The Clinical Significance of O6-Methylguanine-DNA Methyltransferase Promoter Methylation Status in Adult Patients With Glioblastoma: A Meta-analysis

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Background and objective: Promoter status of O6-methylguanine-DNA methyltransferase (MGMT) has been widely established as a clinically relevant factor in glioblastoma (GBM) patients. However, in addition to varied therapy schedule, the prognosis of GBM patients is also affected by variations of age, race, primary or recurrent tumor. This study comprehensively investigated the association between MGMT promoter status and prognosis in overall GBM patients and in different GBM subtype including newly diagnosed patients, recurrent patients and elderly patients.

Methods: A comprehensive search was performed using PubMed, EMBASE, Cochrane databases to identify literatures (published from January 1, 2005 to April 1, 2017) that evaluated the associations between MGMT promoter methylation and prognosis of GBM patients.

Results: Totally, 66 studies including 7,886 patients met the inclusion criteria. Overall GBM patients with a methylated status of MGMT receiving temozolomide (TMZ)-containing treatment had better overall survival (OS) and progression-free survival (PFS) [OS: hazard ratio (HR) = 0.46, 95% confidence interval (CI): 0.41–0.52, p < 0.001, Bon = 0.017; PFS: HR = 0.48, 95% CI 0.40–0.57, p < 0.001, Bon = 0.014], but no significant advantage on OS or PFS in GBM patients with TMZ-free treatment was observed (OS: HR = 0.97, 95% CI 0.91–1.03, p = 0.08, Bon = 1; PFS: HR = 0.76, 95% CI 0.57–1.02, p = 0.068, Bon = 0.748). These different impacts of MGMT status on OS were similar in newly diagnosed GBM patients, elderly GBM patients and recurrent GBM. Among patients receiving TMZ-free treatment, survival benefit in Asian patients was not observed anymore after Bonferroni correction (Asian OS: HR = 0.78, 95% CI 0.64–0.95, p = 0.02, Bon = 0.24, I² = 0%; PFS: HR = 0.69, 95% CI 0.50–0.94, p = 0.02, Bon = 0.24). No benefit was observed in Caucasian receiving TMZ-free therapy regardless of Bonferroni adjustment.

Conclusion: The meta-analysis highlights the universal predictive value of MGMT methylation in newly diagnosed GBM patients, elderly GBM patients and recurrent GBM patients. For elderly methylated GBM patients, TMZ alone therapy might be a more suitable option than radiotherapy alone therapy. Future clinical trials should be designed in order to optimize therapeutics in different GBM subpopulation.

Keywords: O6-methylguanine-DNA methyltransferase, methylation, glioblastoma, prognosis, temozolomide
INTRODUCTION

Glioblastoma (GBM) is the most frequent primary malignant brain tumor with poor prognosis. From 2005, radiotherapy combined with concomitant and adjuvant temozolomide (TMZ) after surgical maximal safe resection, namely STUPP treatment, has been widely used for newly diagnosed GBM patients less than 65 years old (1, 2). A phase III trial showed that tumor treatment fields, a novel cancer treatment modality, had similar efficacy as chemotherapy regimens in recurrent GBM (3). However, limited improvement of the overall survival (OS) has been achieved in patients with GBM (4, 5). Therefore, identification of biomarkers determining tumor response to treatment may help in developing targeted therapy or optimize patients’ management.

O-6-methylguanine-DNA methyltransferase (MGMT) is a ubiquitously expressed DNA repair enzyme. MGMT protein removes alkyl adducts at the O6 position of guanine, thereby neutralizing the cytotoxic effects of alkylating agents such as TMZ (6, 7). High MGMT expression in glioma cells is the predominant mechanism underlying tumor resistance to alkylating agents (8–10). Meanwhile, status of MGMT promoter methylation is associated with tumor response to TMZ therapy (11, 12). MGMT promoter methylation, resulting in transcriptional silencing, correlates well with improved survival in GBM patients exposed to alkylating agents’ treatment (13–15). Results of European Organization for Research and Treatment of Cancer and National Cancer Institute of Canada trial indicated that MGMT promoter methylation was the strongest predictor for outcome and benefit from TMZ (2, 16). Accordingly, this biomarker is currently used for clinical decision-making and stratifying or selecting GBM patients for clinical trials (17).

