Neuro-Oncology Advances

6(1), vdae031, 2024 | https://doi.org/10.1093/noajnl/vdae031 | Advance Access date 1 March 2024

Sex differences in glioblastoma response to treatment: Impact of MGMT methylation

Gino Cioffi, Kristin A. Waite, Mantas Dmukauskas, Michael Glantz, Sonikpreet Aulakh, Theodore Nicolaides, Soma Sengupta, Joanne Xiu, and Jill S. Barnholtz-Sloan

All author affiliations are listed at the end of the article

Corresponding Author: Jill S. Barnholtz-Sloan, PhD, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Shady Grove Campus, 9609 Medical Center Drive, Rockville, MD 20850, USA (jill.barnholtz-sloan@nih.gov)

It has been established that glioblastoma (GBM) survival differs by sex, with females having a significant survival advantage.[1] Another prognostic factor associated with GBM survival is O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, associated with sensitivity to alkylating chemotherapy, such as temozolomide (TMZ), and improved survival.[2,3] *MGMT* methylation status impacts treatment patterns for GBM. Studies suggest that patients with unmethylated tumors should receive only radiotherapy.[4] Here, we investigate, utilizing a large real-world data set, the impact of *MGMT* methylation status on sex-specific survival for different GBM treatment patterns.

Despite aggressive multimodal treatments, GBM remains uniformly lethal. Survival is largely dictated by the extent of surgical resection and response to standard-of-care radiation and TMZ. *MGMT* promoter methylation status has been shown to have prognostic and predictive value.[2,4] It has been postulated that enhanced *MGMT* methylation may predispose an individual to better responses to alkylating treatments and radiotherapy. To analyze the impact of sex, *MGMT* promoter methylation, and treatment modality on survival, we analyzed 2108 adult (>25 years old) individuals with GBM as determined by a combination of annotated histology and *IDH* wild-type status from the CARIS Lifesciences data set from 2013 to 2021 (61.5% male, 38.5% female). *MGMT* promoter methylation analysis was performed by pyrosequencing.[5] Samples with ≥7% and <9% methylation were considered to be equivocal or gray zone results.[5] Real-world overall survival and treatment information was obtained from insurance claims data from payers and calculated from start of any type of GBM treatment to last contact[6]; uninsured patients were not included. Individuals were categorized by *MGMT* promoter methylation status, TMZ treatment at any dosage/time period, and radiation treatment at any dosage. Treatment information was obtained from the insurance claims data. Date of first treatment was determined by the first insurance treatment of interest claims, independent of the tissue collection date.[6] Kaplan–Meier survival curves were evaluated to assess survival differences by sex, stratified by 3 different treatment regimens (TMZ alone, radiation alone, and TMZ plus radiation). Corresponding log-rank test *P*-values are reported ($P < 0.05$ as significant). All analyses were performed using the Caris CODEai data platform. Limitations with the analytical tools within the platform prevented multivariate analyses, and therefore all results are presented stratified by *MGMT* promoter methylation and treatment status. Additional stratifications, including age at diagnosis, were out of this analysis scope.

Within each treatment modality, median survival estimates were generally higher among individuals with *MGMT* promoter methylation compared to unmethylated individuals (Figure 1). This is consistent with other studies that have demonstrated that overall survival is better in individuals with *MGMT* promoter methylation compared to individuals that are unmethylated.[7] Similar results were observed here in individuals treated with standard of care. Previous studies also demonstrated that there is no difference in survival, by sex, in individuals receiving standard of care who have unmethylated *MGMT*. This was observed here, as there were no significant survival differences observed by sex within any treatment group among unmethylated individuals (Figure 1A, C, and E). This larger study does show a direction of lower survival in male survival among the TMZ-only (Figure 1C) and radiation-only (Figure 1E) treatment groups; while this did not reach significance, it may be worth examining in a larger study group. There were no observed sex differences in survival within GBM individuals with *MGMT* promoter methylation receiving either TMZ and radiation (Figure 1B) or TMZ alone (Figure 1D). A notable survival difference was observed among *MGMT*-methylated individuals, with female individuals having significantly lower survival than males (Figure 1F; 13.4 m vs. 24.5 m, respectively, log-rank $P = 0.01$).

MGMT promoter methylation has been implicated as a prognostic biomarker even in the absence of TMZ treatment, suggesting that *MGMT* promoter methylation may be a predictor for overall treatment responsiveness in general.[8] This study, however, did not stratify based upon sex while other studies, due to the rarity of GBM, were carried out in small data sets. In this larger study set here, the observed male:female survival difference among individuals who were treated with only radiation is notable. While not significant, median survival was higher for females among unmethylated patients who received radiation alone (Figure 1E), in contrast to the significant survival difference observed for males when assessing *MGMT*-methylated individuals treated with radiation

