Prognostic value of $O^6$-methylguanine-DNA methyltransferase methylation in isocitrate dehydrogenase mutant gliomas

Keng Lam,†, Blaine S. C. Eldred†, Bryan Kevan, Sean Pianka, Brittany A. Eldred, Serendipity Zapanta Rinonos, William H. Yong, Linda M. Liau, Phioanh L. Nghiemphu, Timothy F. Cloughesy, Richard M. Green, and Albert Lai

Department of Neurology, Kaiser Permanente, Los Angeles Medical Center, Los Angeles, California, USA (K.L., R.M.G.); Department of Neurology, University of California, Los Angeles, California, USA (B.S.C.E, B.K., S.P., S.Z.R., P.L.N., T.F.C., A.L.); Department of Strategic Communications, Sonoma State University, Rohnert Park, California, USA (B.A.E.); Department of Pathology and Laboratory Medicine, University of California, Irvine, California, USA (W.H.Y.); Department of Neurosurgery, University of California, Los Angeles, California, USA (L.M.L.)

†These authors contributed equally to this work.

Corresponding Author: Albert Lai, MD, PhD, Department of Neurology, University of California, 635 Charles E. Young Drive South, NRB Room 555C, Los Angeles, CA 90095, USA (albertlai@mednet.ucla.edu).

Abstract

Background. Patients with isocitrate dehydrogenase ($IDH$) mutant gliomas have been associated with longer survival time than those that are $IDH$ wild-type. Previous studies have shown the prognostic value of $O^6$-methylguanine-DNA methyltransferase ($MGMT$) promoter methylation for glioblastoma multiforme (GBM), which are predominantly $IDH$ wild-type. Little is known of the prognostic value of $MGMT$ methylation status for $IDH$ mutant gliomas.

Methods. We retrospectively identified $IDH$ mutant gliomas patients between 2011 and 2020 that were tested for $MGMT$ promoter methylation. We generated Kaplan–Meier estimator curves and performed Cox proportional hazard models for overall survival (OS) and progression-free survival (PFS) to compare the outcomes of $MGMT$ promoter methylated versus $MGMT$ unmethylated patients.

Results. Of 419 $IDH$ mutant gliomas with $MGMT$ promoter methylation testing, we identified 54 GBMs, 223 astrocytomas, and 142 oligodendrogliomas. 62.3% patients had $MGMT$ methylated tumors while 37.7% were $MGMT$ unmethylated. On Kaplan–Meier analysis, median OS for all $MGMT$ methylated patients was 17.7 years and 14.6 years for unmethylated patients. Median PFS for all $MGMT$ methylated patients was 70 years and for unmethylated patients 5.2 years. After univariate subgroup analysis, $MGMT$ methylation is only prognostic for OS and PFS in GBM, and for OS in anaplastic oligodendroglioma and anaplastic oligodenroglioma for OS. In multivariate analysis, $MGMT$ unmethylated GBM patients carry a higher risk of death (HR 7.72, 95% CI 2.10–28.33) and recurrence (HR 3.85, 95% CI 1.35–10.96).

Conclusions. $MGMT$ promoter methylation is associated with better OS and PFS for $IDH$ mutant GBM. $MGMT$ promoter methylation testing for other $IDH$ mutant glioma subtypes may not provide additional information on prognostication.

Key Points

- Among the patients with GBM $IDH$ mutation, $MGMT$ promoter methylation has a more favorable prognosis in OS and PFS contrasted with unmethylated $MGMT$ promoter status.
- $MGMT$ promoter methylation is not prognostic for $IDH$ mutant astrocytoma and low-grade oligodendroglioma.

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Importance of the Study

The prognostic value of many molecular biomarkers has been elucidated in part thanks to our increased proclivity for molecular testing. We know, for example, that diffuse glioma patients with IDH mutations have longer survival times than those without. More recently, MGMT promoter methylation has also shown promise to be a prognostic factor for patients with GBM.

We conducted a bi-institutional retrospective study to investigate the impact of MGMT methylation on IDH mutants. Our univariate and multivariate analysis support that MGMT methylation is prognostic for only IDH mutant GBM in both OS and PFS and perhaps for anaplastic oligodendroglioma for OS only. Our data analysis did not show benefit of MGMT methylation in the other histopathological subgroups; this is potentially attributable to relative immaturity of survival data.