Although MGMT promoter methylation has a strong influence on response to TMZ and clinical outcome in GBM patients, its prognostic value on GBM patients remains ambiguous. Some studies indicated that it was associated with better outcome in methylated patients receiving TMZ-containing therapy (18, 19). But some studies also showed that it conferred survival benefit in methylated patients receiving TMZ-free therapy (21, 22). So it is necessary to review whether the survival benefit from MGMT methylation is therapy dependent or independent, which will define MGMT promoter methylation as a predictive or prognostic biomarker. In addition to varied therapy schedules, the outcome and survival of GBM patients may be affected by other prognostic variables, including primary or recurrent tumor, age and race. Thus, we conducted a comprehensive and exact analysis on the association between MGMT promoter methylation and prognosis in overall GBM patients as well as in different GBM subpopulation, including newly diagnosed patients, recurrent patients, elderly patients and patients with different races. This meta-analysis will provide an updated and precise review on the clinical value of MGMT promoter methylation on progression-free survival (PFS) and OS in GBM patients.

METHODS

Search Strategy
We performed a systematic review to identify all related articles from PubMed, EMBASE and the Cochrane Library covering the association of MGMT methylation with prognosis and data of hazard ratios (HRs) and 95% confidence intervals (CIs). The articles enrolled in analysis were published between January 1, 2005 and April 1, 2017. The following subject terms were used: (1) “Glioblastoma,” “GBM,” “High-Grade Glioma,” “Astrocytoma,” Grade IV,” “Astrocytomatas, Grade IV,” “Glioblastoma Multiform,” or “Glioblastomas”; (2) “MGMT” or “O-6-methylguanine-DNA methyltransferase.” The eligible studies were restricted to human beings.

Inclusion and Exclusion Criteria
We evaluated the eligible studies only if all the following conditions were met: (1) studies investigated the relation between MGMT promoter methylation and survival in GBM patients; (2) treatment schedules and testing methods were all included; (3) HR and 95% CI for OS and PFS were available directly or calculated using the Kaplan–Meier survival curves; and (4) specific drugs for chemotherapy were introduced.

Study Selection and Data Extraction
Study selection was independently performed by two authors and disagreements were resolved through discussion. The following data were extracted: the author’s name, country, publication year, number of patients, treatment detail, outcomes (including HRs and 95% CIs), the Cox regression model, and study design feature.

Quality Assessment
The bias risk in each study was independently assessed by two authors using a modified domain-based Newcastle-Ottawa Scale (NOS) for non-randomized studies. The assessment included selection bias, performance bias, detection bias, attrition bias and reporting bias. Important prognostic variables, including age, neurologic status, extent of resection, tumor location, primary or recurrent GBM and MGMT promoter status, were added into NOS according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) checklist for a tumor prognostic study (23, 24). The judgment criteria for the modified evaluation were explicitly described in Table S1 in Supplementary Material.

Statistical Analysis
The statistical analysis was performed by STATA 12.0 software. HR and 95% CI were directly extracted or calculated using the Kaplan–Meier survival curves or the methods reported by Tierney et al. (25). To evaluate the association of MGMT promoter methylation with OS and PFS, pooled HRs of methylated GBM patients were compared to those of unmethylated patients. Subgroup analysis was performed to evaluate whether methylated patients benefit from different therapies (TMZ-containing, TMZ-free alkylating agents, or radiotherapy alone). The statistical heterogeneity among studies was assessed by Q-test and I2 statistics (26). If there was no obvious heterogeneity, fixed-effect model was used to estimate the pooled HR (27); otherwise, random-effect model was used (28). Bonferroni method was used for multiple comparison adjustments. Publication bias was assessed by funnel plots and Egger’s test (29), and a trim and fill method was applied to estimate asymmetry in funnel plots.
Sixty-four and 25 studies were included to describe the association between MGMT promoter methylation and survival in overall GBM patients. The characteristics of all studies are summarized in Table 1. Of these 66 studies, 54 studies were related to TMZ-containing chemotherapy (4 studies of radiotherapy alone and 12 studies of TMZ-containing chemotherapy and 12 studies were related to TMZ-free treatment). The characteristics of all studies are summarized in Table 1. Quality assessment showed no apparent variations among the studies in most domains of bias except for selection bias (see Table S1 in Supplementary Material).

RESULTS

Characteristics of Studies

The flow chart of literature selection was presented in Figure 1. Totally, 3,181 articles were screened. Finally, a total of 7,886 patients in 66 studies (four articles comprising two individual trials were extracted as eight individual studies) were identified, including 7 randomized trials, 59 non-randomized trials. Of these 66 studies, 54 studies were related to TMZ-containing chemotherapy and 12 studies were related to TMZ-free treatment (4 studies of radiotherapy alone and 12 studies of TMZ-free alkylating agents chemotherapy). The characteristics of all studies are summarized in Table 1. Quality assessment showed no apparent variations among the studies in most domains of bias except for selection bias (see Table S1 in Supplementary Material).

