# Prognostic Impact of the Combination of *MGMT* Methylation and *TERT* Promoter Mutation in Glioblastoma

Thara Tunthanathip[1] Surasak Sangkhathat[2] Pimwara Tanvejsilp[3] Kanet Kanjanapradit[4]

[1] Division of Neurosurgery, Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand
[2] Department of Surgery and Department of Biomedical Sciences, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand
[3] Department of Pharmacy Administration, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkla Thailand
[4] Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

**Address for correspondence** Thara Tunthanathip, Division of Neurosurgery, Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand (e-mail: tsus4@hotmail.com).

J Neurosci Rural Pract 2021;12:694–703.

## Abstract

**Background** The concept of combinational analysis between the methylation of *O*$^6$*-methylguanine-DNA methyltransferase* (*MGMT*) and *telomerase reverse transcriptase* promoter (*pTERT*) mutation in glioblastoma (GBM) has been reported. The main study objective was to determine the prognosis of patients with GBM based on *MGMT*/*pTERT* classification, while the secondary objective was to estimate the temozolomide effect on the survival time of GBM with *MGMT*/*pTERT* classification.

**Methods** A total of 50 GBM specimens were collected after tumor resection and were selected for investigating *MGMT* methylation and *pTERT* mutation. Clinical imaging and pathological characteristics were retrospectively analyzed. Patients with *MGMT*/*pTERT* classification were analyzed using survival analysis to develop the nomogram for forecasting and individual prognosis.

**Results** All patients underwent resection (total resection: 28%, partial resection: 64%, biopsy: 8%). Thirty-two percent of all cases received adjuvant temozolomide with radiotherapy. Sixty-four percent of the case was found methylated *MGMT*, and 56% of the present cohort found *pTERT* mutation. Following combinational analysis of biomarkers, results showed that the GBMs with methylated *MGMT* and wild-type *pTERT* had a superior prognosis compared with other subtypes. Using Cox regression analysis with multivariable analysis, the extent of resection, postoperative chemoradiotherapy, *MGMT*/*pTERT* classification were associated with a favorable prognosis. Hence, a web-based nomogram was developed for deploying individual prognostication.

**Conclusions** The interaction of *MGMT* methylation and *pTERT* mutation was confirmed for predicting prognosis. The results from the present study could help physicians create treatment strategies for GBM patients in real-world situations.

**Keywords**
- telomerase reverse transcriptase
- O$^6$-methylguanine-DNA methyltransferase
- primary glioblastoma
- survival
- prognosis

**published online**
September 28, 2021

**DOI** https://doi.org/10.1055/s-0041-1735821.
**ISSN** 0976-3147.



Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

## Introduction

Various biomarkers have been used as prognostic factors in malignant brain tumors, including glioblastoma (GBM). GBM is categorized into two groups by *isocitrate dehydrogenase (IDH)* mutation.[1] In prior studies, the *IDH*-wild-type tumor had an inferior prognosis than *the IDH*-mutant tumor. In addition, *IDH* mutation also is one of the key biomarkers that explain the pathogenesis of gliomas.[1,2]

*IDH*-mutant GBMs have been found in just 8 to 10% of cases. Therefore, previous studies have identified various biomarkers that were prognostic factors in the majority of GBMs. For example, the mutation of the *telomerase reverse transcriptase* promoter *(pTERT)* has been reported as a prognostic factor in numerous cancers.[1,3] Simon et al conducted an exploratory study in 192 patients with GBM and reported that GBM patients with *pTERT* mutations were found in 80.3% and significantly related to poor prognosis,[4] while Kim et al found *pTERT* mutation in 59.2% (25/42) of all GBM cases.[5]

