A Pharmacokinetic/Pharmacodynamic Model of Tumor Lysis Syndrome in Chronic Lymphocytic Leukemia Patients Treated with Flavopiridol

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Abstract

Purpose: Flavopiridol, the first clinically evaluated cyclin-dependent kinase inhibitor, shows activity in patients with refractory chronic lymphocytic leukemia, but prevalent and unpredictable tumor lysis syndrome (TLS) presents a major barrier to its broad clinical use. The purpose of this study was to investigate the relationships between pretreatment risk factors, drug pharmacokinetics, and TLS.

Experimental Design: A population pharmacokinetic/pharmacodynamic model linking drug exposure and TLS was developed. Plasma data of flavopiridol and its glucuronide metabolite (flavo-G) were obtained from 111 patients treated in early-phase trials with frequent sampling following initial and/or escalated doses. TLS grading was modeled with logistic regression as a pharmacodynamic endpoint. Demographics, baseline disease status, and blood chemistry variables were evaluated as covariates.

Results: Gender was the most significant pharmacokinetic covariate, with females displaying higher flavo-G exposure than males. Glucuronide metabolite exposure was predictive of TLS occurrence, and bulky lymphadenopathy was identified as a significant covariate on TLS probability. The estimated probability of TLS occurrence in patients with baseline bulky lymphadenopathy less than 10 cm or 10 cm or more during the first 2 treatments was 0.111 (SE% 13.0%) and 0.265 (SE% 17.9%), respectively, when flavo-G area under the plasma concentration versus time curve was at its median value in whole-patient group.

Conclusions: This is the first population pharmacokinetic/pharmacodynamic model of TLS. Further work is needed to explore potential mechanisms and to determine whether the associations between TLS, gender, and glucuronide metabolites are relevant in patients with chronic lymphocytic leukemia treated with other cyclin-dependent kinase inhibitors. Clin Cancer Res; 19(5); 1269–80. ©2012 AACR.
Translational Relevance

Tumor lysis syndrome (TLS) is an oncologic emergency requiring immediate intervention to prevent severe kidney damage, cardiac arrhythmias, and death. Although rare in patients with refractory chronic lymphocytic leukemia (CLL) and despite aggressive prophylactic measures, hyperacute TLS occurs frequently in patients with refractory CLL treated with single-agent cyclin-dependent kinase inhibitors (CDKI), flavopiridol, and dinaciclib. While these agents are impressively active in refractory and cytogenetically high-risk CLL, the prevalent and unpredictable occurrence of TLS limits their broad clinical use. This article presents the first PK/PD model of TLS and explores the unique associations of female gender and metabolite pharmacokinetics with TLS induced by flavopiridol therapy in patients with CLL. This model offers a tool enabling estimation of TLS probability in patients with CLL before therapy with flavopiridol, and it represents a general framework through which the mechanisms of TLS induced by CDKI therapy in patients with CLL can be further studied.

Death (18, 19). TLS is typically rare in CLL, and its prevalence with the pharmacokinetically guided dosing regimen of flavopiridol highlights the exquisite and acute sensitivity of CLL to cyclin-dependent kinase inhibition. Blum and colleagues identified several pretreatment risk factors for TLS in patients with CLL treated with flavopiridol (17). TLS occurrence was associated with advanced disease stage, characterized by bulky tumor burden, elevated white blood cell (WBC) count and β2-microglobulin, and reduced albumin level (17). These were consistent with multiple diseases where tumor bulk and disease stage are general risk factors (19). Overall, these produced ORs for TLS ranging from 1.1 to 2.9. Interestingly, female gender was the most influential of all risk factors identified for TLS occurrence after flavopiridol treatment with an OR of 8.6 [95% confidence interval (CI): 2.6–27.7]. Gender has not been previously reported as a risk factor for TLS. While these factors serve as useful indicators for increased risk of TLS, many patients with CLL who experienced TLS would not have been identified by the combined risk factors.