Association between MGMT Promoter Methylation and Survival in Overall GBM Patients

Sixty-four and 25 studies were included to describe the correlation of MGMT methylation status with OS and PFS in GBM patients, respectively. GBM patients with MGMT promoter methylation had significantly better OS and PFS than those with unmethylated status (OS: HR = 0.52, 95% CI 0.46–0.59, p < 0.001, I² = 86.2%; PFS: HR = 0.51, 95% CI 0.43–0.59, p < 0.001, I² = 70.2%; see Figure S1 in Supplementary Material), indicating the association between methylation and survival benefit in GBM patients. Next, subgroup analysis was conducted to evaluate whether methylated GBM patients could benefit from different therapies. The results of subgroup analysis were summarized in Table 2. Our analysis showed that, among patients exposed to TMZ-containing treatment, methylated patients had longer OS and PFS than unmethylated patients (OS: HR = 0.46, 95% CI 0.41–0.52, p < 0.001, Bon = 0.017, I² = 70.9%, Figure 2; PFS: HR = 0.48, 95% CI 0.40–0.57, p < 0.001, Bon = 0.014, I² = 67.4%, Figure 3). However, no significant OS benefit from TMZ-free treatment was observed in methylated patients by analysis of 12 studies (21, 35, 44, 58, 69, 70, 77, 84, 85) (HR = 0.97, 95% CI 0.91–1.03, p = 0.32, I² = 2.9%, Figure 2). Further analysis showed that methylated patients derived no OS benefit from TMZ-free alkylating agents chemotherapy (HR = 0.97, 95% CI 0.93–1.03, p = 0.41, Bon = 1, I² = 9.1%). Similarly, PFS was not significantly prolonged in methylated patients with TMZ-free alkylating agents chemotherapy (HR = 0.76, 95% CI 0.57–1.02, p = 0.40, Bon = 0.748, I² = 40.8%, Figure 3). These results indicate that MGMT methylation is predictive for better response to TMZ therapy in GBM patients.

Association between MGMT Promoter Methylation and Survival in Newly Diagnosed GBM Subpopulation

There were 54 and 17 studies recruited to assess the impact of MGMT promoter methylation on OS and PFS in newly diagnosed GBM patients, respectively. MGMT promoter methylation in newly diagnosed GBM patients was also associated with improved OS and PFS (OS: HR = 0.49, 95% CI 0.43–0.57, p < 0.001, I² = 87.7%; PFS: HR = 0.50, 95% CI 0.41–0.61, p < 0.001, I² = 73.8%, Figure S2 in Supplementary Material). Subgroup analysis showed that methylated patients receiving TMZ-containing treatment had better OS and PFS than unmethylated patients (OS: HR = 0.45, 95% CI 0.40–0.52, p < 0.001, Bon = 0.017, I² = 69.8%, Figure 4; PFS: HR = 0.47, 95% CI 0.39–0.57, p < 0.001, Bon = 0.014, I² = 66.1%, Figure 5). No significant advantage on OS and PFS was observed in methylated patients receiving TMZ-free treatment (OS: HR = 0.97, 95% CI 0.90–1.04, p = 0.37, Bon = 1, I² = 5.6%, Figure 4; PFS: HR = 0.93, 95% CI 0.70–1.24, p = 0.62, Bon = 1, Figure 5). These observations were similar to those in overall GBM patients, indicating that the beneficial effect of methylation on OS in newly diagnosed patients was also TMZ therapy-dependent.

Association between MGMT Promoter Methylation and Survival in Elderly GBM Subpopulation