In addition, methylation of *$O^6$-methylguanine-DNA methyltransferase (MGMT)* has been studied. The epigenetic methylation predicted the responsiveness of alkylating agents. Esteller et al found that glioma with methylation of *MGMT* interfered with MGMT protein-encoding. Therefore, chemotherapy significantly affected the tumor cells. Gliomas with methylated *MGMT* (*mMGMT*) are called chemosensitivity tumors, while chemo-resistant tumors are gliomas with unmethylated *MGMT* (*uMGMT*), which create $O^6$-methylguanine-DNA methyltransferase to change the structure of the alkylating agents and resist the chemotherapy.[6] Hegi et al studied the association between *mMGMT* and prognosis in patients with GBM. They advised that *mMGMT* was a significantly favorable predictor. Moreover, patients with *mMGMT* who received temozolomide (TMZ) with radiotherapy (RT) had a significantly longer median survival time than patients who received RT alone.[7] Recently, the National Comprehensive Cancer Network has proposed the clinical practice guideline for central nervous system tumor (CNS) treatment. Methylation of *MGMT* was considered for selecting the postoperative management in GBM.[8]

The concept of a combined analysis of biomarkers for predicting prognosis in GBM patients has been proposed in literature reviews. Arita et al studied 453 GBM cases and found the interaction between the methylation of *MGMT* and *pTERT* mutation related to prognosis. In detail, GBMs were categorized into four subgroups as follows: methylated *MGMT* with wild-type *TERT* (*mMGMT-wTERT*), methylated *MGMT* with mutant *TERT* (*mMGMT-wTERT*), *uMGMT* with mutant *TERT* (*uMGMT-wTERT*), *uMGMT* with wild-type *TERT (uMGMT-mTERT*), called *MGMT/pTERT* classification. Hence, *uMGMT-mTERT* GBMs had the poorest prognosis, while *mMGMT-wTERT* and *mMGMT-mTERT* GBMs had the most favorable prognosis. Additionally, *uMGMT-wTERT* GBMs had an average prognosis.[9] Therefore, several prior studies found similar interactions between *MGMT* promoter methylation and *TERT* mutation.[10–12] However, a lack of evidence was mentioned concerning the effect of TMZ on survival in *MGMT/pTERT* classification. The present study aimed to estimate the prognosis of patients with GBM based on *MGMT/pTERT* classification. Also, the secondary objective was to estimate the TMZ effect on the survival time of GBM with *MGMT/pTERT* classification.

## Methods

### Study Design and Population

The Ethics Committee and Institutional Review Board of the Faculty of Medicine, Prince of Songkla University, reviewed and approved the present study (REC 61–065–10–1). All cases were diagnosed as GBMs between 2003 and 2018, and histological slides were reviewed to confirm the diagnosis. Electronic medical records were reviewed for clinical data collection. Moreover, preoperative and postoperative imaging of GBM patients was evaluated by a neurosurgeon.

### DNA Extraction and Molecular Analysis

DNA was extracted according to the manufacturer's instructions. Mutation of *IDH1* and methylation of *MGMT* were investigated, as formerly described.[13] In detail, the methylated *MGMT* was defined as 30% or more methylation.[14] *pTERT* mutations were identified by droplet digital polymerase chain reaction with C228T_113 Assay (Bio-Rad; Assay ID; dHsaEXD72405942) based on the study of Corless et al.[15]

One-hundred and seventy-three patients were treated between January 2003 and December 2018. Therefore, the GBM patients who did not have tumor specimens for the molecular study were excluded. Finally, the remaining 50 patients were completely analyzed for *IDH1* mutation, *MGMT* methylation, and *pTERT* mutation.

### Statistical Analysis

Descriptive statistics were performed to explain the demographic data of the present cohort. The categorical variables were reported as percentages, and mean $\pm$ standard deviation (SD) was used for the continuous variables with normal distribution. Moreover, continuous variables without normal distribution were described by the median and interquartile range (IQR).

Survival analysis was performed for estimating prognosis. The overall survival (OS) and survival probability in each time-point were analyzed. The Kaplan–Meier survival curves were visually analyzed for survival time of each variable. The Cox hazard regression was used for identifying the prognostic factors in univariate analysis and multivariable analysis. In detail, the backward stepwise method was performed to select the final predictive model. A $p < 0.05$ was accepted as being statistically significant. Hence, the final model following multivariable analysis was used for nomogram development, as previously described,[16,17] and the nomogram was deployed as a web-based application for general practice in the future.