Glucuronide conjugation accounts for the majority of metabolic transformation of flavopipridol (20, 21). Glucuronidation can be influenced by factors such as age, gender, cigarette smoking, and obesity (22). In vitro studies showed that isoform UGT1A9 is predominantly responsible for conversion of flavopiridol to 7-O-β-glucopyranuronosyl-flavopiridol (Flavo-7-G), which accounts for 98.5% of glucuronidation product (20, 21). UGT1A1 or UGT1A4 are reported to catalyze formation of 5-O-β-glucopyranuronosyl-flavopiridol (Flavo-5-G), the minor glucuronidation product (20, 21). These metabolites (flavo-G) have been presumed inactive, although studies evaluating their activity have not been reported. Flavo-G conjugates are presumed to be formed predominantly in liver, although glucuronosyltransferase (UGT) activity in other tissues, including lymphocytes, may also contribute. These conjugates, along with parent drug, are eliminated through biliary and renal excretion with the majority of flavopiridol and flavo-G found in bile/feces. Evidence for enterohepatic recirculation of flavopiridol has been reported (23, 24). Glucuronidase activity in the gut may convert flavo-G to flavopiridol, allowing further flavopiridol reabsorption. We previously reported that flavo-G, the primary flavopiridol metabolite, were associated with TLS in the phase I study (14). In the study by Blum and colleagues, this trend was apparent in a subset of 86 of the patients with flavo-G pharmacokinetic data. Interestingly, this analysis also identified the association between flavo-G and gender, where females had higher plasma flavo-G concentrations and areas under the concentration–time curves (AUC).

With the increased development of targeted therapies, TLS is becoming more prevalent and is now observed more commonly in diseases that were previously characterized as low risk for TLS, such as CLL (25–27). Although flavopiridol has shown impressive activity in refractory CLL, the prevalence of TLS has dampened enthusiasm for its broader use in the clinical setting. Recent clinical experience with other CDKIs such as dinaciclib, suggests that TLS may be a class effect in CLL (28). In addition to having similar pharmacodynamic targets, dinaciclib and flavopiridol also have in common a UGT-mediated elimination pathway. Therefore, understanding the relationships between drug and metabolite exposure and occurrence of TLS is imperative to the further development of CDKIs in CLL.

The purpose of this study was to model TLS occurrence in patients with CLL treated with flavopiridol and to explore the relationships and relative contributions of parent drug, glucuronide metabolite, and pretreatment risk factors to TLS occurrence. TLS has not previously been modeled using a pharmacokinetic/pharmacodynamic (PK/PD) nonlinear, mixed effects approach. Herein, we describe the first PK/PD model linking drug and metabolite exposure to TLS.

Materials and Methods

Patients

Subjects included in this study were patients with relapsed, symptomatic CLL or small lymphocytic lymphoma or prolymphocytic leukemia arising from CLL treated with flavopiridol monotherapy in phase I and II studies. Both studies were conducted at The James Cancer Hospital at The Ohio State University (Columbus, Oh). The studies were reviewed and approved by the Institutional Review Boards of The Ohio State University and signed informed consent was obtained from all patients. Each patient received a maximum of 6 cycles with each cycle containing 3 or 4 weekly treatments. Patients were treated at 30 mg/m² half-hour infusion followed by 30 mg/m² as a 4-hour infusion at first dose in cycle 1. Depending on toxicity occurrence, the 4-hour infusion dose was escalated to 50 mg/m² either at the second dose in cycle 1 or at the first dose in cycle 2. Two patients in the phase I study received 40 mg/m² each.
Flavopiridol and flavopiridol glucuronide pharmacokinetic analysis

Plasma samples were collected between 0.5 and 24, 36, or 48 hours after the first dose and/or escalated dose. Flavopiridol and flavo-G concentrations were measured using liquid chromatography-tandem mass spectrometry methods as previously described (14, 29, 30).

Definition, prophylaxis, and management of TLS

TLS was defined as an acute elevation in uric acid, potassium, phosphate, and/or LDH within 24 hours of flavopiridol administration. Prophylaxis for TLS was given to all patients with allopurinol, rasburicase, sodium bicarbonate-containing intravenous hydration, and oral phosphate binder. All patients were monitored for 24 hours after dosing for serum potassium level, and those who experienced hyperkalemia or hyperphosphatemia were treated with sodium polystyrene sulfonate (Kayexalate), furosemide, albuterol, insulin and glucose, calcium, oral phosphate binders, or emergent dialysis (17). Patients who developed TLS or other severe adverse events during the first dose were not dose escalated for subsequent treatments. Hyperacute TLS was designated where dialysis intervention was required within 6 hours of the first dose. They included 6 demographic variables (body weight, height, body surface area, age, sex, and race), 4 baseline variables were collected from all patients before the first dose. They included 6 demographic variables (body weight, height, body surface area, area, age, sex, and race), 4 disease state indices (Rai stage, B2-microglobulin, bulky lymphadenopathy 10 cm or more, and ECOG status), and 10 blood chemistry variables [albumin, WBC, creatinine, potassium, uric acid, phosphate, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and LDH]. Baseline creatinine clearance was estimated using the Cockcroft–Gault equation (31).