Overall survival in elderly GBM patients was assessed on the basis of 11 studies comprising 1,321 patients. Among these studies, elderly was defined as 60 years or older (58), over 65 years old (32, 41, 55, 61, 70, 84), or 70 years or older (39, 62). A significant correlation between MGMT promoter methylation and better OS was observed in elderly GBM patients (HR = 0.58, 95% CI 0.40–0.82, p = 0.002, I² = 83.4%, Figure S3 in Supplementary Material). A significant improvement on OS was also found in methylated elderly patients with TMZ-containing treatment.
| Author | Country | Study type | Cox | Patients (N) | OS HR (95% CI) | Type of cancer | Treatment after resection | Race | Methylation assay method |
|--------|---------|------------|-----|-------------|----------------|----------------|--------------------------|------|-------------------------|
| Arita et al. (31) | Japan | Retrospective | Multivariate | 453 | 0.43 (0.33, 0.56) | GBM | RT + TMZ | Asian | Pyrosequencing |
| Arvold et al. (32) | America | Non-RCT | Univariate | 55 | 0.47 (0.27, 0.81) | GBM | RT + TMZ | Mixed race | NA |
| Azoulay et al. (33) | Canada | Non-RCT | Multivariate | 276 | 0.46 (0.33, 0.64) | GBM | RT + TMZ | Caucasian | NA |
| Brandes et al. (34) | Italy | Non-RCT | Multivariate | 119 | 0.66 (0.47, 0.94) | GBM | RT + TMZ | Caucasian | MSP |
| Clarke et al. (36) | America | RCT | Univariate | 85 | 0.42 (0.13, 1.39) | GBM | RT + TMZ | Mixed race | MSP |
| Chen et al. (35) | China | Non-RCT | Multivariate | 276 | 0.46 (0.33, 0.64) | GBM | RT + TMZ | Caucasian | NA |
| Clarke et al. (36) | America | RCT | Univariate | 276 | 0.46 (0.33, 0.64) | GBM | RT + TMZ | Caucasian | MSP |
| Cominelli et al. (37) | Italy | Non-RCT | Multivariate | 70 | 0.12 (0.01, 0.98) | GBM | RT + TMZ | Caucasian | MSP |
| Etchecoveny et al. (38) | Spain | Non-RCT | Multivariate | 453 | 0.33 (0.24, 0.48) | GBM | RT + TMZ | Caucasian | MSP and Pyrosequencing |
| Gallego Perez-Larraya et al. (39) | France | Non-RCT | Multivariate | 31 | 0.43 (0.20, 0.93) | GBM | TMZ | Caucasian | MSP |
| Gilbert et al. (40) | America | RCT | Univariate | 70 | 0.38 (0.19, 0.76) | GBM | RT + TMZ | Mixed race | MSP |
| Giordana et al. (41) | Germany | Non-RCT | Multivariate | 70 | 0.38 (0.19, 0.76) | GBM | RT + TMZ | Mixed race | MSP |
| Grossman et al. (43) | Germany | Non-RCT | Multivariate | 122 | 0.85 (0.56, 1.31) | GBM | RT + TMZ | Gaussian | MSP |
| Gutenberg et al. (44) | Germany | Non-RCT | Multivariate | 17 | 0.62 (0.43, 0.90) | GBM | RT + TMZ | Caucasian | MSP |
| Han et al. (45) | China | Non-RCT | Multivariate | 85 | 0.42 (0.13, 1.39) | GBM | RT + TMZ | Caucasian | MSP |
| Jungk et al. (46) | Germany | Non-RCT | Multivariate | 276 | 0.46 (0.33, 0.64) | GBM | RT + TMZ | Caucasian | SP |
| Kim et al. (48) | Korea | Non-RCT | Multivariate | 122 | 0.66 (0.44, 0.94) | GBM | RT + TMZ | Caucasian | MSP |
| Lam and Chambers (53) | Canada | Non-RCT | Univariate | 101 | 0.64 (0.38, 1.08) | GBM | BCNU | Caucasian | MSP |
| Liu et al. (51) | America | Non-RCT | Multivariate | 70 | 0.30 (0.14, 0.65) | GBM | RT + TMZ | Caucasian | NA |
| Lai et al. (52) | Czech Republic | Non-RCT | Multivariate | 38 | 0.40 (0.21, 0.70) | GBM | BCNU | Caucasian | MSP |
| Lombardi et al. (55) | Italy | Non-RCT | Multivariate | 243 | 0.30 (0.22, 0.41) | GBM | RT + TMZ | Caucasian | MSP |
| Lombardi et al. (56) | Italy | Non-RCT | Multivariate | 83 | 0.41 (0.22, 0.75) | GBM | RT + TMZ | Caucasian | MSP |
| Malmström et al. (58) | Europe (Multicenter) | RCT | Univariate | 72 | 0.56 (0.34, 0.93) | GBM | TMZ | Caucasian | MSP |
| Metellus et al. (59) | France | Non-RCT | Multivariate | 21 | 0.19 (0.06, 0.77) | GBM | RT + TMZ | Caucasian | MSP |
| Minniti et al. (61) | Italy | Non-RCT | Multivariate | 101 | 0.64 (0.38, 1.08) | GBM | BCNU | Caucasian | MSP |
| Minniti et al. (62) | Italy | Non-RCT | Multivariate | 83 | 0.41 (0.22, 0.75) | GBM | RT + TMZ | Caucasian | MSP |
| Montano et al. (64) | Italy | Non-RCT | Multivariate | 36 | 0.40 (0.19, 0.94) | GBM | RT + TMZ | Caucasian | MSP |
| Motomura et al. (65) | Japan | Non-RCT | Multivariate | 73 | 0.72 (0.37, 1.37) | GBM | RT + TMZ | Caucasian | MSP |
| Murat et al. (66) | Germany | Non-RCT | Multivariate | 42 | 0.06 (0.001, 0.20) | GBM | RT + TMZ | Caucasian | NA |
| Niyazi et al. (68) | Germany | Non-RCT | Multivariate | 300 | 0.39 (0.30, 0.52) | GBM | RT + TMZ | Mixed race | MSP |
| Perry et al. (70) | Canada and Europe | RCT | Univariate | 281 | 0.93 (0.68, 1.21) | GBM | RT + TMZ | Caucasian | MSP |
| Sana et al. (72) | Czech Republic | Non-RCT | Multivariate | 58 | 0.51 (0.29, 0.91) | GBM | RT + TMZ | Caucasian | MSP |

