Population Pharmacokinetics of Temozolomide in Cancer Patients

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Purpose. To evaluate covariate effects on the pharmacokinetics of temozolomide in cancer patients, and to explore the dose-pharmacokinetics-toxicity relationship of temozolomide.

Methods. Non-linear mixed-effects modeling approach was used to analyze the data from 445 patients enrolled in eleven Phase I and Phase II clinical trials. All patients in the phase I trials had advanced cancer. Patients in the phase II trials had anaplastic astrocytoma (AA), glioblastoma multiforme (GBM) or malignant melanoma (MM). A sparse sampling scheme was prospectively developed using Phase I data and was successfully implemented in Phase II trials. Population factors included age, gender, height (HT), weight (WT), body surface area (BSA), serum creatinine (Sr.Cr.), estimated creatinine clearance, serum chemistry data as indices of hepatic function and disease, smoking status, and selected concomitant medications. Descriptive statistics were used to summarize the toxicity and temozolomide dose and exposure relationship.

Results. The pharmacokinetics of temozolomide follows a one-compartment model with first order absorption and elimination. Temozolomide clearance (CL) increased with BSA for both genders. The population mean clearance for GBM or AA patients was 11.2 L/hr for male with BSA equal to 2.0 m^2, and 8.8 L/hr for female with BSA equal to 1.7 m^2. The mean clearance for MM patients was slightly higher. The inter-subject variability in clearance was 15%, and the residual variability was 26%. Other factors investigated in this analysis had little effect on clearance. The overall incidence of neutropenia and thrombocytopenia were 5–8%. Temozolomide dose and AUC did not predict nadir neutrophil and platelet counts due to large variability in counts.

Conclusions. The current dose regimen is administered according to BSA which is the most important factor influencing temozolomide clearance. No further dose adjustment is required.

KEY WORDS: temozolomide; cancer; population pharmacokinetics; nonlinear mixed-effects models; pharmacokinetics-toxicity relationship.

INTRODUCTION

Temozolomide (3,4-Dihydro-3-methyl-4-oxoimidazo-[5,1-d]-1,2,3,5-tetrazin-8-carboxamide) is a broad spectrum anti-tumor agent (1-7) Recently, temozolomide is approved for the treatment of refactory anaplastic astrocytoma (AA) after first relapse in the U.S., of both AA and glioblastoma multiforme (GBM) after first relapse in Europe. Temozolomide is currently undergoing clinical trials in the treatment of other tumors including brain metastasis, lung cancer, and breast cancer.

The objectives of the analysis were (1) to identify and quantify the influence of patient characteristics and other factors on the pharmacokinetics of temozolomide, and (2) to investigate possible influences of patient cofactors and pharmacokinetics on the dose-limiting toxicity of temozolomide. A sparse sampling scheme, which was prospectively developed using phase I data, was successfully implemented in the phase II trials to collect blood samples. There were 380 patients from seven phase II studies. The intensively sampled concentration data from 65 patients enrolled in four phase I studies were used to help model building. The pooled data set in this analysis included 2864 concentrations from 445 patients.

METHODS

Patient Populations

All patients in the analysis were diagnosed with advanced cancer and were to have a life expectancy of greater than 12 weeks, have adequate hematological and renal function, and have a Karnofsky Performance Status (KPS) $\geq$ 70 or ECOG/WHO status class 0, 1 or 2 (8). Two phase II studies enrolled only AA patients, two GBM, and three MM. Patients in the phase I studies had advanced cancer of various types.

Dose Regimen

Phase II patients received temozolomide at either 150 or 200 mg/m^2/day for 5 days every 28-day cycle. Doses were given to patients according to BSA (calculated using WT and HT (9)) and were rounded to 20 mg. Capsules of 20 mg and 100 mg strengths were used. Each daily dose was given with the least number of capsules. Temozolomide were administered after a fast of at least 8 hours; patients remained fasting until at least 2 hours post-dose. Phase I dose ranged from 100 to 200 mg/m^2/day as a 5-day regimen every 28 days or 500 to 1000 mg/m^2 as a single dose every 28 days.