Molecular biomarker testing has become standard practice in the field of neuro-oncology. Not only is it necessary for producing accurate diagnoses, but it also influences determination of optimal treatment regimens. A prime example of this is the discovery of isocitrate dehydrogenase (IDH) mutations in diffuse glioma patients which conferred longer survival times and improved treatment responses when contrasted to their wildtype counterparts. Previous studies have also shown the prognostic value of O\(^6\)-methylguanine-DNA methyltransferase (MGMT) promoter methylation on glioblastoma multiforme (GBM) patients. However, there is still no consensus on the prognostic value of MGMT and whether testing should be routinely conducted for other gliomas, particularly those with IDH mutations. A multivariate analysis from the NRG Oncology/Radiation Therapy Oncology Group 0424 Trial suggests that MGMT methylation may serve as an independent prognostic biomarker for high-risk, low-grade glioma patients receiving temozolomide and radiotherapy. Few studies are available that directly compare the prognosis of MGMT methylated versus unmethylated for IDH mutant gliomas. Thus, we sought to investigate the hypothesis that MGMT has good prognostic value and testing should be utilized routinely for patients with IDH mutants in this retrospective study.

Materials and Methods

Patient Cohort

All study participants were retrospectively identified IDH mutant diffuse gliomas patients with pretreatment MGMT promoter methylation test results. The primary demographics were University of California Los Angeles (UCLA) and Kaiser Permanente Los Angeles (KPLA) adult patients, aged 18 years and older, with tumor samples tested between 2011 and 2020. UCLA and KPLA institutional review board approval and informed patient consent were acquired prior to the collection and analysis of patient data.

Pathological and Molecular Analysis

Tumor samples were assessed by board-certified neuropathologists from our respective institutions and pathologists from outside institutions where a subset of surgeries were performed. On review of the individual pathology reports, we followed the 2016 World Health Organization (WHO) classification criteria of central nervous system tumors when updating obsolete diagnoses such as anaplastic and low-grade mixed gliomas. Our respective institutions used immunohistochemistry, PCR sequencing, or next-generation sequencing (either from Strata or Foundation Medicine) to identify variants in IDH1 or IDH2 genes. The majority of MGMT gene promoter methylation assays was performed by LabCorp or NeoGenomics Laboratories using bisulfite modification of tumor deoxyribonucleic acid (DNA) and polymerase chain reaction (PCR) to detect CpG methylation. Patients with insufficient molecular data to confirm the diagnosis were excluded.

Treatments

For our patient cohort, the extent of resection (EOR) was defined as either biopsy, subtotal resection (STR), or gross total resection (GTR), depending on the results of postoperative head imaging. Radiation therapy may be regional or intensity-modulated radiation therapy (IMRT) adhering to a standard-course radiation therapy regimen (usually 5–6 weeks depending on whether a low-grade or high-grade dose was delivered). Concurrent or adjuvant chemotherapy regimens were diagnosis-dependent and up to the discretion of the treating neuro-oncologists. Regimens commonly included temozolomide (TMZ), procarbazine, CCNU, and vincristine (PCV). Some patients had intervals of surveillance only after resection prior to treatment.

Statistics

We generated univariate Kaplan–Meier estimator curves and performed multivariate Cox regression analyses for both overall survival (OS) and progression-free survival (PFS) to compare the outcomes of MGMT methylated versus unmethylated patients. We defined OS as the time from initial diagnosis to either the “date of death” or “censored at last contact.” The date of diagnosis coincided with the earliest surgical date where the EOR exceeded a biopsy (ie, STR or GTR). We defined PFS as the time from initiation of alkylating chemotherapy (TMZ, CCNU, and BCNU) with or without radiation, until first
imaging confirms recurrence. Important clinical factors including age, gender, Karnofsky Performance Scale (KPS), EOR, and IDH status were adjusted. All statistical analyses were performed in R version 3.6.2 using the packages “survival” and “survminer”.

