

10-22-2022

Germline Polymorphisms in MGMT Associated With Temozolomide-Related Myelotoxicity Risk in Patients With Glioblastoma Treated on NRG Oncology/RTOG 0825

Michael E Scheurer
Baylor College of Medicine

Renke Zhou
Baylor College of Medicine

Mark R Gilbert
National Institutes of Health Clinical Center

Follow this and additional works at: <https://jdc.jefferson.edu/pacbfp>

Part of the [Medical Pathology Commons](#)

Let us know how access to this document benefits you

Laura and Isaac Perlmutter Cancer Center at NYU Langone

Recommended Citation

Scheurer, Michael E; Zhou, Renke; Gilbert, Mark R; Bondy, Melissa L; Sulman, Erik P; Yuan, Ying; Liu, Yanhong; Vera, Elizabeth; Wendland, Merideth M; Youssef, Emad F; Stieber, Volker W; Komaki, Ritsuko R; Flickinger, John C; Kenyon, Lawrence C.; Robins, H Ian; Hunter, Grant K; Crocker, Ian R; Chao, Samuel T; Pugh, Stephanie L; and Armstrong, Terri S, "Germline Polymorphisms in MGMT Associated With Temozolomide-Related Myelotoxicity Risk in Patients With Glioblastoma Treated on NRG Oncology/RTOG 0825" (2022). *Department of Pathology, Anatomy, and Cell Biology Faculty Papers*. Paper 377.
<https://jdc.jefferson.edu/pacbfp/377>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Pathology, Anatomy, and Cell Biology Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Michael E Scheurer, Renke Zhou, Mark R Gilbert, Melissa L Bondy, Erik P Sulman, Ying Yuan, Yanhong Liu, Elizabeth Vera, Merideth M Wendland, Emad F Youssef, Volker W Stieber, Ritsuko R Komaki, John C Flickinger, Lawrence C. Kenyon, H Ian Robins, Grant K Hunter, Ian R Crocker, Samuel T Chao, Stephanie L Pugh, and Terri S Armstrong

Germline polymorphisms in *MGMT* associated with temozolomide-related myelotoxicity risk in patients with glioblastoma treated on NRG Oncology/RTOG 0825

Michael E. Scheurer[®], Renke Zhou, Mark R. Gilbert, Melissa L. Bondy, Erik P. Sulman[†], Ying Yuan[®], Yanhong Liu, Elizabeth Vera[†], Merideth M. Wendland, Emad F. Youssef^{†,‡}, Volker W. Stieber, Ritsuko R. Komaki, John C. Flickinger, Lawrence C. Kenyon, H. Ian Robins, Grant K. Hunter, Ian R. Crocker, Samuel T. Chao, Stephanie L. Pugh, and Terri S. Armstrong

Baylor College of Medicine, Departments of Pediatrics and Medicine, Houston, Texas, USA (M.E.S., R.Z., M.L.B., Y.L.); National Institutes of Health Clinical Center, Bethesda, MD, USA (M.R.G., E.V., T.S.A.); M D Anderson Cancer Center, Brain and Spine Center, Houston, TX, USA (E.P.S., Y.Y., E.V., R.R.K.); National Cancer Institute, Bethesda, MD, USA (M.M.W.); Texas Oncology Cancer Center Sugar Land, Sugar Land, TX, USA (M.M.W.); Arizona Oncology Services Foundation, Tucson, AZ, USA (E.F.Y.); Forsyth Regional Cancer Center, Winston-Salem, NC, USA (V.W.S.); UPMC-Shadyside Hospital, Pittsburgh, PA, USA (J.C.F.); Thomas Jefferson University Hospital, Philadelphia, PA, USA (L.C.K.); University of Wisconsin Hospital, Madison, WI, USA (H.I.R.); Intermountain Medical Center, Murray, UT, USA (G.K.H.); Emory University, Winship Cancer Institute, Atlanta, GA, USA (I.R.C.); Cleveland Clinic Foundation, Cleveland, OH, USA (S.T.C.); NRG Oncology Statistics and Data Management Center, Philadelphia, PA, USA (S.L.P.); Laura and Isaac Perlmutter Cancer Center at NYU Langone, New York, NY, USA (E.P.S.)

[†]At time of study.

[‡]Deceased.

Corresponding Author: Michael E. Scheurer, PhD, Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, MS: BCM305, Houston, TX 77030, USA (scheurer@bcm.edu).

Abstract

Background. We sought to identify clinical and genetic predictors of temozolomide-related myelotoxicity among patients receiving therapy for glioblastoma.

Methods. Patients ($n = 591$) receiving therapy on NRG Oncology/RTOG 0825 were included in the analysis. Cases were patients with severe myelotoxicity (grade 3 and higher leukopenia, neutropenia, and/or thrombocytopenia); controls were patients without such toxicity. A risk-prediction model was built and cross-validated by logistic regression using only clinical variables and extended using polymorphisms associated with myelotoxicity.

Results. 23% of patients developed myelotoxicity ($n = 134$). This toxicity was first reported during the concurrent phase of therapy for 56 patients; 30 stopped treatment due to toxicity. Among those who continued therapy ($n = 26$), 11 experienced myelotoxicity again. The final multivariable clinical factor model included treatment arm, gender, and anticonvulsant status and had low prediction accuracy (area under the curve [AUC] = 0.672). The final extended risk prediction model including four polymorphisms in *MGMT* had better prediction (AUC = 0.827). Receiving combination chemotherapy (OR, 1.82; 95% CI, 1.02–3.27) and being female (OR, 4.45; 95% CI, 2.45–8.08) significantly increased myelotoxicity risk. For each additional minor allele in the polymorphisms, the risk increased by 64% (OR, 1.64; 95% CI, 1.43–1.89).

Conclusions. Myelotoxicity during concurrent chemoradiation with temozolomide is an uncommon but serious event, often leading to treatment cessation. Successful prediction of toxicity may lead to more cost-effective individualized monitoring of at-risk subjects. The addition of genetic factors greatly enhanced our ability to predict toxicity among a group of similarly treated glioblastoma patients.