After nomogram development, the nomogram scoring system was used to estimate the predictive performance, including discrimination, calibration, and internal validation.[18] In detail, Harrell's concordance index (C-index) was

 

estimated for discrimination by Cox hazard regression, and calibration was visually evaluated by closing a 45-degree line. Additionally, internal validation was conducted by the bootstrap validation with 200-time resampling, and the result of the validation was reported as the area under the ROC curves (AUC).[19]

The statistical analysis was performed using the R program version 3.4.0 software (R Foundation, Vienna, Austria). Moreover, a web-based nomogram was developed through https://www.shinyapps.io/.

## Results

### Clinical Characteristics

Fifty patients with GBM were described with baseline clinical characteristics (►**Table 1**). Of them, 58% were male with a median age of 54 years old (IQR: 23). The common signs and symptoms of patients were progressive headache, hemiparesis, and seizure. In addition, the majority of GBM were single and involved the frontal lobe. In addition, more than half of the tumors infiltrated the eloquent area defined according to Lacroix et al.[20]

All patients underwent surgical management. Total tumor resection was performed in 28% of the present cohort. One-third of the cohort refused any further treatment following surgery. TMZ is used postoperatively in 32% of cases in real-

**Table 1** Baseline characteristic of patients with glioblastoma ($n = 50$)

| Factors | *n* (%) |
|---|---|
| Age, years | |
| $< 60$ | 30 (60.0) |
| $\geq 60$ | 20 (40.0) |
| Median age-year (IQR) | 54 (23) |
| Gender | |
| Male | 29 (58.0) |
| Female | 21 (42.0) |
| Signs and symptoms | |
| Progressive headache | 26 (52.0) |
| Motor weakness | 20 (40.0) |
| Seizure | 14 (28.0) |
| Alteration of consciousness | 9 (18.0) |
| Behavior changes | 6 (12.0) |
| Aphasia | 3 (6.0) |
| Ataxia | 1 (2.0) |
| Preoperative Karnofsky performance status | |
| $<80$ | 22 (44.0) |
| $\geq 80$ | 28 (56.0) |
| Number of tumor | |
| Single | 48 (96.0) |
| Multiple | 2 (4.0) |
| Tumor location | |
| Eloquent area[a] | 27 (54.0) |
| Frontal lobe | 19 (38.0) |
| Temporal lobe | 12 (24.0) |
| Corpus callosum | 8 (16.0) |
| Parietal lobe | |
| Occipital lobe | 4 (8.0) |
| Basal ganglion | 3 (6.0) |
| Suprasellar area | |
| Leptomeningeal dissemination | |
| No | 43 (86.0) |
| Brain | 5 (10.0) |
| Brain with spinal cord | 2 (4.0) |
| Mean diameter of tumor-cm (SD) | 5.20 (1.72) |
| Extent of resection | |
| Neuronavigator-guided biopsy | 4 (8.0) |
| Partial resection | 32 (64.0) |
| Total resection | 14 (28.0) |
| Postoperative KPS | |
| $<80$ | 31 (62.0) |
| $\geq 80$ | 19 (38.0) |
| Adjuvant treatment | |
| No adjuvant treatment | 17 (34.0) |
| Radiotherapy alone | 17 (34.0) |
| Temozolomide with radiotherapy | 16 (32.0) |
| *IDH1* mutation | |
| Wild-type *IDH1* | 46 (92.0) |
| Mutant *IDH1* | 4 (8.0) |
| *MGMT* methylation | |
| Unmethylated *MGMT* | 18 (36.0) |
| Methylated *MGMT* | 32 (64.0) |
| *TERT* promoter mutation | |
| Wild-type *TERT* | 22 (44.0) |
| Mutant *TERT* | 28 (56.0) |
| Combined biomarkers | |
| Methylated *MGMT* with wild-type *TERT* | 18 (36.0) |
| Methylated *MGMT* with mutant *TERT* | 14 (28.0) |
| Unmethylated *MGMT* with wild-type *TERT* | 14 (28.0) |
| Unmethylated *MGMT* with mutant *TERT* | 4 (8.0) |

Abbreviations: GBM, glioblastoma; IDH1, isocitrate dehydrogenase1; IQR, interquartile range; KPS, Karnofsky Performance Status; MGMT, $O^6$-methylguanine-DNA methyltransferase; RT, radiotherapy; SD, standard deviation; TERT, telomerase reverse transcriptase; TMZ, temozolomide.
[a]Eloquent area defined tumor involved motor cortex, sensory cortex, visual center, speech center, basal ganglion, hypothalamus, thalamus, brainstem, dentate nucleus.