Results

Cohort Characteristics

We identified a total of 419 IDH mutant gliomas who underwent MGMT promoter methylation testing. Subgroups include 54 GBM (12.9%), 223 astrocytoma (24.8% anaplastic astrocytoma and 28.4% low-grade astrocytoma), and 142 oligodendroglioma (12.9% anaplastic oligodendroglioma and 21.0% low-grade oligodendroglioma). 261 (62.3%) patients had MGMT methylated tumors while 158 (37.7%) were MGMT unmethylated. IDH1 R132H constitutes about 90% of the IDH mutation. 80.4% of the patients were male, median age was 36.7, and the median KPS was 90. Table 1 lists the patient characteristics and the treatments they received.

Among the 419 patients in our cohort, 174 (41.5%) of them received GTR, and 203 (48.5%) underwent STR. 296 of the total patient cohort (70.6%) received TMZ, in which 181 of them (61.1%) are MGMT methylated. 152 (36.3%) of the cohort patients received PCV (42 [56.8%] of them are MGMT methylated). 74 patients (17.7% of the cohort) received bevacizumab. 335 patients (80.0% of the cohort) received bevacizumab. 335 patients (80.0% of the cohort) received radiation therapy. 67 patients (16.0% of the cohort) received no treatments.

Overall Survival Data of MGMT Methylation

The median OS for all MGMT methylated patients was 17.7 years and for unmethylated patients 14.6 years (log-rank P = 0.009) as shown in Kaplan–Meier curves in Figure 1.

For univariate pathological subgroup analysis, MGMT methylation was prognostic for improved OS (log-rank P = 0.002) in IDH mutant GBM. This is not observed for astrocytoma or oligodendroglioma groups as shown in Figure 1 except for anaplastic oligodendroglioma. Similar results are also reached when excluding patients that did not receive any alkylating chemotherapy treatment (Supplemental Figure 1). The median OS for MGMT methylated GBM was not reached due to better survival, while for MGMT unmethylated GBM, median survival was three years. We attempted analysis for patients who did not receive any treatments and found no difference in survival between the MGMT methylated and unmethylated group, but the data were limited by sample size, data immaturity, and selection bias, so these were not reported.

In multivariate analysis, unmethylated MGMT status does not significantly increase the risk of dying compared to methylated MGMT status: with hazard ratio (HR) of 1.34 (95% confidence interval [CI] 0.82–2.19) when excluding GBM patients from the reference group as shown in Table 2. This is also the case for oligodendrogliomas in which the hazard ratios did not reach statistical significance. However, for GBM patient subgroup, MGMT unmethylated patients carry a higher risk of dying (HR 7.72, 95% CI 2.10–28.33).

Progression-Free Survival Data of MGMT Methylation

The median PFS for all MGMT methylated patients was 7.0 years and for unmethylated patients 5.2 years (log-rank P = 0.03) as shown Kaplan–Meier curves in Figure 2.

In univariate pathological subgroup analysis, MGMT methylation was prognostic for improved PFS (log-rank P = 0.02) in IDH mutant GBM. However, similar to OS, this is not observed for other subgroups as shown in Figure 2. The median PFS for MGMT methylated GBM was not reached due to longer time to progression, while the median PFS for MGMT unmethylated GBM was 2.1 years.

In multivariate analysis, unmethylated MGMT status did not significantly increase the risk of progression compared to methylated MGMT status: with HR 1.18 (95% CI 0.81–1.72) when excluding GBM patients from the reference group as shown in Table 3. This is also the case for oligodendrogliomas in which the hazard ratios did not reach statistical significance. However, for the GBM patient subgroup, MGMT unmethylated patients carried a higher risk of recurrence (HR 3.85, 95% CI 1.35–10.96).

Discussion

The current study is just one of a few in the literature that explore the impact of MGMT methylation specifically for gliomas with IDH mutation. At first glance, the MGMT methylated cohort appears to have a more favorable OS, but we found that improved OS occurred mainly within the GBM subgroup, with no differences in other subgroups except for anaplastic oligodendroglioma (OS only). Similarly, MGMT methylation only played a significant role in progestation for GBM patients for recurrence, but this was not the case for other subgroups of glioma (including anaplastic oligodendroglioma) as shown in both Kaplan–Meier and multivariate Cox models.