Blood Sampling Schemes

All concentrations used in the analysis were collected on the first day of therapy. Intensive blood samples ($\geq$ 16 time points) were collected in phase I studies. Sparse sampling schemes were developed using simulations on a phase I study (10). The sparse scheme implemented in the phase II studies was as follows: 1. Pre-dose, 1.5, 2.5, 3.5 and 4 hours post-dose if the patient's last name begins with A-M; 2. Pre-dose, 1.5, 2, 3 and 4 hours post-dose if the patient's last name begins with N-Z.

Few additional samples were collected at 6 hours post-dose in a small number of patients to further assure the reliability of the analysis. The exact dosing and sampling times were recorded on data collection forms, and this information was used in the analysis.

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Assay Method

Temozolomide concentration in blood sample was quantified using an HPLC assay. This assay was determined to be reproducible, sensitive, precise and selective, and was validated over a concentration range of 0.10 to 20.0 μg/mL (Schering-Plough data on file). The limit of quantitation (LOQ) was established at 0.10 μg/mL.

Population Factors

Population factors which were assessed in the analysis included demographic variables (age, gender, WT, HT, BSA, Sr.Cr., and creatinine clearance (CL_{cr}, estimated from age, gender, WT and Sr.Cr.) (11)), serum chemistry data (SGOT, SGPT, alkaline phosphatase, serum bilirubin, serum albumin and total protein) obtained prior to treatment, tobacco use and concomitant medications. Medications commonly used by GBM and AA patients included phenytoin, valproic acid, carbamazepine, dexamethasone, phenobarbital, prochlorperazine, ondansetron and H_2-receptor antagonists. Tumor type was considered as a factor for phase II patients, i.e. brain tumor (GBM & AA) and MM.

Population Pharmacokinetic Model Building

It has been demonstrated in a phase I study that temozolomide is rapidly and completely absorbed and exhibits linear pharmacokinetics with increasing oral dose up to 1000 mg/m^2 (14). The semi-log concentration-time plot of one phase I study in the analysis is shown in Figure 1.1. A one-compartment model with first-order absorption and elimination without lag-time was selected as the pharmacokinetic model for temozolomide.

The observed concentration c_{ij} from patient i at time t_{ij} was modeled as follows:

$$c_{ij} = f_{ij}\,(CL_i, Ke_i, Ka_i; t_{ij})\,(1 + \varepsilon_{ij})$$

where f_{ij} is the function for one-compartment model with first-order absorption and first-order elimination, and ε_{ij} is the random variability terms. ε_{ij} were assumed to be normally distributed with mean zero and variance σ^2. The parameters in the structural model were CL_i, the plasma clearance of temozolomide; Ke_i, the elimination rate constant; and Ka_i, the absorption rate constant.

Individual pharmacokinetic paramenter, e.g. CL_i, was assumed to be log-normally distributed. When there was no covariate, the parameter were modeled as follows:

$$\ln CL_i = \ln \theta_1 + \eta_i^{CL},$$

where θ_1 is the population mean clearance, and η_i^{CL} is the inter-subject variability for clearance. η_i^{CL} was normally distributed with mean zero and variance ω_{CL}^2. Other pharmacokinetic parameters, e.g., Ke_i, and Ka_i, were similarly modeled if necessary.

Continuous covariates were introduced into the model in the fashion of linear additive terms of their log-transformed values. For example, if BSA was a covariate in the model, then the clearance CL_i was modeled as follows:

$$\ln CL_i = \ln \theta_3 + \theta_4 \ln BSA_i + \eta_i^{CL}.$$

Discrete covariates, e.g., gender, were introduced in the model as follows:

$$\ln CL_i = \ln \theta_5 + \eta_i^{CL} \text{ for female,}$$

$$= \ln \theta_6 + \eta_i^{CL} \text{ for male.}$$

Covariates were evaluated in the model building process. The contribution of a covariate in a model was determined by the reduction in the minimum objective function from this model with the covariate removed. This reduction was compared to a Chi-square distribution for statistical significance (α = 0.005). The final population pharmacokinetic model was determined based on both statistical and physiological considerations.