 

world situations, primarily because patients cannot afford the cost of TMZ.

In biomarker profiles, the status of *IDH1* was almost all *IDH*-wild-type GBMs, whereas *mMGMT* was found in 64% of cases. In addition, p*TERT* methylation was frequently found in 56% of cases. When GBM was categorized based on the status of *MGMT* methylation and *pTERT* mutation, tumors were divided into four subgroups as follows: *mMGMT-wTERT*, *mMGMT-mTERT*, *uMGMT-wTERT*, and *uMGMT-mTERT* GBMs. The most common subgroup was *mMGMT-wTERT* GBM, found in 36.0% of cases.

## Survival Analysis

The mean follow-up time was 13.17(SD: 9.6) months, and the median OS of the present study was 11 months (95% confidence interval [CI]: 9.15–12.84), as shown in ►**Fig. 1A**. Additionally, 1, 2, and 3-year survival probabilities were 30.8, 12.6, and 0.42%, respectively. When the Kaplan–Meier survival analysis was performed on each variable, the total tumor resection, adjuvant therapy with TMZ and RT, *mMGMT-wTERT* tumor significantly prolonged survival time, as shown in ►**Fig. 1B–F**.

According to the combined *MGMT/TERT* classification, *mMGMT-wTERT* GBMs had a favorable prognosis with a median OS of 18 months (95% CI: 10.3–25.6), while *uMGMT-mTERT* GBMs had the shortest survival time of 1 month, as shown in ►**Fig. 2**. Moreover, the median OS of *mMGMT-mTERT* and *uMGMT-mTERT* were 9 (95% CI: 7.1–10.8) and 9 months (95% CI: 6.7–11.2), respectively.

We also analyzed the survival impact of TMZ with RT based on the combined *MGMT/TERT* classification, as shown in ►**Table 2**. TMZ with RT significantly benefited *mMGMT-wTERT* GBMs with a median OS of 25 months, whereas other subgroups had a median OS of 12 to 14 months (log-rank test, $p = 0.03$). For subgroup analysis of patients who received TMZ with RT, patients with *mMGMT* seemed to have survival benefit from concurrent chemoradiotherapy.

For prognostication, Cox hazard regression was performed. The extent of resection, TMZ with RT, and *MGMT/TERT* classification were the potential prognostic models for both univariate and multivariable analysis, as shown in ►**Table 3**.

Patients who underwent an operation with total tumor removal had a significantly better prognosis than nontotal tumor removal (hazard ratio [HR]: 0.44, 95% CI: 0.20–0.98). For adjuvant therapy, TMZ treatment had the benefit of prolonging survival time (HR: 0.12, 95% CI: 0.04–0.35). Against a reference of *mMGMT-wTERT* GBMs, other subgroups had a significantly inferior prognosis than the reference. For implication in general practice, we developed the nomogram, a two-dimensional graphic scoring system for individual 1, 2, and 3-year prognostication, as shown in ►**Fig. 3**. Moreover, the web-based nomogram was deployed for ease to predict an individual in general practice via https://thara.shinyapps.io/Nomogram_GBM_PSU/; the code for the prediction tools was linked through https://github.com/Thara-PSU/Nomogram_GBM_PSU.

For evaluation of the predictive nomogram, Harrell's C-index was 0.791 for the model discrimination and the calibration plot was constructed, as shown in ►**Fig. 4**. Moreover, bootstrap validation was performed for the internal validation, wherein the AUC was 0.79.