Millward et al demonstrated through a cohort study of 100 GBM patients treated with chemoradiotherapy that MGMT methylation and IDH1 mutant status are associated with longer OS and PFS than patients with unmethylated MGMT and IDH1 mutation. Published in the same year of 2016, Li et al also concluded through a retrospective cohort study of 157 GBM patients that MGMT methylation and IDH1 mutation cumulatively influenced the overall survival, with median survival of 4.5 years for GBM patients with both MGMT methylation and IDH mutation, compared to 1.3 years without either of these two mutations. Our findings further support the concept that there is strong utility of MGMT promoter methylation testing for all GBM patients for all GBM patients. This is an important note considering that MGMT methylation testing is nationally underutilized with only 13% of GBM tumor samples being tested and reported.
The role of MGMT methylation is less certain for other glioma subtypes, particularly for those with IDH mutation. Recently, a retrospective study from National Cancer Database reviewing more than 1200 patients with grade 3 gliomas and MGMT testing seems to suggest that those with MGMT methylation may have improved OS if they received adjuvant chemoradiation or adjuvant radiation, but not if they received adjuvant chemotherapy or no treatment.13 Unfortunately, their database did not contain IDH mutation status. The study also excluded patients with 1p19q co-deletion, hence effectively excluding the diagnosis of oligodendroglioma, based on 2016 WHO classification. The NOA-04 trial was a phase 3 randomized control trial examining the effect of chemoradiation of anaplastic glioma with PCV or temozolomide. Its long-term analysis indicates that MGMT methylation does not seem to play prognostic or predictive role for IDH mutant tumors (with or without 1p/19q co-deletion).14 This is also consistent with a recent retrospective analysis of 155 patients with grade II glioma, showing that MGMT promoter methylation is only prognostic for IDH wildtype astrocytoma, but not for IDH mutant gliomas, regardless of the 1p19q co-deletion.15

Table 1. Summary of Cohort Characteristics and Treatments

| Characteristics                  | All (n = 419) | MGMT Methylated (n = 261) | MGMT Unmethylated (n = 158) |
|----------------------------------|--------------|--------------------------|-----------------------------|
| Diagnosis; WHO 2016, n (%)       |              |                          |                             |
| GBM                              | 54 (12.9)    | 31 (57.4)                | 23 (42.6)                   |
| AA                               | 104 (24.8)   | 57 (54.8)                | 47 (45.2)                   |
| AO                               | 54 (12.9)    | 49 (90.7)                | 5 (9.3)                     |
| LA                               | 119 (28.4)   | 57 (47.9)                | 62 (52.1)                   |
| LO                               | 88 (21.0)    | 67 (76.1)                | 21 (23.9)                   |
| Age at Dx (y), median (range)    | 36.7 (17.1–78.7) | 40.6 (18.2–78.7) | 33.93 (17.1–63.1)           |
| KPS, median (range)              | 90 (50–100)  | 90 (50–100)              | 90 (50–100)                 |
| Sex, n (%)                       |              |                          |                             |
| Male                             | 253 (60.4)   | 156 (61.7)               | 97 (38.3)                   |
| Female                           | 166 (39.6)   | 105 (63.3)               | 61 (36.7)                   |
| OR, n (%)                        |              |                          |                             |
| GTR                              | 174 (41.5)   | 112 (64.4)               | 62 (35.6)                   |
| STR                              | 203 (48.5)   | 126 (62.1)               | 77 (37.9)                   |
| Biopsy                           | 42 (10.0)    | 23 (54.8)                | 19 (45.2)                   |
| MGMT Methylation, n (%)          |              |                          |                             |
| Methylated                       | 261 (62.3)   | 261 (100.0)              | –                           |
| Unmethylated                     | 158 (37.7)   | –                        | 158 (100.0)                 |
| IDH Mutations                    |              |                          |                             |
| IDH1 R132H                       | 377 (90.0)   | 234 (62.1)               | 143 (37.9)                  |
| IDH1 Other                       | 27 (6.4)     | 15 (55.6)                | 12 (44.4)                   |
| IDH2                             | 15 (3.6)     | 12 (80.0)                | 3 (20.0)                    |
| Treatment, n (%)                 |              |                          |                             |
| RT + TMZ                         | 217 (51.8)   | 131 (60.4)               | 86 (39.6)                   |
| RT + PCV                         | 47 (11.2)    | 32 (68.1)                | 15 (31.9)                   |
| RT (total)                       | 335 (80.0)   | 206 (61.5)               | 129 (38.5)                  |
| TMZ (total)                      | 296 (70.6)   | 181 (61.1)               | 115 (38.9)                  |
| Bevacizumab (total)              | 74 (17.7)    | 42 (56.8)                | 32 (43.2)                   |
| PCV (total)                      | 152 (36.3)   | 101 (66.4)               | 51 (33.6)                   |
| BCNU (total)                     | 5 (1.2)      | 4 (80.0)                 | 1 (20.0)                    |
| None of the above                | 67 (16.0)    | 43 (64.2)                | 24 (35.8)                   |