Key Points

- Toxicity from chemotherapy can be a serious event leading to treatment cessation.
- Genetic variants enhanced prediction of myelotoxicity among glioblastoma patients.
- Individualized genetic monitoring of at-risk subjects could greatly improve outcomes.

Importance of the Study

Based on results from other studies as well as our initial findings, we hypothesized that germline SNPs are logical predictors of chemotherapy toxicity and response, especially when examined in conjunction with clinical risk factors. For cytotoxic regimens like TMZ, variants from DNA repair pathways are likely shared with tumors, making prediction of both toxicity and response possible. We found that clinical variables alone were poor predictors of

myelotoxicity in patients with GBM receiving TMZ; however, the addition of germline variants in the DNA repair gene *MGMT* increased the ability to predict toxicity among these patients. Such findings have the potential to impact the management of patients by predicting toxicity risk and better individualize initial temozolomide dosing and bloodwork monitoring schedules in patients with GBM receiving this drug.

Glioblastoma (GBM) is the most common primary malignant brain tumor in the United States and carries the worst survival for patients with this disease.¹ Currently, temozolomide (TMZ) in conjunction with conventional fractionated external beam radiation therapy followed by adjuvant TMZ is the standard of care for GBM patients.² Despite the widespread acceptance that TMZ is well tolerated, 16% of patients on NRG Oncology's RTOG 0525, a phase III trial comparing conventional adjuvant TMZ with dose-intensive TMZ, experienced grade 3 or higher myelotoxicity during chemoradiation, and 23% of patients had similar levels of myelotoxicity during the standard dose adjuvant phase, typically during the first cycles of the treatment course.³ In addition to contributing to severe infections and bleeding events, grade 3 or 4 myelotoxicities often result in treatment delays or complete cessation of this life-prolonging therapy.^{4–6} The risk is underscored by reports of profound myelotoxicity, including aplastic anemia.^{7–10} For this reason, identifying patients at high risk of developing myelotoxicity would allow for a more suitable starting dose for this sensitive subpopulation. Conversely, it would also allow the remaining population to receive full-dose treatment from the initiation of therapy. Both scenarios potentially increase the therapeutic efficacy of this drug by shifting the therapeutic window and reducing toxicity, thus allowing for continuation of treatment and potentially impacting patient outcomes.

Genomic predictors of treatment-related toxicities have a long history.^{11,12} The seminal discovery of inherited Glucose-6-phosphate dehydrogenase (*G6PD*) deficiency leading to the profound development of hemolytic anemia when exposed to the quinines and other drugs such as

dapsone provided clear evidence that genomic variability in genes that mediate drug metabolism can predict toxicity.¹³ In cancer treatment with irinotecan, there is a significant association between the *UGT1A1* *28/*6 polymorphisms and increased toxicity. This association has demonstrated the clinical relevance of genome-based predictive markers for treatment-related toxicity.¹⁴ More recently, a series of polymorphisms that predict the development of thalidomide-associated peripheral neuropathy^{15,16} was detected in patients treated for multiple myeloma. We also recently reported that several clinical factors as well as specific concomitant medications could be incorporated into a formula to create a “risk score” that was highly correlated with the occurrence of significant myelotoxicity in patients treated with standard dose TMZ at recurrence.¹⁷ Interestingly, the toxicity risk score components varied markedly between male (higher body surface area [BSA], not currently using steroids, and currently using bowel medication) and female (no prior chemotherapy, higher creatinine levels, lower platelet levels, lower BSA, not currently using medication for gastroesophageal reflux disease, and currently using analgesics) patients. Furthermore, a small set of candidate polymorphisms also increased our ability to predict myelotoxicity in this group of GBM patients.

Taken together, these studies strongly support the identification of predictors of treatment toxicity to mitigate the adverse impact of therapy in select populations. For example, if a risk model is validated, patients with newly diagnosed GBM could be screened using a risk calculator for the likelihood of myelotoxicity. Patients at elevated risk of toxicity could then be started at a lower dose of

TMZ (with escalation as tolerated) while the other patients could begin at full dose, thus increasing the likelihood that all patients are able to receive this proven life-extending treatment. We, therefore, conducted a study using a closely monitored patient population who received concurrent TMZ and radiation as a component of a large clinical trial, taking advantage of the trial-related mandatory systematic toxicity reporting and collection of peripheral blood.

Materials and Methods

Study Population

NRG Oncology's RTOG 0825 was a phase III, randomized double-blind placebo-controlled trial comparing standard chemoradiotherapy using TMZ with or without the addition of bevacizumab (BEV) for patients with newly diagnosed GBM.¹⁸ Eligibility criteria included age ≥ 18 years, Karnofsky Performance Status (KPS) ≥ 70 , and adequate hematologic, renal, and hepatic function. Patients with recent or ongoing cardiovascular problems were excluded as well as those with poorly controlled moderate to severe hypertension (baseline systolic blood pressure ≤ 160 mmHg, diastolic ≤ 90 mmHg). Eligibility requirements also included submission of tumor tissue for central pathology review and *MGMT* methylation analysis. A blood sample was requested, although not mandatory. Informed consent for future research was provided as part of the parent trial which was approved by appropriate institutional review boards; de-identified data/samples were analyzed as part of the current study. Approval for the analysis of the de-identified data used in this analysis was received from the University of Texas Health Sciences Center at Houston.

Detailed demographic and clinical characteristics were obtained, including age, height, weight, gender, race/ethnicity, concurrent medications, KPS, baseline laboratory studies, and tumor characteristics. Treatment-specific toxicities graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 were also obtained.

Patients of all races/ethnicities were randomized ($n = 621$) to the clinical trial; however, 591 (95%) of the randomized subjects were non-Hispanic white and had complete clinical data. Therefore, we limited our analyses to non-Hispanic whites to minimize any confounding due to population structure in the genetic analyses. Blood samples were collected on 367 non-Hispanic white patients and were available for inclusion in the genetic analyses.

Processing of Blood Samples and Genotyping

Blood was collected as an amendment to the trial protocol. Germline DNA was extracted from blood using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Germline DNA samples were genotyped using the HumanOmni2.5Exome beadchip (Illumina, San Diego, CA), according to manufacturer's protocol.