(Continued)
| Author                          | Country          | Study type | Cox            | Patients (N) | OS HR (95% CI) | Type of cancer | Treatment after resection | Race       | Methylation assay method |
|--------------------------------|------------------|------------|----------------|--------------|----------------|----------------|---------------------------|------------|--------------------------|
| Saraiva-Esperon et al. (73)    | America          | Non-RCT    | Multivariate   | 159          | 0.52 (0.36, 0.73) | GBM            | RT + TMZ                  | Caucasian  | MSP                      |
| Saraiva-Esperon et al. (73)    | Australia        | Non-RCT    | Multivariate   | 144          | 0.42 (0.28, 0.63) | GBM            | RT + TMZ                  | Mixed race | Pyrosequencing            |
| Schallh et al. (74)            | Germany          | Non-RCT    | Multivariate   | 61           | 0.88 (0.36, 2.15) | GBM            | RT + TMZ                  | Caucasian  | MSP                      |
| Schaub et al. (75)             | Germany          | Non-RCT    | Univariate     | 143          | 1.13 (0.77, 1.66) | Recurrent GBM  | RT + BEV + CPT-11         | Caucasian  | NA                       |
| Shenouda et al. (76)           | Canada           | Non-RCT    | Univariate     | 48           | 0.40 (0.19, 0.77) | GBM            | RT + TMZ                  | NA         | NA                       |
| Soffietti et al. (77)          | Italy            | Non-RCT    | Multivariate   | 38           | 0.82 (0.38, 1.74) | Recurrent GBM  | BEV + FTM                | Caucasian  | MSP                      |
| Stummmer et al. (78)           | Germany          | Non-RCT    | Univariate     | 79           | 0.23 (0.10, 0.52) | GBM            | RT + TMZ                  | Caucasian  | MSP                      |
| Butt et al. (79)               | Europe (multicenter) | Non-RCT    | Univariate     | 55           | 0.44 (0.21, 0.91) | GBM            | RT + TMZ + Cilengtide      | Caucasian  | MSP                      |
| Thon et al. (80)               | Germany          | Non-RCT    | Multivariate   | 56           | 0.31 (0.16, 0.58) | GBM            | RT + TMZ (unresectable)   | NA         | NA                       |
| Vaisio et al. (81)             | America          | Non-RCT    | Multivariate   | 86           | 0.11 (0.04, 0.28) | GBM            | TMZ                       | Mixed race | NA                       |
| Van Meighem et al. (82)        | Belgium          | Non-RCT    | Multivariate   | 112          | 0.70 (0.27, 1.8) | GBM            | RT + TMZ                  | Caucasian  | MSP                      |
| Lee et al. (83)                | Korea            | Non-RCT    | Multivariate   | 340          | 0.54 (0.41, 0.70) | GBM            | RT + TMZ                  | Asian      | MSP                      |
| Weller et al. (84)             | Europe (multicenter) | Non-RCT    | Univariate     | 105          | 0.55 (0.44, 0.68) | GBM            | RT + TMZ                  | Caucasian  | MSP                      |
| Wick et al. (85)               | Europe (multicenter) | RCT     | Univariate     | 101          | 0.96 (0.56, 1.63) | GBM            | RT                        | Caucasian  | MSP                      |
| Wick et al. (86)               | Europe (multicenter) | RCT     | Univariate     | 108          | 0.44 (0.27, 0.72) | GBM            | TMZ                       | Caucasian  | MSP                      |
| Yang et al. (87)               | China            | Non-RCT    | Multivariate   | 206          | 0.78 (0.57, 1.04) | GBM            | RT + BCNU                 | Asian      | MSP                      |
| Yang et al. (88)               | China            | Non-RCT    | Multivariate   | 238          | 0.59 (0.37, 0.95) | GBM            | RT + TMZ                  | Asian      | Pyrosequencing            |
| Zhang et al. (89)              | China            | Non-RCT    | Multivariate   | 154          | 0.24 (0.15, 0.39) | GBM            | RT + TMZ                  | Asian      | NA                       |

Studies enrolled for OS analysis. TMZ, temozolomide; RCT, randomized control trial; RT, radiotherapy; BCNU, carmustine; FTM, fotemustine; BEV, bevacizumab; CCNU, lomustine; ELE, interferon-β; CPT-11, irinotecan; MSP, methylation-specific PCR; NA, not available.