The software NONMEM (Nonlinear Mixed-Effects Model) (12, 13, 14) was used for model building. SAS (15) was used for data manipulation and statistical analysis before and after modeling. S-PLUS (16) was used to generate graphical displays. All NONMEM runs were performed on a DEC AlphaServer 2100 at Schering-Plough Research Institute, Kenilworth, New Jersey.

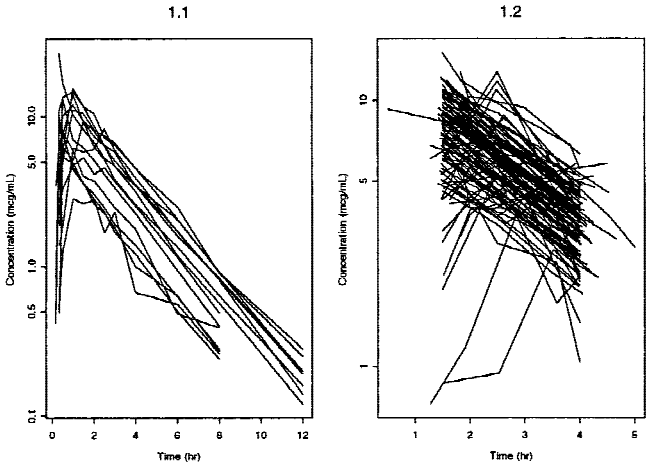

Fig. 1. The concentration-time data of two studies. Figure 1.1 is from a phase I study, and Figure 1.2 is from a phase II study. The vertical axis is in logarithmic scale.

RESULTS

The demographics of the 445 patients are summarized in Table 1. There were 65 patients in the phase I studies. In the phase II studies, there were 178 patients with GBM, 116 patients with AA, and 86 patients with MM. Approximately 40% (166/445) were female patients. Age, WT, HT, BSA, and Sr.Cr. profiles were similar among studies. The majority of patients (95%) were Caucasians. Smoking status was available only for GBM and AA studies, and approximately 20% were smokers. The concentration-time plots of two studies from each phase are displayed in Figure 1.

Population Pharmacokinetic Models

The initial covariate evaluation based on phase I data indicated that temozolomide clearance was influenced by BSA, WT, HT and gender. Therefore, only clearance models that involved these covariates were examined in the combined phase I and II data. Very few phase II patients had samples collected during absorption phase. Therefore, the absorption rate constant (Ka) for the phase II patients was mod-

Table 1. Summary of Patient Demographics

Patient	Gender[a]	Age (yr)[b]	WT (kg)[b]	BSA (m^2)[b]	HT (cm)[b]	Sr.Cr. (mg/dL)[b]	Race[c]	Smoking[d]
Phase I	F: 26, M: 39	53 (25–78)	72 (45–108)	1.83 (1.41–2.28)	171 (150–193)	0.95 (0.5–1.5)	12	—
GBM	F: 65, M: 113	52 (24–77)	78 (41–137)	1.90 (1.36–2.71)	172 (150–197)	0.87 (0.4–1.5)	2	35 (142)
AA	F: 47, M: 69	43 (19–76)	78 (45–145)	1.90 (1.43–2.51)	172 (146–190)	0.84 (0.4–1.6)	4	24 (92)
MM	F: 28, M: 58	53 (20–82)	78 (39–112)	1.90 (1.37–2.40)	173 (154–192)	0.90 (0.5–1.4)	0	—
ALL	F: 166, M: 279	50 (19–82)	77 (39–145)	1.89 (1.36–2.71)	172 (146–197)	0.88 (0.4–1.6)	18	59 (234)

[a] F: Female, and M: Male. [b]Numbers are Mean (Min–Max). [c]Number of non-Caucasian patients. [d]Numbers are Smokers (Non-smokers).

eled as a fixed constant, which equals to the mean Ka from the phase I patients. Tumor type was considered as a covariate for the phase II patients, i.e. brain tumor (AA & GBM) and MM.