Statistical Methods

We defined cases as patients with severe TMZ-related myelotoxicity using CTCAE version 3.0 for grade 3 and higher leukopenia, neutropenia, and/or thrombocytopenia, using the earliest event for each patient. Controls were defined as patients with grade 0–2 toxicity. The risk modeling was conducted in two steps: first, we built a model including only clinical factors; and second, we extended that model to include genetic polymorphisms. Clinical risk factors with a P value < 0.15 in the univariable logistic regression model were included for variable selection in the multivariable model. The final clinical risk model for toxicity was built using the backward selection method at P -value $< .05$. Where appropriate, a test for trend was used to assess significant trends in the association analysis. Next, we conducted a genome-wide association study (GWAS) of myelotoxicity. All of the samples had call rates $\geq 95\%$. Among available polymorphisms included on the beadchip ($n = 2\,583\,651$), we included in the analysis autosomal SNPs having call rates $\geq 95\%$, showing minimal departure from Hardy-Weinberg equilibrium (HWE; $P > 10^{-5}$ in controls) and minor allele frequencies (MAF) $\geq 5\%$ ($n = 1\,257\,515$). We dropped from further analysis all loci in strong linkage disequilibrium (LD) with another marker ($D' \geq 0.99$) to reduce redundant information. We used a standard GWAS nominal P -value $\leq 10^{-7}$ to select the most significant SNPs associated with myelotoxicity. We estimated the main effect of each genotype using the allele-based Chi-squared association test. Polymorphisms significantly associated with myelotoxicity in the main effects analyses were then included in the final multivariable regression models of clinical risk factors. We also conducted a joint effect analysis to test the hypothesized dose-response relationship between genotype and myelotoxicity, by summing the number of at-risk alleles (minor allele) identified from the main effect analysis. We used a 30-fold cross-validation of the entire process to check the performance of the final prediction model.¹⁹ We calculated the area under curve (AUC) for the receiver operating characteristic (ROC) curve as a way to assess the performance of the prediction models. We used PLINK v.1.9 to conduct the GWAS, and R v.3.2.3 (packages: *genetics* and *haplo.stats*) to perform all cross-validation and haplotype analyses. We conducted all other analyses using SAS v.9.4.

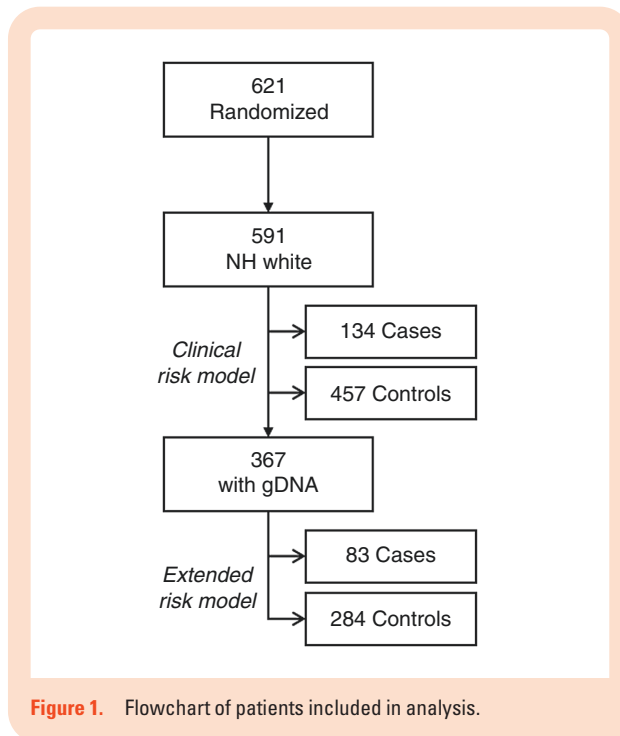
Data Availability

The data sets generated and/or analyzed during this study, as well as the computer code used to perform statistical analysis, are available from the corresponding authors on reasonable request. The datasets from the parent trial are available in dbGaP.

Results

Clinical Myelotoxicity Risk Model

Clinical characteristics and univariable logistic regression outcomes of the participants in this analysis (Figure 1) are



listed in Table 1. There were 134 cases with myelotoxicity (23%) and 457 controls; median ages at diagnosis were 59 and 58 years, respectively. Overall, 61% of the subjects were male; however, there were more females among the cases (61%) compared to controls (33%). In addition, those receiving combination therapy (60%) were more likely to experience myelosuppression than those who received TMZ alone (40%).

Of the 134 cases with myelotoxicity, 66 (49%) were first reported during the concurrent phase; an average of 40 days from the start of treatment (SD = 14 [range: 8–77]). Thirty-eight of these patients (57%) stopped treatment due to toxicity. Treatment termination due to any toxicity during the concurrent phase was more common among patients with myelotoxicity (60%) than those without myelotoxicity (20%) ($P < .001$). One patient continued to report myelotoxicity within 5 months of treatment termination, and one patient died within 1 month of treatment termination. Twenty-eight (42%) were able to continue to adjuvant treatment, 8 of which again experienced myelotoxicity; an average of 145 days from the start of treatment (SD = 91 [range: 91–364]) and an average of 111 days from the first event (SD = 91 [range: 61–333]). Sixty-seven (50%) of the remaining cases were first reported to have significant myelotoxicity during the adjuvant/follow-up phase; occurring an average of 194 days from the start of treatment (SD = 125 [range: 78–566]).

Before any adjustment, patients receiving both TMZ and BEV were 60% more likely to develop myelotoxicity compared to those patients who only received TMZ (OR, 1.6; 95% CI, 1.01–2.40; P value, .015). Females were 3.26 times more likely to have myelotoxicity compared to males (OR, 3.26; 95% CI, 2.19–4.86; P value, $< .0001$). Patients with impaired KPS had higher risk of myelotoxicity (KPS = 70: OR, 2.45; 95% CI, 1.2–4.99. KPS = 80: OR, 2.09; 95% CI, 1.12–3.92.