Studies enrolled for PFS analysis. TMZ, temozolomide; RCT, randomized control trial; RT, radiotherapy; BCNU, carmustine; FTM, fotemustine; BEV, bevacizumab; ACNU, nimustine; CCNU, lomustine; ELE, interferon-β; CPT-11, irinotecan; MSP, methylation-specific PCR; NA, not available.
therapy, so we further assess whether elderly patients with MGMT methylation could benefit from TMZ alone or radiotherapy alone therapy. Compared to unmethylated elderly patients, prolonged OS was observed in methylated elderly patients receiving TMZ alone therapy but not in those receiving radiotherapy alone (TMZ alone: HR = 0.48, 95% CI 0.35–0.66, p < 0.001, \( I^2 = 0\% \)); Radiotherapy alone: HR = 1.02, 95% CI 0.83–1.25, p = 0.83, \( I^2 = 0\% \), Figure 6). These results indicated the strong correlation between MGMT methylation and better response to TMZ therapy in elderly GBM patients.

**Association between MGMT Promoter Methylation and Survival in Recurrent GBM Subpopulation**

Eleven studies were included to analyze the association between MGMT promoter methylation and survival in recurrent GBM
patients (19, 21, 22, 44, 46, 56, 60, 63, 75, 77, 89). A significant improvement on OS and PFS was observed in methylated recurrent patients (OS: HR = 0.70, 95% CI 0.56–0.88, \( p < 0.001 \), \( I^2 = 61.4\% \); PFS: HR = 0.54, 95% CI 0.42–0.70, \( p < 0.001 \), \( I^2 = 54.8\% \), Figure S4 in Supplementary Material). Subgroup analysis showed TMZ-containing therapy conferred a survival benefit in methylated recurrent patients (OS: HR = 0.59, 95% CI 0.44–0.78, \( p < 0.001 \), Bon = 0.017, \( I^2 = 65\% \), Figure 9; PFS:...
HR = 0.49, 95% CI 0.34–0.70, \( p = 0.001 \), Bon = 0.014, \( I^2 = 66\% \), Figure 10). In contrast, TMZ-free therapy did not improve OS (HR = 0.92, 95% CI 0.70–1.19, \( p = 0.52 \), Bon = 1, \( I^2 = 16.4\% \), Figure 9) or PFS (HR = 0.66, 95% CI 0.49–0.88, \( p = 0.005 \), Bon = 0.065, \( I^2 = 0\% \), Figure 10) in methylated recurrent patients.

**Association between MGMT Promoter Methylation and Survival in GBM Patients with Different Races**

There were 42 studies for Caucasian (European, Canadian, Australian), 16 studies for Asian (Chinese, Japanese, Korean), and 8 studies for mixed race (American). Compared to unmethylated patients, both OS and PFS were improved in methylated patients (OS: Asian: HR = 0.54, 95% CI 0.44–0.65, \( p < 0.001 \), \( I^2 = 61.1\% \); Caucasian: HR = 0.53, 95% CI 0.45–0.63, \( p < 0.001 \), \( I^2 = 86.8\% \); Mixed race: HR = 0.48, 95% CI 0.38–0.62, \( p < 0.001 \), \( I^2 = 67.7\% \); PFS: Asian: HR = 0.53, 95% CI 0.43–0.65, \( p < 0.001 \), \( I^2 = 31.4\% \); Caucasian: HR = 0.49, 95% CI 0.37–0.65, \( p < 0.001 \), \( I^2 = 77.8\% \); Mixed race: HR = 0.51, 95% CI 0.40–0.65, \( p < 0.001 \), \( I^2 = 61\% \), Figure S5 in Supplementary Material). Among GBM patients with TMZ-containing treatment, MGMT methylation benefited to both Caucasian and Asian (Asian OS: HR = 0.48, 95% CI 0.42–0.54, \( p < 0.001 \), Bon = 0.017, \( I^2 = 43.8\% \); PFS: HR = 0.49, 95% CI 0.41–0.59, \( p < 0.001 \), Bon = 0.014, \( I^2 = 0\% \); Caucasian OS: HR = 0.46, 95% CI 0.39–0.55, \( p < 0.001 \), Bon = 0.017, \( I^2 = 75.5\% \); PFS: HR = 0.46, 95% CI 0.34–0.63, \( p < 0.001 \), Bon = 0.014, \( I^2 = 76.2\% \), Figure S6 in Supplementary Material). Among patients receiving TMZ-free treatment, survival benefit in Asian patients was not observed anymore after Bonferroni correction (Asian OS: HR = 0.78, 95% CI 0.64–0.95,
$p = 0.02$, Bonferroni adjustment. The impact of MGMT promoter methylation in mixed race was not evaluated since data in TMZ-free group was not available.