**Abbreviations:** AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; BCNU, carmustine; Dx, diagnosis; EOR, extent of resection; GBM, glioblastoma multiforme; GTR, gross-total/near-total resection; IDH, Isocitrate Dehydrogenase; KPS, Karnofsky performance status; LA, low-grade astrocytoma; LO, low-grade oligodendroglioma; MGMT, O6-methylguanine-DNA methyltransferase; PCV, procarbazine-lomustine (CCNU)-vincristine; RT, radiation therapy; STR, subtotal resection; TMZ, temozolomide; WHO 2016, 2016 World Health Organization classification of central nervous system tumors.
MGMT is a DNA repair protein known to cause tumor resistance to alklation chemotherapy by removing alkyl adducts from the O6-position of guanine.\textsuperscript{16} MGMT promoter methylation allows the silencing of the repair protein, rendering tumor cells more vulnerable to chemotherapy that induces DNA damage. The mechanism remains
unclear, on the other hand, of how glioma patients remain unclear, on the other hand, how glioma patients with IDH mutations respond better to therapies and have longer survival. One leading theory is that IDH mutation can cause increased levels of 2-hydroxyglutarate, leading to genome-wide DNA methylation, including the MGMT promoter, thereby interfering with tumor cell survival.17 However, MGMT methylation also frequently exists in the context of wild-type gliomas, so it remains uncertain how MGMT methylation occurs in these gliomas.17 MGMT methylation can be found at multiple CpG sites, with presumptive epigenetic regulation.18 Nevertheless, it remains unclear why patients with IDH mutant GBM do better if they also have MGMT methylation, when this may not be the case for lower-grade gliomas. Chai et al illustrate that OS and PFS of GBM IDH mutant seem to differ by the extent of methylation of MGMT at CpG sites.19 A larger number of methylated CpG sites has also shown to be associated with favorable outcome for low-grade gliomas.15 Taken together, a possible proposal is that within lower-grade IDH mutant gliomas, comparing MGMT methylated vs. MGMT unmethylated tumors, the extent of methylation may be similar quantitatively, despite being qualitatively different in our testing methods. This hypothesis will require further confirmation. Additionally, discordance between MGMT methylation and expression has been described in the literature for GBM, with one study describing 41.2% of methylated tumors with high MGMT expression despite correlation was still observed between MGMT methylation and survival.20 21 We are unaware of large studies that suggest any discordance between MGMT methylation and expression for other gliomas. Some advocate that MGMT methylation has predictive value for IDH mutant GBM, but with a higher pyrosequencing cutoff value (≥30%).19 It is certainly plausible that changing the cutoff value may result in more statistically significant results for our IDH mutant gliomas, but that remains to be studied, but that remains to be studied in the future. Another hypothesis is that genome-wide changes caused by the 2-hydroxyglutarate from IDH mutation for lower-grade gliomas already confers enough survival benefits, such that any additional advantage from MGMT methylation would not make any significant difference. Given these multiple possible explanations, more investigation is needed at this juncture.

This study comes with several limitations. First, because we only analyzed patients who tested for both IDH mutations and MGMT methylation, there is potential for selection bias. Second, our tumor classification is based on the 2016 WHO. The 2021 WHO classification was not published until after the completion of our project, which would have changed some nomenclatures. For example, IDH mutant glioblastoma would be classified as “astrocytoma, IDH-mutant, WHO grade 4” instead, with the term “anaplastic” also falling out of favor.22 Because the new classification would require biomarkers that were not analyzed at the time of our data collection, reclassification would not be possible for many tumor samples. Furthermore, the number of survival events may be limited, especially for OS; hence, we recognize the possibility of an immature dataset. Finally, this is a retrospective study and findings should be further validated prospectively.