KPS = 90: OR, 1.44; 95% CI, 0.79–2.62. Trend P value, .0356). Increased creatinine, BSA and TMZ dose were associated with a lower risk of myelotoxicity. The final multivariable clinical factor model included treatment arm, gender, and anticonvulsant status (Table 2). The AUC for the ROC curve was 0.672, which represented a low prediction accuracy for clinical factors alone. When limiting the dataset to the 367 patients with genotype data, the AUC for the ROC curve for the model was 0.68, which was not statistically different than the AUC in the full dataset ($P = .77$).

Genetic Main Effects on Myelotoxicity

Among 367 patients with genotype information, 5 SNPs reached genome-wide statistical significance (Table 3). Four of the five SNPs were in *MGMT*, and the MAF of each SNP ranged from 0.46 to 0.59 among cases and 0.25 to 0.36 among controls. The strongest association with myelotoxicity was observed for rs1008982 with unadjusted P value 1.36×10^{-10} (OR, 3.12; 95% CI, 2.19–4.46 in unadjusted model; OR, 4.55; 95% CI, 2.81–7.35 in adjusted model including significant clinical factors). The final significant SNP, rs1549102, is located in *ANKS1B* with unadjusted P value 3.62×10^{-7} (OR, 2.52; 95% CI, 1.75–3.62 in unadjusted model; OR, 2.50; 95% CI, 1.71–3.66 in adjusted model including significant clinical factors).

Multivariable Model of Myelotoxicity

We compared demographic and clinical characteristics and toxicity outcome incidence between participants with ($n = 367$) and without ($n = 224$) genotype data to ensure comparability between these groups. No significant differences between the two sets of patients were found for any variables (data not shown), including those in the final clinical model (Table 2).

Initially, we aimed to fit our original clinical model developed for those receiving TMZ at recurrence¹⁷ to this sample; however, that model did not adequately fit the current data from newly diagnosed patients (data not shown). Therefore, we initiated an agnostic model building process in the current dataset as outlined above. Table 2 summarizes the results of multivariable regression model including both selected clinical and genetic risk factors.

Receiving BEV in addition to TMZ increased myelotoxicity risk 80% (OR, 1.82; 95% CI, 1.02–3.27; adjusted P -value, .0441). Females were about 4.5 times more likely to develop toxicity (OR, 4.45; 95% CI, 2.45–8.08; adjusted P -value, $< .0001$). Taking anticonvulsants increased risk, but not statistically. However, because anticonvulsant use influenced the overall fit of the model (P -value for likelihood ratio test $< .05$), we included it in the final clinical model. For each additional at-risk (minor) allele in any of the top 5 SNPs, the risk increased by 64% (OR, 1.64; 95% CI, 1.43–1.89; adjusted P -value, $< .0001$). The AUC of the final model increased to 0.827 after including the SNP dose effect, indicating a dramatic improvement in prediction accuracy. Figure 2 shows the ROC curve for the cross-validated model; the AUC is 0.807.

Table 1. Characteristics and Univariable Logistic Regression Outcomes of Myelotoxicity

Categorical Characteristics	Cases (N = 134)		Controls (N = 457)		OR	95% CI	P-value
	No.	%	No.	%			
Treatment arm							.015
TMZ+placebo	54	40.3	239	52.3	1		
TMZ+BEV	80	59.7	218	47.7	1.6	1.01–2.40	
Gender							<.0001
Male	52	38.8	308	67.4	1		
Female	82	61.2	149	32.6	3.26	2.19–4.86	
KPS							.0356
70	23	17.2	52	11.4	2.45	1.20–4.99	
80	42	31.3	111	24.3	2.09	1.12–3.92	
90	52	38.8	200	43.7	1.44	0.79–2.62	
100	17	12.7	94	20.6	1		
Surgery							.365
Subtotal	55	41	157	34.4	1.33	0.49–3.68	
Gross total	76	56.7	288	63	1		
Other	3	2.3	12	2.6	0.95	0.26–3.44	
Tumor MGMT Methylation Status							.423
Unmethylated	90	68.7	321	72.3	1		
Methylated	41	31.3	123	27.7	1.19	0.75–1.85	
BMI							.4839
Underweight & Normal	39	29.1	116	25.4	1.36	0.81–2.29	
Overweight	60	44.8	197	43.1	1.24	0.77–1.98	
Obese	35	26.1	142	31.1	1		
Missing	0	0	2	0.4			
Anticoagulant agents							.3368
No	128	95.5	426	93.2	1.55	0.63–3.80	
Yes	6	4.5	31	6.8	1		
Proarrhythmic potential agents							.2178
No	130	97	431	94.3	1.96	0.67–5.72	
Yes	4	3	26	5.7	1		
Herbal							.6509
No	128	95.5	432	94.5	1.24	0.50–3.08	
Yes	6	4.5	25	5.5	1		
Corticosteroids							.3701
No	32	23.9	127	27.8	1		
Yes	102	76.1	330	72.2	1.23	0.79–1.92	
Anticonvulsants							.0901
No	29	21.6	133	29.1	1		
Yes	105	78.4	324	70.9	1.49	0.94–2.35	
EIAEDs							.1646
No	117	87.3	374	81.8	1.49	0.85–2.62	
Yes	17	12.7	81	17.7	1		
Missing	0	0	2	0.5			
NEIAEDs							.0472
No	77	57.5	304	66.5	1		
Yes	57	42.5	151	33	1.49	1.01–2.21	
Missing	0	0	2	0.5			