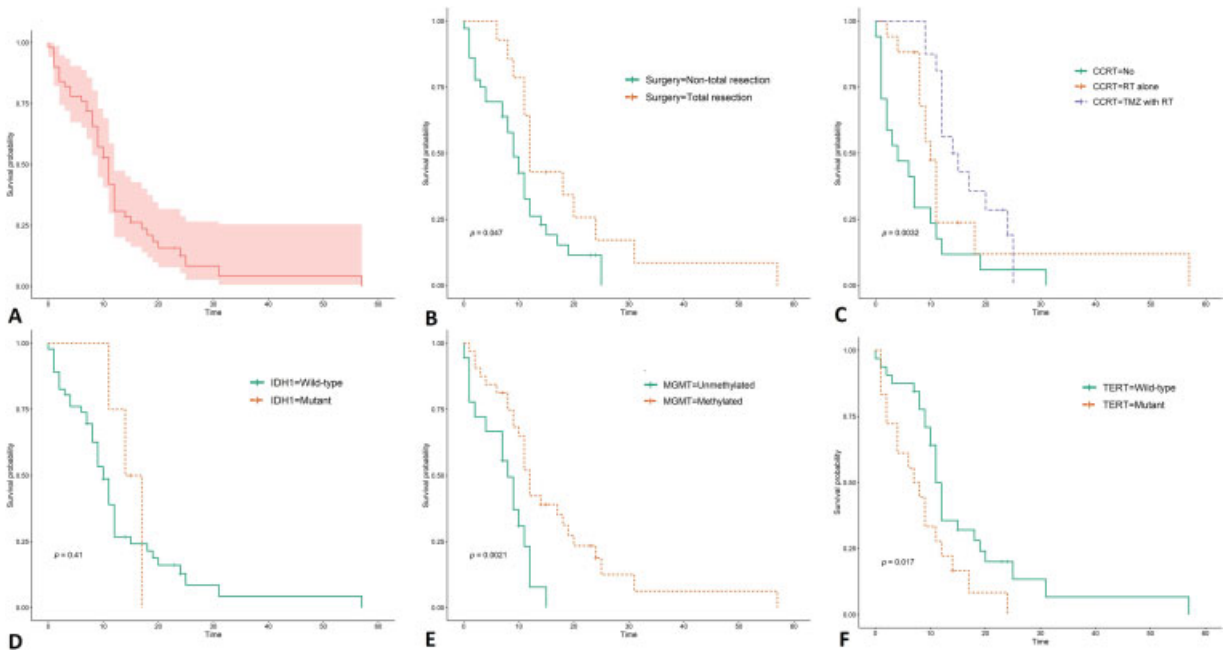


**Fig. 1** Survival curves of patients with glioblastoma. (**A**) Overall, (**B**) extent of resection, (**C**) concurrent chemoradiotherapy (CCRT), (**D**) isocitrate dehydrogenase *(IDH1)* mutation, (**E**) $O^6$-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, and (**F**) *telomerase reverse transcriptase* (*TERT)* promoter mutation. TMZ, temozolomide.

 