Covariates

Baseline variables were collected from all patients before the first dose. They included 6 demographic variables (body weight, height, body surface area, age, sex, and race), 4 disease state indices (Rai stage, B2-microglobulin, bulky lymphadenopathy 10 cm or more, and ECOG status), and 10 blood chemistry variables [albumin, WBC, creatinine, potassium, uric acid, phosphate, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and LDH]. Baseline creatinine clearance was estimated using the Cockcroft–Gault equation (31).

Population pharmacokinetic modeling

The population pharmacokinetics of flavopiridol and flavo-G were evaluated using nonlinear, mixed effect modeling as implemented in NONMEM (version 7 Level 1.2, ICON Development Solutions) with Intel Visual Fortran compiler (version 11.1.060, Intel Corporation). Data were fit using a log transform both sides (LTBS) approach. The first-order conditional estimation (FOCE) method was used throughout the model-building process. The minimum objective function value (OFV) was used to compare nested models. A decrease of 6.64 or more in OFV was considered statistically significant at $P < 0.01$ for nested models. A pharmacokinetic model for flavopiridol was developed first. The pharmacokinetic model for flavo-G was built with the parameters for flavopiridol fixed to their final values. Once the flavo-G model was established, the parameters for both parent drug and metabolite were simultaneously estimated.

Standard model building approaches were used for both analytes. To establish a base model, 1-, 2-, and 3-compartment models were tested. A parameter estimating the fraction of parent drug converted to metabolite ($F_{m}$) was described using logit transformation to constrain the value between (0, 1) and was used to link the parent drug and metabolite compartments. Interindividual (IIV) and interoccasion variability (IOV) were estimated for the pharmacokinetic parameters using an exponential error model. According to the study design, patients started on a low total dose (30 mg/m² bolus + 30 mg/m² infusion dose) and were escalated to a higher total dose (30 mg/m² bolus + 50 mg/m² infusion dose) if the low dose was tolerated. Because pharmacokinetic sampling occurred during the first administration of each dose level, IOV was tested on the 2 total dose levels in the model of 60 or 80 mg/m². Terms describing correlations between the IIV random effects were included where feasible (e.g., where the correlation was estimable, the model converged successfully, and the OFV was reduced). Residual variability was described using an additive error model (which is proportional after back transformation of the data).

Covariate analysis was conducted by comparing hierarchical models based on the likelihood ratio test. Continuous covariates were normalized to the median value, which was assumed for missing covariate data. Initially, each covariate was plotted against individual estimates of IIV variability (e.g., eta values) for screening. Covariate–parameter relationships that displayed a visual trend in the graphical assessment were then introduced singly into the base model separately using equations (1) and (2) for continuous and dichotomous covariates, respectively.

$$TVCL_i = \theta_{CL} \times (NCov_i)^{\eta_{CL}}$$

Equation (1)

$$TVCL_i = \theta_{CL} \times e^{\eta_{COV_i}}$$

Equation (2)

In equation (1), $TVCL_i$ is the typical value of clearance adjusted with the normalized continuous covariate, $NCov_i$. $NCov_i$ represents the continuous covariate for individual $i$ divided by the median value of that covariate. $TVCL$ and $\theta_{CL}$ are equivalent when the continuous covariate takes the median value (i.e., when $NCov_i = 1$). $\theta_1$ denotes the estimate of influence of the continuous covariate. In equation (2), $TVCL_i$ is the typical value of clearance when the dichotomous covariate takes the value of 0, for example, $SEX = 0$ refers to male patients. $COV_i$ is the dichotomous covariate for individual $i$. $\theta_2$ is the proportional change in clearance when the dichotomous covariate takes the value of 1.