Table 1. Continued

Categorical Characteristics	Cases (N = 134)		Controls (N = 457)		OR	95% CI	P-value
	No.	%	No.	%			
Urine protein/creatinine ratio							.9768
≤0.5	115	85.8	393	86	1		
>0.5	18	13.4	61	13.3	1.01	0.57–1.78	
Missing	1	0.8	3	0.7			
Continuous Characteristics	Median	IQR	Median	IQR			
Age, years	59	51–64	58	51–65	1.00	0.98–1.02	.9693
Creatinine, mg/dL	0.78	0.65–0.90	0.83	0.70–0.95	0.14	0.05–0.42	.0005
Platelet, 10 ³ /mm ³	240.5	192–302	235	193.0–283.5	1.00	0.99–1.01	.5535
ANC, 10 ² /mm ³	60.4	35.6–90.0	63	41.0–90.4	1.00	0.99–1.01	.6629
Hemoglobin, g/dL	13.4	12.3–14.2	13.5	12.5–14.4	0.94	0.82–1.09	.4145
WBC, 10 ² /mm ³	86	60–119	85	64–112	1.00	0.99–1.01	.7684
BUN, mg/dL	17	13–22	17	14–21	0.99	0.96–1.03	.7739
Total Bilirubin, mg/dL	0.4	0.3–0.6	0.5	0.3–0.6	0.84	0.42–1.71	.6364
SGOT, u/dL	20	16–26	21	16–26	0.99	0.98–1.01	.6985
SGPT, u/dL	31.5	23–47	36	25–50	0.99	0.98–1.01	.2498
PT, seconds	11.3	10.2–12.7	11.1	10.2–12.8	0.97	0.91–1.04	.3752
PT/INR	1	0.9–1.0	1	0.9–1.0	0.50	0.13–1.88	.3017
TMZ dose, 10 ² mg	30.5	27.3–33.6	31.5	28.5–34.7	0.97	0.95–0.99	.0439
BSA	1.87	1.72–2.08	2	1.84–2.18	0.15	0.06–0.34	<.0001

Abbreviations: ANC, absolute neutrophil count; BEV, bevacizumab; BSA, body surface area; BUN, blood urea nitrogen; CI, confidence interval; EIAEDs, enzyme-inducing anti-epileptic drugs; INR, international normalized ratio; IQR, inter-quartile range; KPS, Karnofsky Performance Status; NEIAEDs, non-enzyme-inducing anti-epileptic drugs; OR, odds ratio; PT, prothrombin time; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; TMZ, temozolomide; WBC, white blood cell count.

Bold values represent factors with a univariable *p* value <0.15 and were considered for the multivariable model.

Haplotype Block Structure and LD Analysis among SNPs from MGMT

Because four of the top five SNPs were in *MGMT*, we constructed haplotypes to better understand the contribution of variation across *MGMT* on toxicity. Haplotype block structures were defined using the LD confidence intervals method.²⁰ The association between toxicity and each common haplotype (frequency > 0.05) was evaluated by logistic regression analysis adjusting for the selected clinical risk factors. Figure 3 shows a plot of the pairwise LD (*D'*) values for the 13 significant SNPs within *MGMT* at 10⁻⁷ level. Two blocks (both intronic) with high LD were identified: block 1, containing selected SNPs rs1711667 and rs10466114; block 2, containing selected SNPs rs12266634 and rs1008982. We examined the association between the common haplotypes in *MGMT* and toxicity (Table 4). Two risk haplotypes were identified in Block 1: CA (adjusted OR, 3.96; 95% CI, 2.45–6.40) and CG (adjusted OR, 2.98; 95% CI, 1.62–5.49). One risk haplotype was identified in Block 2: GG (adjusted OR, 4.98; 95% CI, 2.99–8.29). The global score test showed significant differences in the haplotype profile for both Blocks 1 and 2 (*P* < .0001). Finally, we explored the haplotype association between toxicity and the four selected SNPs globally using a block-free approach. Consistent with the genotype analysis, the most common

haplotype AGCA was also the most favorable haplotype, containing zero risk alleles. When we used this favorable haplotype as the reference group, haplotype CAGG (containing all four risk alleles) showed statistically significant increased toxicity (adjusted OR, 6.40; 95% CI, 3.59–11.43).

Discussion

The occurrence of myelotoxicity during concurrent chemoradiation with TMZ is an uncommon but serious event. In this study, as with others, occurrence was idiosyncratic, occurring early in treatment rather than after cumulative exposure. As expected,²¹ incidence was higher in those on combination therapy, but surprisingly dose and BSA did not predict occurrence. The significance of the early toxicity in this study is noteworthy. The majority of patients who experienced myelotoxicity stopped their treatment and several experienced myelotoxicity months afterwards. Patients who continued onto adjuvant therapy also were likely to subsequently experience significant myelotoxicity.

Gender was the only demographic factor associated with myelotoxicity in this study. Females were 4.5 times as likely to experience myelotoxicity than males. Gender as a

Table 2. Multivariable Logistic Regression Model with Significant Clinical Risk Factors and Dose Effect of Top SNPs

Characteristics	Cases		Controls		OR	95% Wald CI		P-value
	No.	%	No.	%				
Final Clinical Model (<i>n</i> = 591)								
Treatment arm								.0243
TMZ+placebo	54	40.3	239	52.3	1			
TMZ+BEV	80	59.7	218	47.7	1.60	1.06	2.38	
Gender								<.0001
Male	52	38.8	308	67.4	1			
Female	82	61.2	149	32.6	2.98	2.00	4.45	
Anticonvulsants								.0386
No	29	21.6	133	29.1	1			
Yes	105	78.4	324	70.9	1.65	1.03	2.65	
Final Clinical+SNP Model (<i>n</i> = 367)								
Treatment arm								.0441
TMZ+placebo	30	36.1	153	53.9	1			
TMZ+BEV	53	63.9	131	46.1	1.82	1.02	3.27	
Gender								<.0001
Male	31	37.4	194	68.3	1			
Female	52	62.7	90	31.7	4.45	2.45	8.08	
Anticonvulsants								.1442
No	20	24.1	88	31.1	1			
Yes	63	75.9	196	69.0	1.64	0.85	3.18	
Top SNPs dose effect, number at-risk alleles	Median = 6, IQR = 4–7		Median = 3, IQR = 1–5		1.64	1.43	1.89	<.0001

Abbreviations: BEV, bevacizumab; CI, confidence interval; IQR, inter-quartile range; OR, odds ratio; SNP, single nucleotide polymorphism; TMZ, temozolomide.