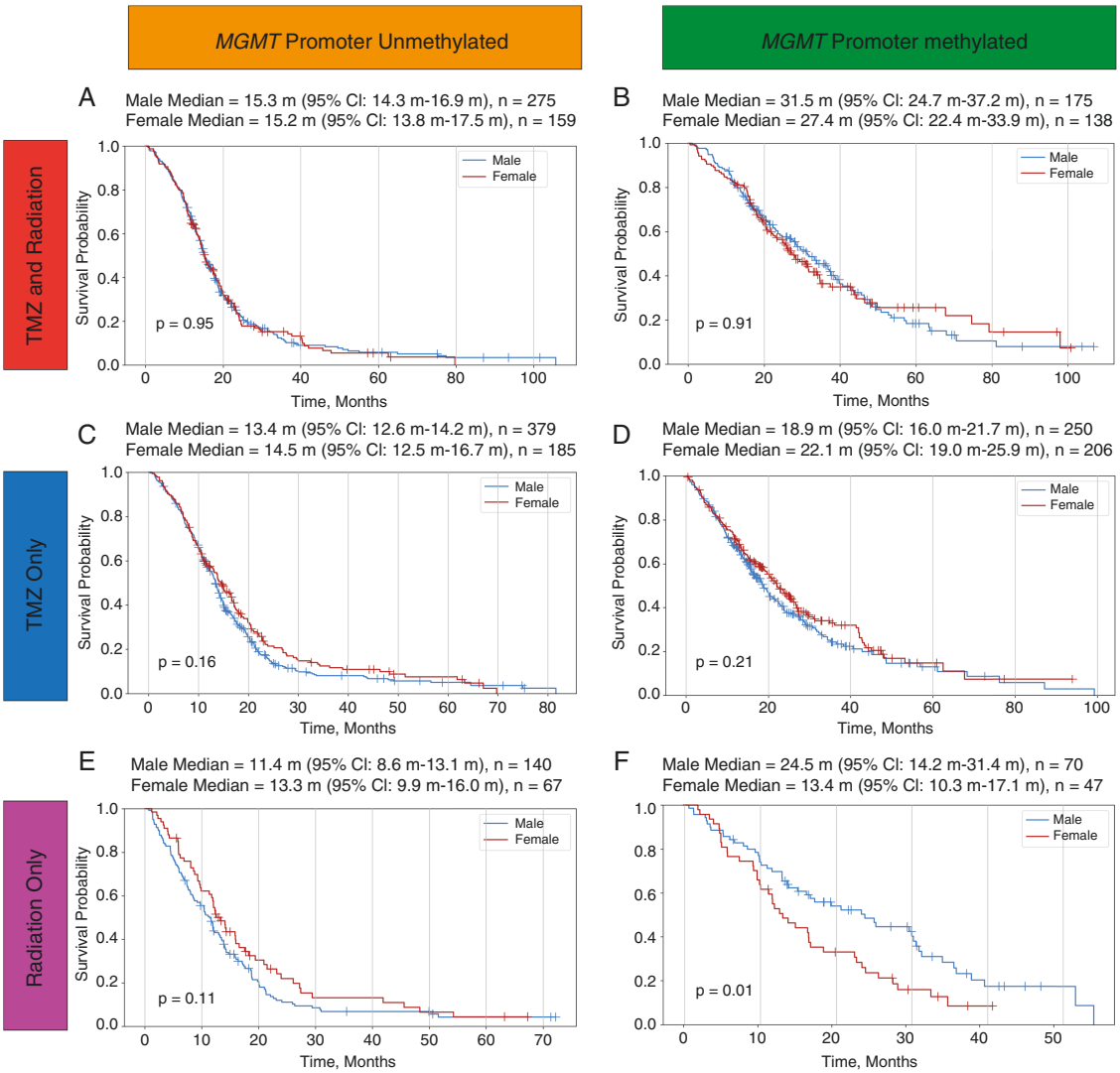

Figure 1. Sex-specific Kaplan–Meier survival curves for (**A**) *MGMT* promoter-unmethylated glioblastoma (GBM) cases treated with temozolomide (TMZ) and radiation, (**B**) *MGMT* promoter-methylated GBM cases treated with TMZ and radiation, (**C**) *MGMT* promoter-unmethylated GBM cases treated with TMZ only, (**D**) *MGMT* promoter-methylated GBM cases treated with TMZ only, (**E**) *MGMT* promoter-unmethylated GBM cases treated with radiation only, and (**F**) *MGMT* promoter-methylated GBM cases treated with radiation only. *P*-values of <.05 were determined to be statistically significant.

alone (Figure 1F). Female GBM individuals with *MGMT* promoter methylation treated with radiation alone had a median survival that was almost half that of male GBM individuals with *MGMT* promoter methylation treated with radiation alone. Methylated females who received only radiation appear to have little to no survival benefit relative to their unmethylated counterparts, unlike the other treatment groups in this study. Current clinical recommendations suggest the use of concurrent TMZ and radiation for treatment of *MGMT*-methylated GBM, and the results here may suggest that this course of treatment is particularly important for females.[2] Further research into this would be strengthened by the inclusion of other prognostic factors for GBM, such as extent of surgical resection, and Karnofsky Performance status which were unavailable in this data set. Due to the unavailability of these data, we cannot rule out that potential differences in clinical outcomes may be driven by covariates not captured in this analysis.