Covariates were added to the base model if the decrease in OFV was at least 3.8 units ($P < 0.05$). Clinical relevance was also considered when determining inclusion of a statistically significant covariate in that the effect of a covariate had to change the parameter value by at least 20% over the range of covariate values in the database (selection criteria of 20% was based on standard considerations for bioequivalency;
A logistic regression model (the full model) and a stepwise backward deletion procedure was conducted, using the criteria of \( P < 0.01 \) based on the likelihood ratio test.

**Population PK/PD Modeling**

As with the population pharmacokinetic modeling, NONMEM (with the same compiler and installation) was used for population PK/PD modeling with FOCE- and LAPLACE-like options. Clinical grading of TLS according to the Common Terminology Criteria for Adverse Events is either 0 (no TLS), 3 (present), 4 (life-threatening consequences; urgent intervention indicated), or 5 (death). All patients whose PK/PD data were used in our study were graded 0, 3, or 4. Therefore, logistic regression was used to characterize the probability of the pharmacodynamic effect, TLS occurrence (grades 3 or 4), and to evaluate its relationship with drug or metabolite exposure. TLS occurrence during cycle 1 was therefore modeled as a dichotomous outcome variable. TLS presents as metabolic abnormalities secondary to spontaneous or most commonly cytotoxic treatment-induced rapid tumor cell death. When tumor burden is high before the treatment and tumor cells are responsive to the first treatment, rapid cell death causes mass cellular debris released into blood circulation and may lead to elevation of electrolyte levels in blood. Following repeated doses, the decreased tumor burden, and potentially decreased sensitivity to drug results in a decreased likelihood of TLS. In these 2 studies, we observed that incidence of TLS was highest following the first dose at each dose level, and then decreased to lower or no incidence at later doses. TLS was rarely observed in subsequent cycles, particularly if patients had experienced TLS in the first cycle. Given the low rate of occurrence at later cycles, only TLS during cycle 1 was considered, thus the data included one to 4 observations per subject. Similarly within cycle 1, the probability of TLS decreased over time. Thus, the effect of time on TLS within cycle 1 was tested by several functions, including exponential, cubic, step, and Bateman functions. Both the maximum concentrations (\( C_{\text{max}} \)) and AUC of both compounds at each dose level for each patient was estimated by known dosing amount and post hoc parameters from the final population pharmacokinetic model. AUC values were obtained up to 168 hours after each dose. After drug effect was established, the remaining covariates were examined. Continuous variables were added to the model in linear function with addition of term \( \gamma_i \times (\text{Cov}_{ik} - \text{MedDrugExp}_i) \) into equation (3). Gamma (\( \gamma_i \)) is the covariate factor where \( \gamma_i \) is estimated in day 1 or 8 and \( \gamma_i \) is fixed as 0 in day 15 or 22. \( \text{Cov}_{ik} \) is the covariate value for the \( k \)th patient and \( \text{MedDrugExp}_i \) is the median covariate value. Dichotomous variables were evaluated with separate estimation of the drug exposure factor between 2 statuses. In equation (3), term \( \beta_{ij} \times (\text{DrugExp}_{ij} - \text{MedDrugExp}_{ij}) \) is used, for example, where drug exposure effect was separately estimated in male (\( j = 0 \)) and female (\( j = 1 \)) patients. \( \text{Eta}(\eta) \) is the IV random effect that was fixed to zero in this model as the data were too limited to estimate variability.

**Model evaluation**

Model evaluation was conducted on the final models. The parameters of the fixed and random effects were examined for reasonable estimation, SE, shrinkage, and correlation. Goodness-of-fit plots were graphed to evaluate model appropriateness. Normality assumption of the random effects was visually checked by histogram and quantile-quantile plots. Visual predictive check (VPC) of the pharmacokinetic models were generated from 1,500 simulations. The pharmacodynamic model was evaluated by comparing observed TLS probability and 95% CI of predicted TLS probability. Predicted probability was calculated for each patient in each day using bootstrap parameters from 1,000 bootstrap runs. These 1,000 calculated probabilities were used to construct 95% CI of the probability of TLS for a range of drug exposures in day 1 or 8. The observed probabilities of TLS were compared with the predicted values.