In summary, MGMT promoter methylation is associated with better OS and PFS for IDH mutant GBM. Hence, routine testing for MGMT promoter methylation status should
Figure 2. Progression-free survival (PFS) outcomes of patients stratified by MGMT promoter methylation status. (A) PFS of the entire patient cohort. (B) PFS of patients diagnosed with glioblastoma multiforme (GBM). (C) PFS of patients diagnosed with anaplastic astrocytoma (AA) and low-grade astrocytoma (LA). (D) PFS of patients diagnosed with anaplastic oligodendroglioma (AO) and low-grade oligodendroglioma (LO). (E) PFS of patients diagnosed with AA. (F) PFS of patients diagnosed with LA. (G) PFS of patients diagnosed with AO. (H) PFS of patients diagnosed with LO.
be considered for all IDH mutant GBM patients. Testing for other IDH mutant glioma subtypes may not provide additional information on prognostication.

Supplementary Material
Supplementary material is available at Neuro-Oncology Advances online.

Keywords
glioblastoma | IDH | MGMT

Table 3. Multivariate Analysis of PFS in Various Pathological Subgroups

| Variable (PFS) | All (Ref. AO + LO) (n = 346) | All (Ref. Not GBM) (n = 346) | GBM (n = 53) |
|---------------|-----------------------------|-----------------------------|--------------|
|               | HR  | P-value | 95% CI | HR  | P-value | 95% CI | HR  | P-value | 95% CI |
| Age at intervention | 1.00 | .60 | [0.98, 1.01] | 0.99 | .40 | [0.98, 1.01] | 1.01 | .70 | [0.96, 1.07] |
| KPS ≤ 70 | 1.51 | .20 | [0.77, 2.97] | 1.51 | .20 | [0.77, 2.96] | 1.74 | .60 | [0.22, 14.01] |
| Gender (male) | 1.09 | .60 | [0.78, 1.52] | 1.10 | .60 | [0.79, 1.54] | 0.59 | .30 | [0.23, 1.52] |
| EOR (biopsy ref.) | 0.80 | .40 | [0.46, 1.40] | 0.83 | .50 | [0.48, 1.44] | 2.79 | .30 | [0.48, 16.35] |
| GTR | 0.86 | .60 | [0.51, 1.46] | 0.88 | .60 | [0.52, 1.48] | 1.44 | .70 | [0.29, 7.17] |
| STR | 1.46 | .10 | [0.93, 2.31] | 1.00 | – | – | – | – | – |
| Gender (male) | 0.92 | .80 | [0.42, 2.00] | 0.74 | .40 | [0.35, 1.53] | – | – | – |
| Unmethylated MGMT | 1.58 | .30 | [0.71, 3.52] | 1.18 | .40 | [0.81, 1.72] | 3.85 | .01* | [1.35, 10.96] |
| MGMT (U) × (AO + LO) | 1.00 | – | – | 1.00 | – | – | – | – | – |
| MGMT (U) × (AA + LA) | 0.63 | .30 | [0.26, 1.54] | 1.00 | – | – | – | – | – |
| MGMT (U) × GBM | 2.06 | .20 | [0.62, 6.85] | 2.76 | .04* | [1.05, 7.30] | – | – | – |
| Events | 149 | 149 | 20 | 149 | 149 | 20 | 149 | 149 | 20 |

Abbreviations: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; Dx, diagnosis; EOR, extent of resection; GBM, glioblastoma multiforme; HR, hazard ratio; KPS, Karnofsky performance status; LA, low-grade astrocytoma; LO, low-grade oligodendroglioma; PFS, progression-free survival; U, unmethylated. 95% CI, 95% confidence interval.
* ≥95% significance; ** ≥99% significance. Of note, age at intervention is analyzed as a continuous variable.

Author Contributions

Authorship statement: K.L., B.S.C.E., B.K., and A.L. designed the study. K.L., B.S.C.E., B.K., S.P., W.Y., L.L., P.L.N., T.F.C., R.M.G., S.Z.R, and A.L. collected the data. K.L., B.S.C.E., B.K., S.P., R.M.G., and A.L. analyzed the data. K.L., B.S.C.E., B.A.E., S.Z.R., and A.L. drafted and edited the manuscript. All authors approved the final manuscript.

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