Previous research suggested *MGMT* promoter methylation impacts survival of individuals with GBM receiving standard-of-care treatment (TMZ and concurrent radiation) in a sex-dependent manner.[9] We did not observe a significant survival difference, by sex, among individuals who received only TMZ or TMZ and concurrent radiation in this larger study. The prominent sex difference observed among individuals with *MGMT* promoter methylation who received only radiation treatment may suggest that there is a potential sex bias on the impact of *MGMT* promoter methylation and survival prognosis post treatment. This potential sex bias may be related not to the established role in sensitivity to alkylating chemotherapy,

but rather to underlying mechanisms of tumor biology that remain to be elucidated. *MGMT* promoter methylation has been correlated with increased occurrence with K-Ras mutation in female colorectal cancer individuals,[10] it remains to be determined if similar pathways are involved with GBM.

Keywords

MGMT methylation | overall survival | primary brain tumors | sex differences

Funding

The research performed by G.C., K.A.W., M.D., and J.S.B.-S. was provided by the Division of Cancer Epidemiology and Genetics (DCEG) of the National Cancer Institute (NCI). G.C. and K.A.W. are full-time contractors of the NCI. M.D. is a full-time research fellow of DCEG at NCI. J.S.B.-S. is a full-time employee of NCI and is supported through intramural funds.

Conflict of interest statement

The authors have no conflict of interest to declare. J.S.B.-S. is a full-time employee of the NIH/NCI. G.C. and K.A.W. are full-time contractors of the NIH/NCI. M.D. is a full-time postdoctoral fellow of the NIH/NCI.

Authorship statement

G.C.: conceptualization, investigation, formal analysis, visualization, writing original draft and editing; K.A.W.: funding acquisition, investigation, writing original draft and editing, project administration, resources; M.D.: conceptualization, investigation, methodology, visualization, writing original draft and editing, supervision; J.X.: data curation, formal analysis, investigation, methodology, reviewing final draft; J.S.B.-S.: conceptualization, investigation, project administration, resources, supervision, reviewing final draft.

Data availability

Deidentified data were analyzed and summarized. Additional data can be requested from CARIS Life Sciences.

Affiliations

Trans Divisional Research Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA (G.C., K.A.W., M.D., J.S.B.-S.); Departments of Neurosurgery and Oncology, Penn State College of Medicine-Hershey Medical Center, Hershey, Pennsylvania, USA (M.G.); Section of Hematology-Oncology, Department of Medicine, West Virginia University School of Medicine, Morgantown, West Virginia, USA (S.A.); Caris Life Sciences, Phoenix, Arizona, USA (T.N., J.X.); Department of Neurology/Neurosurgery, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina, USA (S.S.); Center for Biomedical Informatics and Information Technology, National Cancer Institute, Bethesda, Maryland, USA (J.S.B.-S.)

References

1. Ostrom QT, Rubin JB, Lathia JD, Berens ME, Barnholtz-Sloan JS. Females have the survival advantage in glioblastoma. *Neuro Oncol*. 2018;20(4):576–577.
2. Riemenschneider MJ, Hegi ME, Reifenberger G. MGMT promoter methylation in malignant gliomas. *Target Oncol*. 2010;5(3):161–165.
3. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352(10):997–1003.
4. Mansouri A, Hachem LD, Mansouri S, et al. MGMT promoter methylation status testing to guide therapy for glioblastoma: refining the approach based on emerging evidence and current challenges. *Neuro Oncol*. 2019;21(2):167–178.
5. Darabi S, Xiu J, Samec T, et al. Capicua (CIC) mutations in gliomas in association with MAPK activation for exposing a potential therapeutic target. *Med Oncol*. 2023;40(7):197.
6. Abraham JP, Magee D, Cremolini C, et al. Clinical validation of a machine-learning-derived signature predictive of outcomes from first-line oxaliplatin-based chemotherapy in advanced colorectal cancer. *Clin Cancer Res*. 2021;27(4):1174–1183.
7. Franceschi E, Tosoni A, Minichillo S, et al. The prognostic roles of gender and O^6-methylguanine-DNA methyltransferase methylation status in glioblastoma patients: the female power. *World Neurosurg*. 2018;112:e342–e347.
8. Rivera AL, Pelloski CE, Gilbert MR, et al. MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. *Neuro Oncol*. 2010;12(2):116–121.
9. Smits A, Lysiak M, Magnusson A, et al. Sex disparities in MGMT promoter methylation and survival in glioblastoma: further evidence from clinical cohorts. *J Clin Med*. 2021;10(4):556.
10. Huang CC, Chien WP, Wong RH, et al. NAT2 fast acetylator genotype and MGMT promoter methylation may contribute to gender difference in K-RAS mutation occurrence in Taiwanese colorectal cancer. *Environ Mol Mutagen*. 2009;50(2):127–133.