**Publication Bias**
Publication bias was evaluated by Egger’s test. Publication bias was observed in OS and PFS analysis in overall GBM patients (OS: $p < 0.001$; PFS: $p = 0.04$). More results were presented in Table 2. Therefore, we performed the trim and fill analysis to estimate the publication bias. However, those results remain...
unchanged after introducing the trim and fill method to correct the publication bias.

**Sensitive Analysis**

Sensitivity analysis was conducted by sequentially omitting individual studies to assess whether a single study might significantly affect the overall results. Sensitivity analysis showed one study (41) predominantly contributed to heterogeneity in elderly GBM subpopulation, especially in TMZ-containing group (Figure S7 in Supplementary Material). Further sensitivity analysis revealed that other results did not show any apparent variations in pooled HRs for OS or PFS, supporting the robustness of the primary findings.

**DISCUSSION**

Although MGMT has been widely established as a clinically relevant biomarker in GBM patients, its clinical implication has not been definitely confirmed. A prognostic factor is a clinical or biologic characteristic that is objectively measured and provides information on likely outcome of the cancer disease independent of treatment, while a predictive factor is a clinical or biologic characteristic providing information on likely benefits from one specific treatment rather than another (90). Which one is more appropriate to describe the relationship between MGMT promoter methylation and GBM prognosis? Among overall GBM patients, MGMT methylation conferred a survival benefit to patients with TMZ-containing treatment, but not to those with TMZ-free treatment. It seems that MGMT methylation has a predictive value for GBM patients exposed to TMZ-containing treatment. However, considering the differentiation of prognostic variables among patients, including primary or recurrent GBM, age and race, the universality of predictive value of MGMT methylation in different GBM subgroups should be profoundly validated. Therefore, we further assess its clinical significance in newly diagnosed patients, recurrent patients, elderly patients, and Asian and Caucasian patients.

In newly diagnosed and recurrent GBM patients, MGMT methylation was associated with improved OS and PFS with TMZ-containing treatment, but not in those with TMZ-free treatment. Then MGMT methylation is predictive for a benefit from TMZ-containing chemotherapy in newly diagnosed and recurrent patients.

In elderly GBM patients, MGMT methylation also conferred an OS benefit in patients with TMZ-containing treatment, but not in those with TMZ-free treatment. Therefore, MGMT methylation in elderly patients is likely to have a similar predictive value as in newly diagnosed and recurrent GBM patients.
patients are often clinically unable to tolerate multimodality therapy, thus TMZ or radiotherapy alone is commonly used. This meta-analysis showed that elderly patients with methylated status exposed to TMZ alone had improved OS than those exposed to radiotherapy alone, while such difference was not observed in those with unmethylated status. Our results highlight that TMZ alone therapy might be a more effective option than radiotherapy alone therapy for elderly GBM patients with methylated MGMT status. But the optimal radiotherapy regimen for elderly and/or frail patients with newly diagnosed GBM remains to be defined (91). A recent study showed that short-course radiation (40 Gy in 15 fractions) plus TMZ conferred a survival advantage over...
radiotherapy alone in elderly patients (65 years of age or older) with newly diagnosed GBM, especially in those with methylated MGMT status (70). Due to the lack of a uniform definition for elderly, different cutoff age was employed in different studies. Patients aged more than 70 years were excluded from Stupp study (87). In this meta-analysis, patients aged 60 or more were enrolled for analysis. Our results showed that patients aged over 70 years with MGMT methylation also benefit from TMZ-containing therapy. The definition of cutoff age for the elderly are closely linked to prognosis, therapeutic goals, or patterns of care, so further research in this field should standardize the cutoff age for enrollment eligibility (92).
Another interesting issue is the clinical value of MGMT methylation in Asian and Caucasian patients. A previous study showed that MGMT methylation correlated with better OS and PFS in Caucasian patients and only better OS in Asian patients regardless of therapeutic intervention (93). But the benefit of different therapies in methylated patients was not investigated in the study. In our analysis, survival benefit in Asian patients with TMZ-free treatment was not observed anymore after Bonferroni adjustment. Bonferroni correction can avoid false positives, and then the risk of false negatives would be increased. So the finding in Asian patients should be cautiously interpreted. It must be noted that only four studies (519 patients) for OS and a single study (137 patients) for PFS were enrolled for this subgroup analysis. Therefore, our finding on patients with different races needs to be further verified by more clinical studies. Furthermore, recent studies also give a hint about the different regulation of MGMT methylation in different ethnic background. Single nucleotide polymorphisms (rs16906252) in MGMT promoter-enhancer is a key determinant in the acquisition of MGMT methylation (94). The genotype of rs16906252 varies among different ethnic groups (95), which may result in different MGMT methylation status. In addition to promoter methylation, other molecules are also involved in regulation of MGMT expression or function. For example, miR-181d can bind to the 3′ untranslated region of MGMT transcripts, then decrease its mRNA stability and/or reduce protein translation (96). Further studies on ethnically genetic variations are necessary.