Table 3. Top SNPs with *P* Values at Least 10^{-7} Levels Identified by Genotyping

SNP	Gene	Location	Chr: Position	Allele	Minor Allele Freq.		Unadjusted <i>P</i> value	Unadjusted			Adjusted ^a		
					Cases	Controls		ORs	95% CI		ORs	95% CI	
rs1008982	MGMT	Intron	10: 131445731	G/A	0.560	0.290	1.36×10^{-10}	3.12	2.19	4.46	4.55	2.81	7.35
rs12266634	MGMT	Intron	10: 131412659	G/C	0.512	0.267	2.76×10^{-9}	2.88	2.02	4.12	3.78	2.40	5.95
rs1711667	MGMT	Intron	10: 131278410	C/A	0.590	0.357	7.52×10^{-8}	2.60	1.82	3.70	3.47	2.23	5.37
rs10466114	MGMT	Intron	10: 131367368	A/G	0.458	0.253	3.84×10^{-7}	2.50	1.74	3.58	2.95	1.94	4.49
rs1549102	ANKS1B	Intron	12: 99282746	C/A	0.663	0.438	3.62×10^{-7}	2.52	1.75	3.62	2.50	1.71	3.66

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusting for the selected clinical risk factors: treatment arm, gender, and anticonvulsants.

predictor of toxicity was also shown in our earlier study of recurrent disease,¹⁷ as well as a smaller study of the concurrent phase of treatment.²² The propensity for females to experience more drug toxicity is increasingly being recognized. Overall, the degree to which gender relates to drug toxicity is not known, but it was reported that up to 7% of new drug applications that include gender analysis showed at least a 40% difference in pharmacokinetics between males and females.²³ The underlying molecular

mechanism is probably multifactorial, including increased drug exposure by hormone-dependent regulation of proteins in drug metabolism pathways and/or by direct action of sex hormones on the drug target.²⁴

As noted by Church and colleagues,¹² the burden of chemotherapy-associated toxicity is recognized but there are limited tools to increase the precision of prescribing chemotherapy to reduce risk. They coined the term “toxgnostics” as the systematic agnostic study of

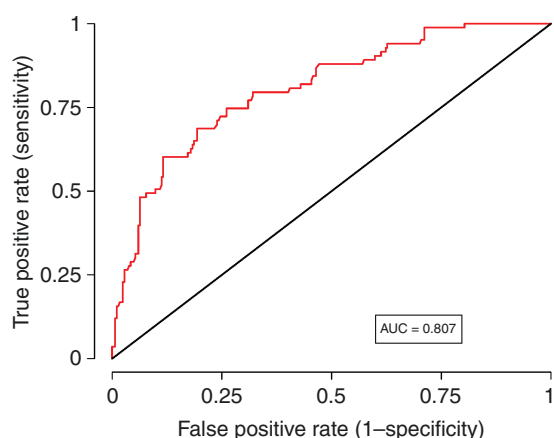


Figure 2. Receiver-operator characteristics (ROC) curve and area under the curve (AUC) metrics of the multivariable risk prediction model with both clinical and genetic factors conducting 30-fold cross-validation. The AUC of 0.807 indicates that the model provides excellent discrimination between those with and without toxicity.

genetic predictors of such toxicity. Key elements framing these analyses were: analysis embedded within large, prospective, randomized, controlled clinical trials; phenotype clearly defined and clinically relevant; analysis using the maximum relevant genomic diversity; and comparison of the performance of individual variants to that of a combined risk score, which may outperform individual variants. We followed these key tenants in our study.

In this report, for every additional at-risk allele carried by a patient, the risk of myelotoxicity increased by 64%. Further, the inclusion of the SNP data in our risk prediction model resulted in a cross-validated AUC of 0.807. Interestingly, four of our five top SNPs are located in *MGMT*. In our study, there was no association between tumor *MGMT* promoter methylation status and myelotoxicity. This is not a surprising finding as the promoter methylation, to our knowledge, is restricted to tumor cells; therefore, reduction of *MGMT* enzyme activity in hematopoietic cells could be due to the polymorphisms rather than expression changes due to the methylation status of the promoter region of the gene. Furthermore, there were no associations between our top SNPs and *MGMT* promoter methylation status (all *P*-values > .226). However, two recent studies reported increased risk of myelotoxicity to be