**Results**

There were 111 unique patients for pharmacokinetic analysis from phase I and II studies, including 8 patients who were retreated after relapse. Eighty-one patients received escalated infusion doses of 50 mg/m² (70–134.5 mg). There were 1,374 and 781 plasma concentration observations of flavopiridol and flavo-G available for modeling, respectively. Flavo-G concentrations were
measured in 85 of 111 patients. The patient population was primarily Caucasian American with a median age of 60 years, and was 70% male. There was a wide range of body weight in this patient group (45.1–153.7 kg). Most patients were at late stage of disease and had variable WBC count. There was 1 patient (0.9%) with missing data for race, ECOG status, total bilirubin, and AST, 2 patients (1.8%) with missing data for potassium and ALT, 3 patients (2.7%) with missing uric acid data, and 13 patients (11.7%) with missing phosphate data. Forty-three percent (43%) of patients had TLS in the first cycle. A summary of the demographics of the patients in the database is presented in Supplementary Table S1.

The overall scheme for the pharmacokinetic model of flavopiridol and flavopiridol glucuronide is presented in Fig. 1. A 2-compartment pharmacokinetic model with first-order elimination described the disposition of flavopiridol well, as previously reported (14, 29). Individual ETAs for clearance and volume were highly correlated (\( r > 0.9 \)), so a shared ETA was used with an estimated scale factor applied to the shared ETA for volume of distribution [i.e., \( \text{CL} = TVCL \times \exp(\text{ETA1}) \) and \( V = TVV \times \exp(\text{THETA}(n) \times \text{ETA1}) \)], where \( \text{THETA}(n) \) is the estimated variance scale factor. The population parameter estimates for flavopiridol are presented in Table 1. The clearance, central volume of distribution, distribution clearance, and peripheral volume of distribution were 34.1 L/hour, 75.8 L, 6.77 L/hour, and 91.8 L, respectively. The parameters were estimated with good precision (less than 20%–30%) and shrinkage (less than 20%). In the final model, an allometric function using normalized body weight was applied to clearance parameters with a fixed factor of 0.75 and to volume parameters with a fixed factor of 1. Flavopiridol pharmacokinetic parameters including this body size factor were consistent with our previous model that used body surface area as a covariate (14). We also observed a significant effect in univariate analysis from sex with females having 12.3%
lower CI compared with males (OFV = −7.9). However, as sex and body weight were correlated, we used normalized body weight, which had better precision and lower shrinkage compared with sex. No other covariates met the cut-off criteria for inclusion in the final flavopiridol model. Plots of the observed concentrations versus the population and individual predictions are presented in Fig. 2, and residual plots are presented as Supplementary Data. The VPC plot in Fig. 2 shows that the 95% CI and prediction intervals (PI) adequately describe the observed concentrations at each time point with no notable bias. VPC plots provided as Supplementary Data, with patients stratified by weight (<80 kg and ≥80 kg), show similar flavopiridol CI and PI between the 2 categories.

A 2 compartment-model was also selected to describe flavo-G plasma concentration–time profiles. To address identifiability limitations, as clearance of parent drug in metabolic or nonmetabolic pathways could not be distinguished by plasma sampling of metabolite alone, a parameter for the fraction of parent drug converted to metabolite, $F_{\text{m}}$, was estimated using 0.5 as an initial estimate based on preliminary metabolism and pharmacokinetic data. During model development, pharmacokinetic parameters of both parent drug and metabolite were simultaneously estimated, and the model successfully converged. However, $F_{\text{m}}$ was fixed during covariate analysis to avoid terminated runs. This approach for estimating $F_{\text{m}}$ has been presented in recent literature (33). This parameter did not include a term for IIV variability. Random effects on flavo-G volumes of distribution were also unidentifiable and were therefore not included. Using the base model with both flavopiridol and glucuronide metabolite, sex was the only significant covariate on metabolite clearance, suggesting that the clearance of flavo-G in female patients was approximately half the clearance of male patients. Population parameter estimates of flavo-G are shown in Table

Figure 2. Observed concentrations versus the population (A) or individual (B) predictions and VPC (C) plots using the population pharmacokinetic model for flavopiridol. For C, open circles, observed data; gray solid line, median of observed data at nominal time; gray dashed line, 95% CI of observed data at nominal time; black solid line, median of simulated data at nominal time; black dashed line, 95% PI of simulated data at nominal time; gray area, 95% CI around median or 95% PI.
1. Figure 3 shows goodness-of-fit plots of the pharmacokinetic model of flavo-G and its VPC stratified by sex. Residual plots for flavo-G are provided as Supplementary Data.