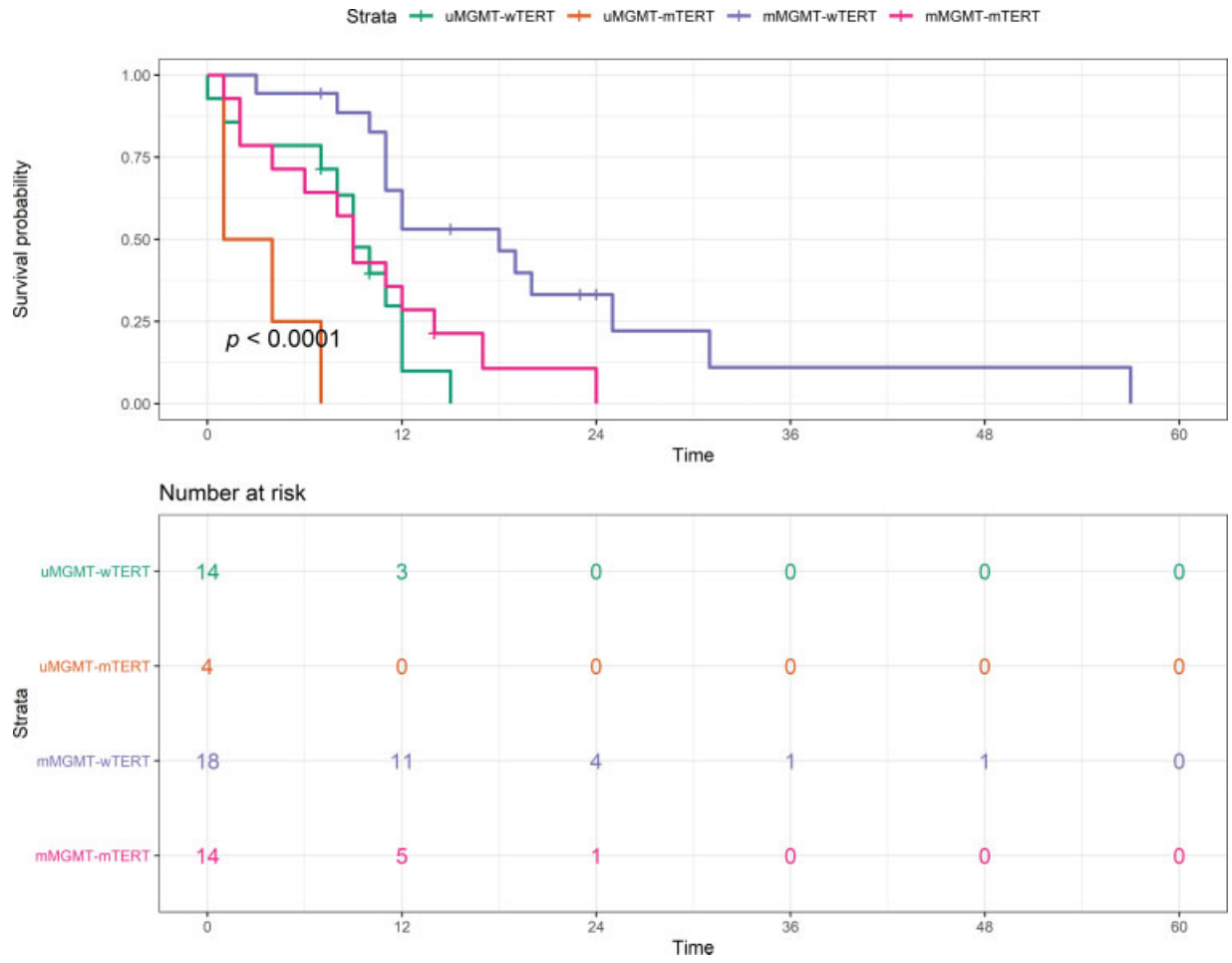


**Fig. 2** Survival curves of the *O*[6]*-methylguanine-DNA methyltransferase/promotor telomerase reverse transcriptase (MGMT/pTERT)* classification. mMGMT, methylated O[6]-methylguanine-DNA methyltransferase; mTERT, methylated telomerase reverse transcriptase; uMGMT, unmethylated O[6]-methylguanine-DNA methyltransferase; wTERT, wild-type telomerase reverse transcriptase.

**Table 2** Median survival time and Cox regression analysis of GBM patients who received temozolomide with radiotherapy based on the *MGMT*/*TERT* classification

| The combined *MGMT*/*TERT* classification | Median survival time (95% CI)[a] | Hazard ratio (95% CI) |
|---|---|---|
| Methylated *MGMT* with wild-type *TERT* ($n = 5$) | 25 (10.3–25.6) | Ref |
| Methylated *MGMT* with mutant *TERT* ($n = 7$) | 14 (8.8–19.1) | 4.5 (0.84–23.5) |
| Unmethylated *MGMT* with wild-type *TERT* ($n = 0$) | 12 (9.4–14.5) | 8.5 (1.2–56.8) |
| Unmethylated *MGMT* with mutant *TERT* ($n = 4$) | – | – |

Abbreviations: CI, confidence interval; GBM, glioblastoma; MGMT, O[6]-methylguanine-DNA methyltransferase; TERT, telomerase reverse transcriptase.
[a]*p*-Value of log-rank test = 0.03.