These models for combined phase I and II patients were summarized in Table 2. Model 4 (BSA) resulted in the smallest minimum objective function among all single covariate models (Models 2–4). The combinations of two covariates, a continuous covariate and gender, were evaluated in Models 6–8. All models showed significant reductions in their objective functions ($p \leq 0.002$) indicating gender had significant contribution as a covariate. Although the improvement in objective function over Model 6 (WT+Gender) was marginal, Model 8 (BSA+Gender) resulted in the smallest objective function value and was selected as a temporary population model for further investigation.

This temporary model was chosen as the final population model after some failed attempts for refinement. One was the adding of a random effect to Ke for phase II patients. The individual CL estimates obtianed from a model run including inter-subject variability of Ke for phase II patients were compared with that of the temporary model. A high correlation ($R^2 = 0.996$) was found between these two estimates indicating that the individual Ke was not needed. These individual Ke estimates obtained in this run were examined against covariates and no influence of any covariates was found.

The final population model was again run to determine parameter estimates using the data that included an additional patient whose WT was missing and BSA was available. Diagnostic plots of this run are displayed in Figure 2. Agreement is shown between predicted and observed concentrations, and there is no peculiar trend between residuals and predicted concentrations. Hence, the model fitted the data well.

The parameter estimates in the final model are listed in Table 3. The clearance of temozolomide was an increasing function of BSA for both genders. For a female patient with BSA = 1.7 m^2 and a male patient with BSA = 2.0 m^2 the model predicted mean clearances are given in the last column of Table 3. The inter-subject variability for CL was 15%. The population mean of Ka was estimated at 2.66 hr^{-1} for phase I patients and the inter-subject variability was 115%. Ka of the phase II patients was fixed at the mean of phase I patients. The population means of Ke were estimated at 0.397 hr^{-1} and 0.363 hr^{-1} for phase I and phase II patients, respectively. The inter-subject variability of Ke was 6% in the phase I patients. The residual variability was 26%. The relationships between temozolomide clearance and BSA for both genders are graphically displayed in Figure 3.

The effects of smoking status and concomitant medication were evaluated based on the individual estimates and are summarized in Table 4. Tobacco use and seven out of eight co-medications, except valproic acid, did not affect the clearance of temozolomide ($p \geq 0.095$). Valproic acid use in the male patients resulted in a statistically significant decrease in clearance ($p = 0.044$). However, this difference may be a true false positive. A contributing factor to the false positive result may be the highly unbalanced sample size between patients with co-medication and those without. However, this apparent 4% decrease in clearance was not considered to be clinically important.

Serum albumin and total protein levels were used as indicators of patient's hepatic function; SGOT, SGPT, bilirubin and alkaline phosphatase were used as indices of hepatocellular disease. The influence of hepatic function on temozolomide clearance was evaluated by examining for trends in the

Table 2. Population Pharmacokinetic Models for the Phase I and Phase II Patients

Model	Covariate	Minimum objective function	Reference model	Δdf	p-value
1	None	3784	—	—	—
2	WT	3623	1	1	<0.0001
3	HT	3658	1	1	<0.0001
4	BSA	3594	1	1	<0.0001
5	Gender	3675	1	3	<0.0001
6	WT + Gender	3580	2	6	<0.0001
7	HT + Gender	3637	3	6	0.002
8	BSA + Gender	3573	4	6	0.002

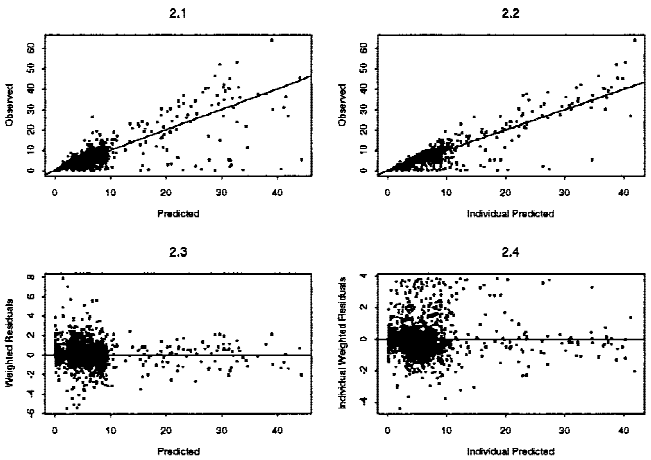

Fig. 2. The diagnostic plots of the final model. The lines in Figure 2.1and 2.2 are line of identity.