The estimated parameters for the model describing the probability of TLS are shown in Table 2. Because most TLS events occurred in day 1 or 8, a step function with time effect provided the best estimate of TLS probability within this dataset. A linear function was used to evaluate the relationship between drug exposure and TLS probability on day 1 or 8. Inclusion of flavopiridol $C_{\text{max}}$ or AUC did not improve the model, whereas inclusion of flavo-G $C_{\text{max}}$ or AUC led to a significant decrease in OFV ($P < 0.01$), suggesting that exposure to flavo-G was predictive of TLS. Successful convergence was obtained when flavo-G AUC was included, and it was therefore selected for use in the final model.

We evaluated the potential influence of patient dropout during cycle 1 of the study. Among 7 patients who dropped out after day 1, 4 patients dropped out due to TLS and 3 due to other toxicities. Patients who dropped out at day 15 or 22 did so for reasons other than TLS. Compared with number of patients having TLS at day 1 ($n = 25$) or day 8 ($n = 26$), the number of patients who dropped out due to TLS was low. Overall, dropouts in this study were deemed to post no significant influence and minimal impact.

For pharmacodynamic model covariate analysis with 6 risk factors, inclusion of $\beta_2$-microglobulin ($\Delta$OFV = $-16.05$), WBC ($\Delta$OFV = $-10.41$), bulky lymphadenopathy 10 cm or more ($\Delta$OFV = $-9.54$), or creatinine clearance ($\Delta$OFV = $-8.34$) into the model resulted in significant decreases in OFV ($P < 0.01$) with successful convergence. The other factors evaluated [sex ($\Delta$OFV = $-3.25$) or albumin ($\Delta$OFV = $-1.55$)] did not result in significant reductions of OFV and were not considered further. When both $\beta_2$-microglobulin and bulky lymphadenopathy status were...
Table 2. Population pharmacodynamic parameter estimates for a logistic regression model for probability of TLS occurrence

<table>
<thead>
<tr>
<th>Model Term</th>
<th>Parameter Estimate (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept* (Day 1 or 8, bulky lymphadenopathy &lt; 10 cm)</td>
<td>( \theta_1 ) = -2.02 (13.0)</td>
</tr>
<tr>
<td>Intercept* (Day 1 or 8, bulky lymphadenopathy ( \geq 10 ) cm)</td>
<td>( \theta_2 ) = -1.03 (17.9)</td>
</tr>
<tr>
<td>Slope of drug effect (Day 1 or 8, metabolite AUC, regardless of bulky disease status)</td>
<td>( \theta_3 ) = 0.0281 (20.4)</td>
</tr>
<tr>
<td>Intercept (Day 15 or 22, regardless of bulky disease status)</td>
<td>( \theta_4 ) = -30 (43.7)</td>
</tr>
<tr>
<td>Slope of drug effect (Day 15 or 22, regardless of bulky disease status)</td>
<td>( \theta_5 ) = 0, fixed</td>
</tr>
<tr>
<td>IV</td>
<td>( \eta ) = 0, fixed</td>
</tr>
</tbody>
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*When drug exposure is equal to median drug exposure.

Discussion

In patients with refractory CLL, flavopiridol has shown significant efficacy with TLS as the dose-limiting toxicity. Although TLS reflects rapid and high activity for flavopiridol to destroy CLL tumor cells, a recent analysis of clinical outcomes data indicated TLS was not associated with objective response (17). Furthermore, the probability of TLS was not associated with flavopiridol pharmacokinetics. While the probability of TLS was associated with expected pretreatment variables, including bulky disease and WBC, it was also associated with unexpected variables, including gender and flavo-G pharmacokinetics. Outside of flavopiridol in CLL, TLS had not been previously associated with gender or glucuronide metabolite levels. We therefore sought to further explore the apparent links between TLS, gender, and flavo-G.