Due to the limited number of trials recruited for analysis, the presented information about PFS in patients with TMZ-free treatment, especially in newly diagnosed and recurrent subgroups, should be interpreted carefully. It should be acknowledged that we did not obtain any data of PFS in elderly patients exposed to TMZ-free treatment. Therefore, the predictive or prognostic value of this biomarker for PFS is far from identified in our analysis. In fact, clinical measurement of PFS may be a critical challenge in GBM trials. It is well known that GBM patients suffer inevitably recurrence despite integrated therapy (97). Pseudoprogression, also denoted as radiotherapy-introduced necrosis, exhibits contrast enhancement similar to early tumor progression on magnetic resonance imaging. Primary GBM patients receiving concurrent and adjuvant TMZ-based chemoradiotherapy have a high likelihood of developing pseudoprogression (98, 99), which occurs mainly within 3 months after completion of chemoradiotherapy. However, no technique has been proven to reliably differentiate between tumor recurrence and pseudoprogression. Additionally, both entities might coexist in the same patient at the same time in different areas of the tumor. The misdiagnosis of pseudoprogression as tumor recurrence may lead to a record of shorter PFS. Interestingly, MGMT promoter methylation was associated with a high incidence of pseudoprogression in newly diagnosed GBM patients undergoing TMZ-based chemoradiotherapy (100). In addition, GBM patients with the occurrence of pseudoprogression had a longer OS than those without pseudoprogression (98, 101), indicating that pseudoprogression may be a predictor for better response to therapy. Therefore, it is critically important to develop imaging techniques and biomarkers to discriminate pseudoprogression from early progression.

We also noticed the methodological diversity of measurement of MGMT promoter methylation. MGMT promoter methylation was detected by methylation-specific polymerase chain reaction (MSP), pyrosequencing, and methylation-sensitive high-resolution melting (MS-HRM) in 48, 6, and 2 studies, respectively. Additionally, various cutoff values for methylated positivity were used in these studies. However, there were few studies that have
compared the merits and disadvantages of these MGMT testing methods (17). Further efforts should standardize the MGMT methylation testing methods and cutoff point.

Limitations of this study should be acknowledged. Firstly, heterogeneity existed in the pooled analysis for PFS and OS either in overall population or in subgroup analysis. Heterogeneity may result from different techniques of defining MGMT promoter status and varied therapy schedule. Different chemotherapy and radiotherapy schedules may influence the prognosis of GBM patients, thus analysis of the correlation between a single treatment schedule and MGMT promoter status was not conducted in this meta-analysis. Second, considering the scarce number of multivariate studies in some of subgroup analysis, univariate studies were also included in our analysis. We also performed analysis using only multivariate studies and similar findings were observed (Table S3 in Supplementary Material). Third, due to the limited number of original documents on PFS, there was not enough power to identify the impact of MGMT methylation on PFS, especially in patients receiving TMZ-free therapy. Fourth, quality assessment was performed by a modified domain-based NOS (102, 103), which was proposed as a potential helpful and practically method for assessment of tumor prognostic studies. However, this novel NOS has not been fully validated and results should be interpreted with caution. Fifth, Egger’s test showed that publication bias existed in pooled analysis for OS, but the trim and fill analysis upheld the reliability of our results.

In conclusion, our results highlight the universal predictive value of MGMT methylation in newly diagnosed GBM patients, elderly GBM patients and recurrent GBM patients. For elderly methylated GBM patients, TMZ alone therapy might be a more suitable option than radiotherapy alone therapy. This study may be helpful to optimize therapeutics in different GBM subpopulation.

AUTHOR CONTRIBUTIONS

Y-HZ and C-JC contributed to the conception of the experiments and manuscript preparation. Y-HZ, C-SX, X-TZ, J-LL, JL, and KL contributed to data research and review. Y-HZ and HW performed data analysis. Z-FW and Z-QL contributed to interpretation and discussion of the results.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found at https://www.frontiersin.org/articles/10.3389/fneur.2018.00127/full#supplementary-material.

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