Table 3. Parameter Estimates and Standard Errors of the Final Model

Phase	Gender	ln CL (L/hr)	Ke (hr^{-1})	Ka (hr^{-1})	ω_{CL}	ω_{Ke}	ω_{Ka}	σ	CL (L/hr)
I	Female	ln 6.26 + 1.05 ln BSA	0.397	2.66	15%	6%	115%	26%	10.1
		(0.69)[a] (0.21)	(0.005)	(0.46)					
	Male	ln 4.87 + 1.14 ln BSA							13.9
		(1.15) (0.26)							
II GBM & AA	Female	ln 4.87 + 1.09 ln BSA	0.363	2.66	15%	—	—		8.8
		(0.45) (0.16)	(0.007)	(0.46)					
	Male	ln 7.01 + 0.66 ln BSA							11.2
		(0.76) (0.15)							
II MM	Female	ln 4.31 + 1.45 ln BSA							9.4
		(0.60 (0.25)							
	Male	ln 5.81 + 1.13 ln BSA							12.9
		(1.28) (0.32)							

[a] The number in parentheses is the standard error of the estimate.

plots against individual clearances. No trend was found indicating none of these variables (across the range tested) had an effect on the clearance of temozolomide. Individual clearances were examined against Sr.Cr. and CL_{cr} as indicators of renal function. No influence of renal function on the clearance of temozolomide was found.

Dose Limiting Toxicity of Temozolomide

Myelosupression is the primary dose-limiting toxicity of temozolomide. Nadir neutrophil counts and nadir platelet counts during the first treatment cycle were chosen as indices of hematological toxicity. Patients who had hematology tests performed greater than or equal to 13 days after the first dose were included. There were 270 patients with available neutrophil counts and 284 patients with platelet counts. Dose and the area under concentration-time curve (AUC), estimated by the ratio of total daily dose and CL, were used as measures of temozolomide exposure.

The incidences of neutropenia (absolute neutrophil count <500/μL) and thrombocytopenia (absolute platelet count <20,000/μL) in the first treatment cycle vs. patient characteristics and temozolomide exposure are summarized in Table 5. The overall incidence of neutropenia was 7.4%, or