The final pharmacokinetic model estimated unexplained proportional residual error of 39.6% and 55.8% in parent compound and metabolite concentration estimates, respectively. Additional covariates that can explain such high variability are yet to be identified. With such high residual error, sequential modeling of parent drug and metabolite were conducted first to estimate pharmacokinetic parameters for each compound, followed by simultaneous modeling with these parameter estimates as initial estimates to ensure convergence. We recently reported that pharmacogenetic factors significantly contribute to flavopiridol and flavo-G (29), and such factors will likely improve the model. However, pharmacogenetic data were not available for a large proportion of patients in this dataset. While imputation methods are available for genotype data, these methods require either larger datasets and/or prior validated information relating genotype and phenotype or use of linkage disequilibrium to infer genotypes from determined genotypes (34–38). We therefore concluded evaluation of pharmacogenetics as a covariate in this model to be impractical. Other missing covariates were imputed with the median value, which is a common imputation method to deal with limited missing data. The disadvantage to this method of imputation is that covariate distribution may be clustered around the median value thus underestimating the covariate effect. We determined this impact should be minimal as the percentage of missing data is less than 12% in one covariate and less than 3% for all other covariates used.

For development of the pharmacokinetic model, body weight and gender were determined to improve the models for flavopiridol and flavo-G clearance, respectively. Other groups have shown that flavo-G elimination is primarily due to biliary and fecal excretion (21, 23). In support of this, our results suggest that flavo-G deposited into urine represents less than 5% of the total dose of
flavopiridol (data not shown). Because of the inability to robustly estimate $F_m$, we were not able to effectively determine the impact of gender or other covariates on the fraction of flavopiridol converted to flavo-G. Therefore, the gender effect may be more appropriately attributed to flavo-G formation as opposed to flavo-G clearance. Gender differences in UGT enzyme expression and/or activity have been observed in animal models and in humans (39–44). However, data are not clear with respect to gender-specific expression and activity for UGT1A9. Future studies are needed to better define how gender may influence flavo-G formation and/or elimination.

Pharmacodynamic modeling of TLS probability used a logistic regression model. The sharp decrease in TLS probability after the first and second doses was handled with a step function based on time. As the most significant risk factor in a multivariate analysis (17), gender was investigated in the covariate analysis for the pharmacodynamic model. However, it was not shown to be a significant pharmacodynamic covariate in this analysis. This may be explained by our use of gender as a covariate on flavo-G pharmacokinetics with females having higher exposure. Because flavo-G AUC was the drug exposure factor used to scale TLS probability, the gender effect was already present and thus was diminished in covariate analysis of the pharmacodynamic model. It is important to note, however, that the exclusion of gender from the current pharmacodynamic model, which does not necessarily indicate gender, is only related to TLS through metabolite pharmacokinetics. It is still possible that females have an increased probability of developing TLS by some unknown mechanism not related to pharmacokinetics.

Inclusion of β2-microglobulin level gave the highest OFV changes, although it gave the widest CIs for TLS probability along with days in cycle 1, stratified by male (A), female (B), and all (C) patients. VPC of TLS probability with increasing metabolite AUC (D). For D, data were first sorted by observed metabolite AUC and divided into 13 bins with approximately 17 observations (AUC/TLS pair) in each bin. The median metabolite AUC was calculated for each bin, and observed incidence of TLS events was determined for each bin. One thousand bootstrap replicates were generated from the final TLS model and probability of TLS was calculated within each bin from the vectors of the bootstrap parameters. Median and 95% boundaries were then calculated from the 1,000 predicted probabilities at each AUC bin. The median probability is reflective of the typical probability of TLS occurring at a given AUC, whereas the top and bottom boundaries reflect the 95% CI of the probability curve.

Figure 4. Visual predictive check plot of TLS probability along with days in cycle 1, stratified by male (A), female (B), and all (C) patients. VPC of TLS probability with increasing metabolite AUC (D). For D, data were first sorted by observed metabolite AUC and divided into 13 bins with approximately 17 observations (AUC/TLS pair) in each bin. The median metabolite AUC was calculated for each bin, and observed incidence of TLS events was determined for each bin. One thousand bootstrap replicates were generated from the final TLS model and probability of TLS was calculated within each bin from the vectors of the bootstrap parameters. Median and 95% boundaries were then calculated from the 1,000 predicted probabilities at each AUC bin. The median probability is reflective of the typical probability of TLS occurring at a given AUC, whereas the top and bottom boundaries reflect the 95% CI of the probability curve.
probability. Inclusion of bulky lymphadenopathy status produced a significant decrease in OFV and showed the narrowest CIs in modeling TLS probability with respect to flavopiridol glucuronide AUC. The different behaviors of these 2 covariates may be due to the different modeling structures for continuous versus dichotomous covariates. Given the strong correlation between these 2 covariates, we selected only bulky lymphadenopathy status for inclusion in our final model. However, further consideration for both covariates should be given in future models and in larger datasets.

