropenia.

Data on the occurrence of hematologic toxicity were available for all patients who were included in the pharmacogenetic substudy. Eight patients had grade 4 neutropenia at cycle 1: three Japanese and five Caucasian patients (Fig. 2). Two Japanese patients who received the highest dose level of 900 mg/m$^2$ indisulam had grade 4 neutropenia. Both patients had the CYP2C19*2 mutation; one patient was homozygous and had a nadir neutrophil count as low as $0.018 \times 10^9$/L, and the other patient was heterozygous. A third Japanese patient with a neutrophil count below $0.5 \times 10^9$/L was heterozygous for CYP2C19*2 and was treated with 800 mg/m$^2$ indisulam. Two Caucasian patients had received 800 mg/m$^2$ indisulam; one was homozygous for the CYP2C9*3 mutation and had severe dose-limiting neutropenia during 2 weeks, whereas the other patient had a wild-type genotype and a neutrophil count below $0.5 \times 10^9$/L at a single occasion. Clinical data are depicted in Fig. 2. At the higher dose levels, nadir neutrophil counts decreased with increasing dose level and with an increasing number of influential mutations. Due to small patient numbers, significant relationships between CYP2C polymorphisms and observed nadir neutrophil counts could not be shown. For the same reason, relative risks of severe neutropenia

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Effect</th>
<th>Effect size (%)</th>
<th>95% CI* (%)</th>
<th>$P$ $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>No significant effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>Reduction of $V_{\text{max}}$</td>
<td>27</td>
<td>13-40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>Reduction of CL</td>
<td>38</td>
<td>31-45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>Insufficient data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*4</td>
<td>Insufficient data</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The 95% confidence interval was established by likelihood profiling.

$^1$ The log-likelihood ratio test was used to calculate the $P$ value.
for the various mutations could not adequately be determined using clinical data.

**Simulation study of dose-limiting neutropenia.** The hypothesis that CYP2C9*3, CYP2C19*2, and CYP2C19*3 polymorphisms may cause a higher risk of dose-limiting neutropenia was confirmed by the results of the simulation study listed in Table 4. These results show that the risk of dose-limiting neutropenia was increased by 40% or more in simulated patient groups with a single polymorphism. Homozygous mutations or combinations of multiple heterozygous mutations may result in a relative risk of serious toxicity of more than 2.

**Dose adaptation.** The CYP2C19*2 and CYP2C19*3 mutations caused a larger increase of the risk of dose-limiting neutropenia than CYP2C9*3. Consequently, the dosage of indisulam should be adapted to a larger extent for CYP2C19*2 and CYP2C19*3 than for CYP2C9*3. Each CYP2C19 mutation required a dose reduction of 100 mg/m². The recommended dose reduction for a CYP2C9*3 mutation was 50 mg/m². A simulation study showed that this guidance for dose adaptation resulted in the normalization of the relative risk of severe neutropenia (Table 4).

**Table 4.** Simulated relative risk of dose-limiting neutropenia after administration of indisulam in a 1-h infusion and the 95% confidence intervals (in brackets)

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Caucasian heterozygous</th>
<th>Homozygous</th>
<th>Japanese heterozygous</th>
<th>Homozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard dose: 700 mg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>1.39 (1.21-1.77)</td>
<td>2.24 (1.45-3.27)</td>
<td>1.47 (1.11-1.60)</td>
<td>NA</td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>1.86 (1.69-2.08)</td>
<td>NA</td>
<td>1.97 (1.73-2.33)</td>
<td>4.51 (3.31-6.25)</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>1.04 (0.78-1.06)</td>
<td>NA</td>
<td>1.07 (0.86-1.20)</td>
<td>1.01 (0.69-1.62)</td>
</tr>
<tr>
<td>Adjusted dose: 700 mg/m²—recommended dose reduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>1.03 (0.82-1.29)</td>
<td>1.30 (0.81-2.18)</td>
<td>1.09 (0.79-1.24)</td>
<td>NA</td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>1.04 (0.78-1.06)</td>
<td>NA</td>
<td>1.07 (0.86-1.20)</td>
<td>1.01 (0.69-1.62)</td>
</tr>
</tbody>
</table>

Note: Each subpopulation consisted of at least 10,000 simulated patients. These genotypes were not observed in the study population. Abbreviation: NA, not applicable.

**Discussion**

In the current study, the relationships between various polymorphisms of two CYP450 enzymes (CYP2C9 and CYP2C19) and the pharmacokinetics of indisulam were assessed. It was shown that the elimination rate of indisulam was significantly decreased by CYP2C9*3, CYP2C19*2, and CYP2C19*3 polymorphisms. These CYP2C mutations caused an increased risk of dose-limiting neutropenia.