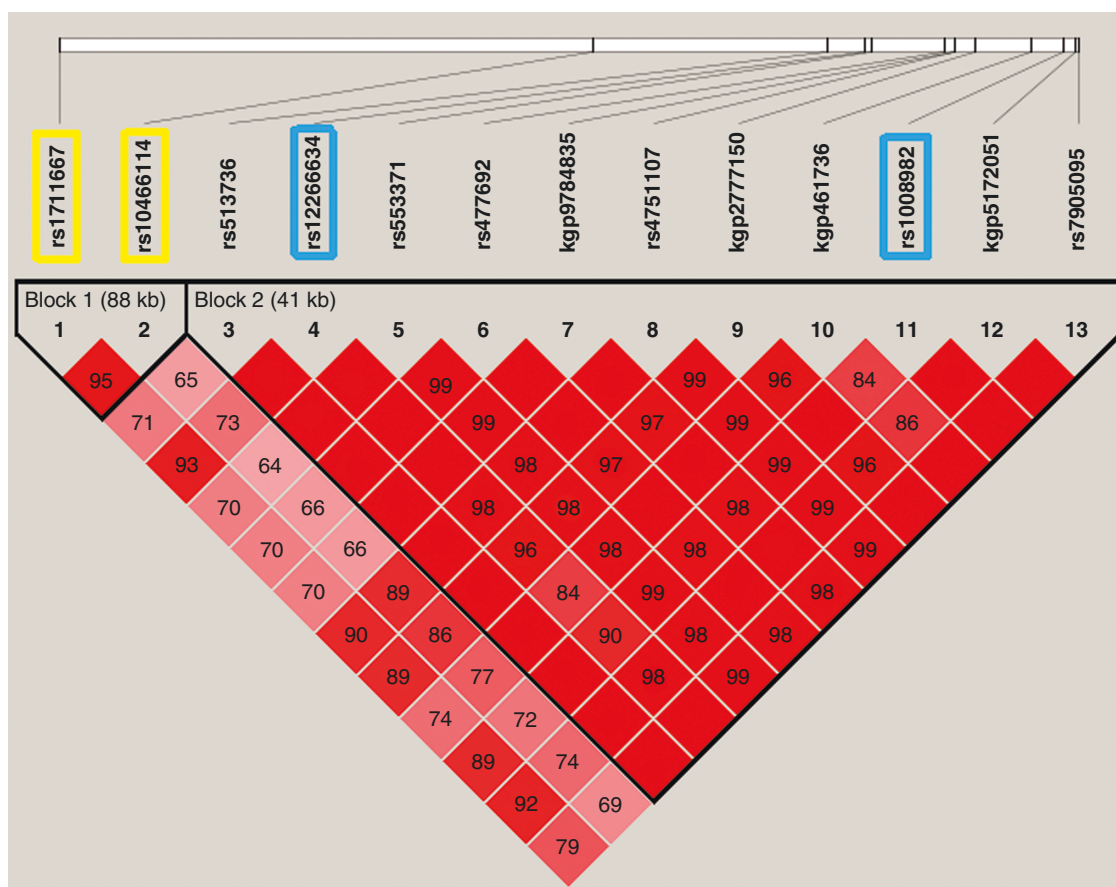


Figure 3. Linkage disequilibrium (LD) plot of *MGMT* SNPs identified at E-7 level and haplotype-block structure. The values indicate the LD relationship between each pair of SNPs; darker shading denotes a greater extent of LD between SNPs. The SNPs selected for inclusion in the haplotype analysis sufficiently capture the variation represented in this region of *MGMT*.

Table 4. Association between Haplotypes in MGMT and Myelotoxicity

Blocks	Haplotype ^a	Cases (N=83)		Controls (N=284)		Total %	OR ^b	95% Wald CI		Global P-value
		No.	%	No.	%					
Block 1	AG	32	38.8	182	64.0	58.4	1.00			<.0001
	CA	36	43.6	70	24.8	29.1	3.96	2.45	6.40	
	CG	13	15.5	31	10.8	11.8	2.98	1.62	5.49	
Block 2	CA	34	40.7	197	69.5	63.0	1			<.0001
	GG	40	47.9	71	24.9	30.1	4.98	2.99	8.29	
Block free	AGCA	30	36.0	178	62.8	56.7	1			<.0001
	CAGG	32	39.0	56	19.7	24.2	6.40	3.59	11.43	

Note: Loci for Block 1: rs1711667 and rs10466114; Block 2: rs12266634 and rs1008982; block free: rs1711667, rs10466114, rs12266634, and rs1008982.

^aHaplotypes with frequency less than 0.05 were excluded from the analysis.

^bLogistic regression model adjusting for treatment arm, gender, and anticonvulsant use.

associated with female gender and with *MGMT* promoter methylation. Becker-Schiebe et al.²⁵ identified the methylated *MGMT* promoter in the tumor tissue of 4/5 (80%) patients with grade 4 myelotoxicity compared to 19/64 (30%) among those without grade 4 toxicity. Lombardi et al.²² detected *MGMT* promoter methylation in peripheral blood DNA from 4/4 (100%) patients with grade 3–4 myelotoxicity and 0/9 (0%) patients without myelotoxicity. They also reported that the distribution of genotypes in 3 candidate SNPs in *MGMT* was not statistically different between patients with and without myelotoxicity; however, several SNPs in various drug metabolism genes were associated with myelotoxicity.

The differences between our study and previous ones are three-fold. First, our study is based on a large randomized, controlled clinical trial rather than institutional series of patients. Second, due to the multi-institutional nature of the parent trial, our study is much larger, providing the ability to build multivariable risk prediction models for toxicity. Third, our genetic analyses considered first genome-wide variation rather than candidate SNPs or pathways. One primary limitation to our study was the inability to validate our findings in an independent sample of patients. However, given the unique treatment combination provided under the parent trial, there was no existing comparable population. For this reason, we employed a highly robust cross-validation of our findings, as described in the methods above. This approach provided the opportunity to validate our results using a sophisticated statistical approach in the absence of an external validation population.

This unique collaborative research effort allows us to maximize power for the discovery of novel associations between clinical variables, germline polymorphisms, and myelotoxicity in this at-risk population. Our group was among the first to examine the effects of individual genomic variation on treatment toxicity and response among GBM patients. Our preliminary studies were limited by the retrospective nature of our single institutional repository and the application of our risk model for recurrent GBM. The current expansion of these efforts to perform analyses using the prospectively collected data and samples from RTOG 0825 has allowed for a more robust analysis using

modern treatment paradigms. The findings of this report are limited in clinical utility, until further test development can be completed and integrated into clinical care. Use of this model for prediction of risk of toxicities has the potential to provide more cost-effective individualized monitoring of at-risk subjects and allow better individualized initial temozolomide dosing and bloodwork monitoring schedules in patients with GBM receiving this drug. In order to implement this as a clinical tool, development of a assay system to evaluate the pertinent polymorphisms would be necessary, as has been successfully done for other chemotherapy-associated toxicities, such as to predict against fluoropyrimidine toxicity.²⁶

Keywords

genetic association | glioblastoma | myelotoxicity | risk prediction | temozolomide

Funding

This work was supported by National Cancer Institute (U10CA180868 (NRG Operations), U10CA180822 (NRG SDMC), UG1CA189867 (NCORP), U24CA196067 (NRG Biospecimen Bank)); National Institutes of Health (1R01NR013707, K07CA181480) by a research grants from the Voices Against Brain Cancer Foundation and Genentech.

Acknowledgments

We kindly thank Philip J Stella, MD for collecting/assembly data and reading and reviewing this manuscript. In addition to the work he did accruing patients under Michigan Cancer Research Consortium CCOP. Presented: 2015 Society for Neuro-Oncology Meeting, November 19–22, 2015.