Our observed set of associated factors, including gender and glucuronide metabolite pharmacokinetics, may indicate unique mechanisms that are involved with flavopiridol-induced TLS in CLL. Our final and current model suggests that gender may play a role in TLS development through drug metabolite exposure, although the mechanism for this is not clear. We have previously determined flavo-G metabolites were not cytotoxic to CLL cells ex vivo nor were these metabolites formed at detectable levels within human whole blood or in CLL cells ex vivo (data not shown). Therefore, flavo-G is unlikely involved in direct CLL cell killing. Importantly, TLS is a function of both the rate of tumor cell death and the rate of elimination of toxic cellular debris from systemic circulation. Therefore, if flavo-G does contribute to TLS, it may do so by interfering with renal or hepatic clearance of cellular debris through unknown mechanisms. It should be noted, however, that while we have observed the association between flavo-G, gender, and TLS in the 2 independent trials, the pharmacokinetic concentration–profiles of flavo-G and flavopiridol overlap considerably, thus preventing us from discerning their individual contributions to the occurrence of TLS.

Diversity in enzyme activities and expression of genes involved in flavopiridol disposition may influence its efficacy and toxicity. A previous study by Ramirez and colleagues has shown significant interpatient variability of flavopiridol glucuronidation in human hepatic microsomes (45). Many factors, including polymorphism effects of UGT genes on flavopiridol disposition are limited. Zhai and colleagues reported a lack of association of UGT1A1*28 and flavopiridol pharmacokinetics (45), and separate reports have shown a lack of 1A7 and 1A9 polymorphic effects on gene expression, flavopiridol transformation in vitro, and no changes in flavopiridol disposition in patients (46, 47). Our group previously observed a lack of significant associations of UGT1A1*28 and UGT1A9*22 in multivariable analyses, although these polymorphisms were associated with flavopiridol and/or flavo-G pharmacokinetics in univariate analyses (29). In light of our findings in this current study, a thorough evaluation of polymorphisms in UGT enzymes, particularly UGT1A9, would be warranted in future clinical studies with flavopiridol. If UGT pharmacogenetics proved to be a significant factor contributing to flavo-G disposition and TLS, prospective genotyping may reduce risk for patients with CLL receiving flavopiridol therapy.

This study represents the first population PK/PD approach for modeling TLS. As with other TLS models previously published (48, 49), our model may not be generalizable to other diseases and therapies. Rather than modeling a drug exposure–TLS relationship, which may change with each drug therapy, modeling of a biomarker–TLS relationship may be preferred, if such a biomarker could be identified to be rapidly modulated after flavopiridol therapy and correlate with TLS occurrence and either drug or metabolite levels. However, aside from the current markers used to declare TLS occurrence (potassium, uric acid, phosphate, and LDH), no such marker has been identified. Pretreatment bulky lymphadenopathy status and ß2-microglobulin were correlated with TLS, but these markers were not altered by therapy on a time scale that would be useful for establishing drug exposure–biomarker and biomarker–TLS relationships in a predictive model. Thus, we were ultimately left with the glucuronide metabolite as the best observed “biomarker” associated with TLS. The observance of hyperacute TLS in CLL is rare outside of CDKI trials (28, 50). Interestingly, TLS is also observed in patients with CLL treated with dinaciclib, a second generation CDKI (28). Like flavopiridol, dinaciclib is also eliminated through excretion and metabolism. Although the metabolic and transport pathways have not been elucidated, glucuronidation is clearly important in dinaciclib clearance (unpublished data). As new trials with dinaciclib are conducted in CLL, it will be important to monitor the association of gender and dinaciclib on TLS probability.

**Disclosure of Potential Conflicts of Interest**

J.C. Byrd has ownership interest in a patent. No potential conflicts of interest were disclosed by the other authors.

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