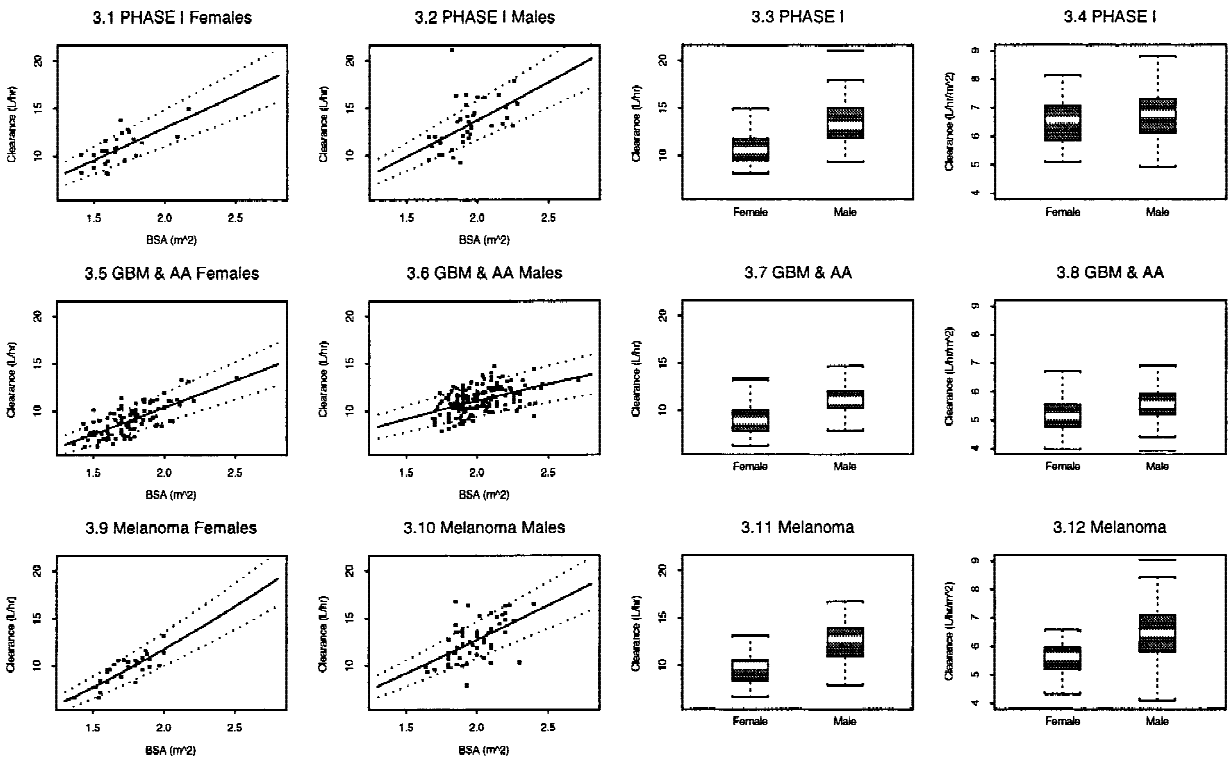

Fig. 3. Temozolomide CL vs. Covariates. The solid lines in scatter plots are the mean clearance, and the broken lines are one standard deviation from the mean. In box plots, the central (shaded) box contains 50% of the data, and the white line indicates the median. The whiskers connected the central box contains approximately 95% of the data, and the values outside the whiskers are plotted individually by horizontal lines.

Table 4. Comparison of Mean (S.E.) BSA-Adjusted Clearance of Temozolomide (L/hr/m^2)

Tobacco Use:	Non-smoker	Smoker	p-value[a]
Female	5.45 (0.06) 94[b]	5.34 (0.15) 18	0.760
Male	5.55 (0.05) 140	5.68 (0.07) 41	0.136
Concomitant Medication	NO	YES	p-value[a]
Dexamethasone:			
Female	5.28 (0.08) 59	5.32 (0.08) 52	0.936
Male	5.65 (0.06) 88	5.50 (0.06) 91	0.095
Phenytoin:			
Female	5.25 (0.06) 78	5.41 (0.13) 33	0.422
Male	5.58 (0.05) 116	5.57 (0.07) 63	0.869
Phenobarbital:			
Female	5.30 (0.06) 100	5.26 (0.18) 11	0.824
Male	5.57 (0.04) 161	5.57 (0.14) 18	0.907
Carbamazepine:			
Female	5.31 (0.07) 898	5.24 (0.11) 23	0.724
Male	5.55 (0.05) 134	5.64 (0.09) 45	0.367
Valproic acid:			
Female	5.33 (0.06) 96	5.06 (0.16) 16	0.178
Male	5.60 (0.04) 156	5.37 (0.12) 23	0.044
H-2 receptor antagonists:			
Female	5.28 (0.07) 72	5.32 (0.10) 39	0.998
Male	5.59 (0.05) 118	5.54 (0.07) 61	0.485
Ondansetron:			
Female	5.34 (0.09) 44	5.27 (0.07) 67	0.553
Male	5.50 (0.07) 65	5.61 (0.05) 114	0.124
Prochlorperazine:			
Female	5.29 (0.06) 105	5.43 (0.18) 6	0.453
Male	5.56 (0.04) 167	5.68 (0.13) 12	0.586

[a] Wilcoxon rank-sum test. [b]Mean (S.E.) N.

20/270. Older female patients who received higher dose (per m^2) tended to have a greater chance of developing neutropenia. Temozolomide exposure in terms of AUC was 12% higher (35.81 vs. 32.03 hr·μg/mL) in the patients who developed neutropenia. This difference in mean AUCs was primarily due to the larger proportion of patients who received high doses (200 mg/m^2/day) in the patients who developed neutropenia (17/20 : 120/250).