The activity of the *3-mutated CYP2C9 enzyme was shown to be reduced for S-warfarin *in vitro* by Haining et al. (33). This polymorphism was also associated to poor tolbutamide metabolism *in vivo* (34). In the current pharmacogenetic study, the *3 mutation in the CYP2C9 gene reduced the Michaelis-Menten elimination rate of indisulam. Thus, the saturable elimination pathway may correspond to hydroxylation of indisulam by CYP2C9 (19).

De Morais et al. (23, 24) showed that the *2 and *3 mutations in the CYP2C19 gene created a premature stop codon, resulting in a truncated nonfunctional CYP2C19 protein. These CYP2C19 mutations were related to poor metabolism of the CYP2C19 substrate (S)-mephenytoin (23, 24) and to a reduction of the clearance of indisulam. Consequently, the linear elimination pathway of indisulam may represent hydroxylation by CYP2C19 (19).

The CYP2C9*5 mutation results in lower enzymatic activity and that CYP2C19*4 represents a defective allele (30, 35). Therefore, the CYP2C9*5 and CYP2C19*4 polymorphisms may be related to reduced indisulam clearance. The AUC of indisulam in a patient who was heterozygous for the CYP2C9*5 mutation was indeed substantially higher than the AUC of three Caucasian wild-type patients who had received the same indisulam dose. Due to low allelic frequency, statistical significance of the effects of the CYP2C9*5 and CYP2C19*4 polymorphisms could not be shown in the present study.

In our population of Japanese and Caucasian patients, six CYP2C polymorphisms were observed, which are located on chromosome 10. The occurrence of multiple polymorphisms on the same allele is indicated by the haplotype. Multiple polymorphisms may not be fully independent. It may therefore be preferable to consider the haplotype rather than the genotype of individual polymorphisms for data analysis. This
strategy was, however, not suitable for the current analysis because haplotype frequencies were too low.

The CYP2C19 polymorphisms occurred at much higher frequencies in the Japanese population compared with the Caucasian population (CYP2C19*2: 36% versus 9.8%; CYP2C19*3: 7.1% versus 0%). Because these CYP2C19 variants significantly impaired indisulam clearance, Japanese patients typically have a lower CL than Caucasians. Indeed, in the previously published population pharmacokinetic model, Japanese patients had a 3.4-fold lower value for CL than Caucasian individuals (16). This difference was not statistically significant upon inclusion of CYP2C19 genotype in the drug elimination model. This confirms that the lower linear clearance of indisulam in Japanese patients is due to genomic differences between the Caucasian and Japanese patient populations.

Mixed-effects modeling was used to assess the effects of CYP2C mutations on pharmacokinetic parameters. From a statistical standpoint, this method is superior to more commonly used methods in pharmacogenetic studies, where standard pharmacokinetic two-stage analyses are followed by conventional statistical tests in order assess differences between genetic subgroups (36).

It was shown that CYP2C9*3, CYP2C19*2, and CYP2C19*3 mutations led to decreased indisulam clearance and increased drug exposure. A clear relationship has been established between indisulam pharmacokinetics and its dose-limiting toxicities, neutropenia and thrombocytopenia. Based on the results of the present pharmacogenetic study and a semiphysiologic pharmacokinetic-pharmacodynamic model, it was expected that the risk of hematologic side effects is higher for patients with variant CYP2C9 and/or CYP2C19 alleles than for wild-type patients. A simulation study showed that the relative risk of dose-limiting neutropenia may be 2.2 for a homozygous Caucasian CYP2C9*3 mutant and 4.5 for a homozygous Japanese CYP2C19*2 mutant after administration of the recommended dosage of 700 mg/m² indisulam. Patient groups in the study population were too small to verify these estimated relative risks of dose-limiting neutropenia with observed data on hematologic toxicity. Nevertheless, the current clinical data imply that the CYP2C9*3 polymorphism is predictive for the occurrence of hematologic toxicity: the relative risk of grade 4 neutropenia was 2 in a group of 12 patients who were treated at the 600 mg/m² dose level and a patient with a homozygous CYP2C9*3 genotype had more severe neutropenia than a wild-type patient after administration of 800 mg/m². Furthermore, the lowest neutrophil nadir was observed in a Japanese homozygous CYP2C19*2 mutant patient.

The pharmacogenetic effects may be relevant for the treatment of patients with indisulam. Pretreatment genetic screening will permit planning of appropriate initial dosing for individual patients to achieve an optimal therapeutic effect. A reduced initial dosage for patients with high-risk CYP2C2 mutations is recommended. This recommendation is based on a retrospective study of limited sample size and therefore should be carefully interpreted. Haplotype frequencies in the study population were insufficient to provide a dosing strategy for patients with variant alleles of both CYP2C9 and CYP2C19.

In conclusion, CYP2C9*3, CYP2C19*2, and CYP2C19*3 polymorphisms were related to a decreased elimination rate of indisulam. Screening for these CYP2C polymorphisms may assist in the selection of an optimized initial indisulam dosage. It seems warranted to perform a prospective study to define solid recommendations for pharmacogenetically guided dose adjustments.

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References


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