Conflict of interest statement. None: Dr(s). Armstrong, Bondy, Crocker, Flickinger, Gilbert, Hunter, Kenyon, Komaki, Liu, Robins, Scheurer, Stella, Stieber, and Vera. Dr. Chao discloses honoraria from Varian, Zeiss, and Abbvie, and a consulting or advisory role from Novocure. Dr. Pugh discloses research funding from Millennium. Dr. Sulman discloses honoraria from Merck, consulting or advisory roles from Merck, AbbVie, and Novocure, research funding from AbbVie and Novocure, and travel, accommodations, or expenses from Merck, AbbVie, and Novocure. Dr. Wendland discloses employment from US Oncology. Dr. Youssef discloses stock or other ownership with Gammatile, and patents, royalties, or other intellectual property with Gammatile. Dr. Yuan discloses honoraria from Vertex Pharmaceuticals and Ono Pharmaceuticals, consulting or advisory roles from Akros Pharma Inc., and travel, accommodations, or expenses from Vertex Pharmaceuticals.

Authorship Statement. Conception and design- YY, EPS, MES, MRG, MLB, TSA. Collection and assembly of data- YY, EFY, MMW, EPS, MES, HIR, SLP, RRK, LCK, GKH, MRG, IRC, STC, TSA. Data analysis and interpretation- RZ, YY, EV, VWS, MES, SLP, YL, MRG, JCF, TSA. Drafting the work or revising it critically for important intellectual content- MES, RZ, MRG, MLB, EPS, YY, YL, EV, MMW, EFY, VWS, RRK, JCF, LCK, HIR, GKH, IRC, STC, SLP, TSA. Read and Approved the final version- MES, RZ, MRG, MLB, EPS, YY, YL, EV, MMW, EFY, VWS, RRK, JCF, LCK, HIR, GKH, IRC, STC, SLP, TSA.

References

- Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. *Neuro Oncol.* 2015; 17(Suppl 4):iv1-iv62.
- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005; 352(10):987-996.
- Gilbert MR, Wang M, Aldape KD, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. *J Clin Oncol.* 2013; 31(32):4085-4091.
- Gerber DE, Grossman SA, Zeltzman M, Parisi MA, Kleinberg L. The impact of thrombocytopenia from temozolomide and radiation in newly diagnosed adults with high-grade gliomas. *Neuro Oncol.* 2007; 9(1):47-52.
- Singhal N, Selva-Nayagam S, Brown MP. Prolonged and severe myelosuppression in two patients after low-dose temozolomide treatment- case study and review of literature. *J Neurooncol.* 2007; 85(2):229-230.
- Noronha V, Berliner N, Ballen KK, et al. Treatment-related myelodysplasia/AML in a patient with a history of breast cancer and an oligodendroglioma treated with temozolomide: case study and review of the literature. *Neuro Oncol.* 2006; 8(3):280-283.
- Comez G, Sevinc A, Sever ON, et al. An unusual case of aplastic anemia caused by temozolomide. *Case Rep Med.* 2010; 2010:975039.
- Oh J, Kutas GJ, Davey P, Morrison M, Perry JR. Aplastic anemia with concurrent temozolomide treatment in a patient with glioblastoma multiforme. *Curr Oncol.* 2010; 17(4):124-126.
- Jalali R, Singh P, Menon H, Gujral S. Unexpected case of aplastic anemia in a patient with glioblastoma multiforme treated with Temozolomide. *J Neurooncol.* 2007; 85(1):105-107.
- Morris EB, Kasow K, Reiss U, Ellison D, Broniscer A. Bone marrow transplantation for severe aplastic anemia secondary to temozolomide. *J Neurooncol.* 2009; 91(2):237-239.
- Huang RS, Ratain MJ. Pharmacogenetics and pharmacogenomics of anticancer agents. *CA Cancer J Clin.* 2009; 59(1):42-55.
- Church D, Kerr R, Domingo E, et al. "Toxgnostics": an unmet need in cancer medicine. *Nat Rev Cancer.* 2014; 14(6):440-445.
- Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007; 447(7145):661-678.
- Iyer L, Das S, Janisch L, et al. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J.* 2002; 2(1):43-47.
- Johnson DC, Corthals SL, Walker BA, et al. Genetic factors underlying the risk of thalidomide-related neuropathy in patients with multiple myeloma. *J Clin Oncol.* 2011; 29(7):797-804.
- Cibeira MT, de Larrea CF, Navarro A, et al. Impact on response and survival of DNA repair single nucleotide polymorphisms in relapsed or refractory multiple myeloma patients treated with thalidomide. *Leuk Res.* 2011; 35(9):1178-1183.
- Armstrong TS, Cao Y, Scheurer ME, et al. Risk analysis of severe myelotoxicity with temozolomide: the effects of clinical and genetic factors. *Neuro Oncol.* 2009; 11(6):825-832.
- Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med.* 2014; 370(8):699-708.
- Molinaro AM, Simon R, Pfeiffer RM. Prediction error estimation: a comparison of resampling methods. *Bioinformatics.* 2005; 21(15):3301-3307.
- Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science.* 2002; 296(5576):2225-2229.
- Kurtin S. Myeloid toxicity of cancer treatment. *J Adv Pract Oncol.* 2012; 3(4):209-224.
- Lombardi G, Rumiato E, Bertorelle R, et al. Clinical and genetic factors associated with severe hematological toxicity in glioblastoma patients during radiation plus temozolomide treatment: a prospective study. *Am J Clin Oncol.* 2015; 38(5):514-519.
- Anderson GD. Sex and racial differences in pharmacological response: where is the evidence? Pharmacogenetics, pharmacokinetics, and pharmacodynamics. *J Womens Health (Larchmt).* 2005; 14(1):19-29.
- Nicolson TJ, Mellor HR, Roberts RR. Gender differences in drug toxicity. *Trends Pharmacol Sci.* 2010; 31(3):108-114.
- Becker-Schiebe ME, Wetzel M, Wetzel F, Christansen H, Hoffmann W. Hematologic toxicity of temozolomide and radiation in glioblastoma patients - correlation with clinicopathological factors. *Clin Med J.* 2015; 1:63-69.
- Del Re M, Di Paolo A, van Schaik RH, et al. Dihydropyrimidine dehydrogenase polymorphisms and fluoropyrimidine toxicity: ready for routine clinical application within personalized medicine? *EPMA J.* 1(3):495-502.