The overall incidence of thrombocytopenia was 5.3%, or 15/284. Older female patients who received higher dose (per m^2) tended to have a greater chance of developing thrombocytopenia. Temozolomide exposure in terms of AUC was slightly higher (8% or 34.78 vs. 32.16 hr·μg/mL) in the patients who developed thrombocytopenia.

DISCUSSION

This report provides the first description of the influence of population factors on the pharmacokinetics of temozolomide in advanced cancer patients. The population mean clearance of temozolomide increased with BSA and associated with small inter-subject variability (15%). This small variability was expected since the metabolism of temozolomide is primary via non-enzymatic pH dependent degradation (1,17–19). By the same token, it was not surprising that smoking status, hepatic function and disease, renal function and co-medications showed little effect on clearance. Administration of valproic acid in male patients was associated with a 4% decrease in the clearance. These data are compatible with the small role that hepatic metabolism plays in the clearance of temozolomide and with the inhibitory effect of valproic acid on oxidative metabolism (19).

Table 5. Incidences of Neutropenia and Thrombocytopenia by Patient Characteristics and Temozolomide Exposure

	Nadir Neutrophil Count				Nadir Platelet Count			
Variable	<500/μL	≥500/μL	%	p-value	<20,000/μL	≥20,000/μL	%	p-value
Age:								
≤29	1	14	6.7	0.312[a]	1	16	5.9	0.089[a]
30–39	3	48	5.9		0	52	0	
40–49	5	65	7.1		4	69	5.5	
50–59	4	78	4.9		4	81	4.7	
60–69	5	39	11.4		4	43	8.5	
≥70	2	6	25.0		2	8	20.0	
All	20	250	7.4		15	269	5.3	
Gender:								
Female	12	89	11.9	0.029[b]	10	100	9.1	0.024[b]
Male	8	161	4.7		5	169	2.9	
Dose:								
≤150 mg/m^2/day	3	130	2.3	0.001[b]	5	134	3.6	0.164[b]
200 mg/m^2/day	17	120	12.4		10	135	6.9	
Dose (by Gender):								
≤150 mg/m^2/day								
Female	3	43	6.5	0.040[b]	4	46	8.0	0.056[b]
Male	0	87	0		1	88	1.1	
200 mg/m^2/day								
Female	9	46	16.4	0.187[b]	6	54	10.0	0.182[b]
Male	8	74	9.8		4	81	4.7	
AUC: (μg · hr/mL)								
Female	35.36 (1.68) 12	33.55 (0.65) 89	—	0.287[c]	35.58 (2.03) 10	33.50 (0.59) 100	—	0.277[c]
Male	36.48 (1.28) 8	31.18 (0.42) 161	—	0.007[c]	33.16 (3.73) 5	31.37 (0.40) 169	—	0.363[c]
ALL	35.81 (1.11) 20	32.03 (0.36) 250	—	0.003[c]	34.78 (1.79) 15	32.16 (0.34) 269	—	0.071[c]

[a] Fisher's exact test, two-sided. [b]Fisher's exact test, one-sided. [c]Wilcoxon rank-sum test. [d]Mean (S.E.) N.

The incidences of neutropenia and thrombocytopenia during the first treatment cycle were low and tended to associate with patient's age, gender, and temozolomide exposure. However, neither total daily dose nor AUC were a predictor of the nadir counts due to large variability in the nadir counts and relatively narrow ranges for dose and AUC. The relationships with other pharmacokinetic parameters such as C_{max} and the duration of exposure above a certain concentration threshold were not examined.

In conclusion, the population pharmacokinetics of temosolomide has been reported in patients with advanced cancer. The clearance of temozolomide was affected only by BSA and gender. Neither hepatic function, renal function, nor commonly used co-medications had a clinically significant impact on the clearance of temozolomide. This finding supported the current dose regimen, which is already adjusted according to BSA. No further dose adjustment is required.

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