## Discussion

In the present study, we found the key biomarkers that significantly predicted prognosis, while the interaction of *MGMT* methylation status and *pTERT* mutation was also detected. The unmethylation of *MGMT* promoter and mutation of *TERT* influenced the shortened survival time in the present study. Therefore, patients with *mMGMT-wTERT* GBM had the longest survival time, while the poorest prognosis was for patients with *uMGMT-mTERT* GBM. These findings were in accordance with prior studies. Purkait et al stratified the subgroups of GBMs based on both biomarkers and reported that patients with *uMGMT-mTERT* GBM had the shortest survival time (HR: 11.12, 95% CI: 1.99–61.99).[10] Similarly, Sasaki et al studied these biomarkers for predicting prognosis in elderly GBM patients and found that the poorest prognosis was the *uMGMT-mTER* subtype.[12]

According to the CNS tumor classification, GBMs are categorized by *IDH* mutation.[1] The wild-type *IDH1* GBMs were commonly found in the primary GBM and associated

 

**Table 3** Univariate and multivariable analysis of Cox proportional hazard regression for death

| | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|
| **Factors** | **Hazard ratio (95% CI)** | ***p*-Value** | **Hazard ratio (95% CI)** | ***p*-Value** |
| Age, years | | | | |
| $<60$ | Ref | | | |
| $\geq 60$ | 1.59 (0.86–2.94) | 0.13 | | |
| Gender | | | | |
| Male | Ref | | | |
| Female | | | | |
| Preoperative Karnofsky performance status | | | | |
| $< 80$ | Ref | | | |
| $> 80$ | 0.70 (0.38–1.29) | 0.25 | | |
| Tumor location | | | | |
| Eloquent area [a,b] | 1.44 (0.78–2.67) | 0.23 | | |
| Temporal lobe[a] | 1.10 (0.53–2.27) | 0.78 | | |
| Corpus callosum[a] | 1.17 (0.51–2.65) | 0.70 | | |
| Frontal lobe[a] | 0.59 (0.31–1.14) | 0.11 | | |
| Parietal lobe[a] | 1.58 (0.69–3.62) | 0.27 | | |
| Occipital lobe[a] | 0.59 (0.17–1.99) | 0.39 | | |
| Diameter of maximum tumor, cm | | | | |
| $< 5$ | Ref | | | |
| $> 5$ | 0.90 (0.48–1.66) | 0.90 | | |
| Extent of resection | | | | |
| Nontotal resection | Ref | | Ref | |
| Total resection | 0.51 (0.25–0.99) | 0.05 | 0.44 (0.20–0.98) | 0.05 |
| Postoperative Karnofsky performance status | | | | |
| $<80$ | Ref | | | |
| $>80$ | 0.54 (0.28–1.03) | 0.06 | | |
| Adjuvant treatment | | | | |
| No adjuvant treatment | Ref | | Ref | |
| Radiotherapy alone | 0.50 (0.23–1.05) | 0.06 | 0.57 (0.24–1.30) | 0.18 |
| Temozolomide with radiotherapy | 0.31 (0.14–0.65) | 0.002 | 0.12 (0.04–0.35) | $<0.001$ |
| *IDH1* mutation | | | | |
| Wild-type *IDH1* | Ref | | | |
| Mutant *IDH1* | 0.62 (0.19–2.00) | 0.44 | | |
| *MGMT* promoter methylation | | | | |
| Methylated *MGMT* | Ref | | | |
| Unmethylated *MGMT* | 0.37 (0.18–0.73) | 0.004 | | |
| *TERT* promoter mutation | | | | |
| Wild-type *TERT* | Ref | | | |
| Mutant *TERT* | 2.22 (1.14–4.35) | 0.01 | | |
| Combined biomarkers | | | | |
| Methylated *MGMT* with wild-type *TERT* | Ref | | Ref | |
| Methylated *MGMT* with mutant *TERT* | 2.91 (1.27–6.65) | 0.01 | 9.52 (3.29–27.53) | $<0.001$ |
| Unmethylated *MGMT* with mutant *TERT* | 3.74 (1.54–9.08) | 0.004 | 6.99 (2.55–19.15) | $<0.001$ |
| Unmethylated *MGMT* with wild-type *TERT* | 16.99 (4.47–64.60) | $<0.001$ | 14.38 (3.43–60.27) | $<0.001$ |

Abbreviations: CI, confidence interval; IDH1, isocitrate dehydrogenase1; MGMT, $O^6$-methylguanine-DNA methyltransferase; TERT, telomerase reverse transcriptase.

[a]Data show only "yes group" while reference groups (no group) are hidden.

[b]Eloquent area defined tumor involved motor cortex, sensory cortex, visual center, speech center, basal ganglion, hypothalamus, thalamus, brainstem, dentate nucleus.

 

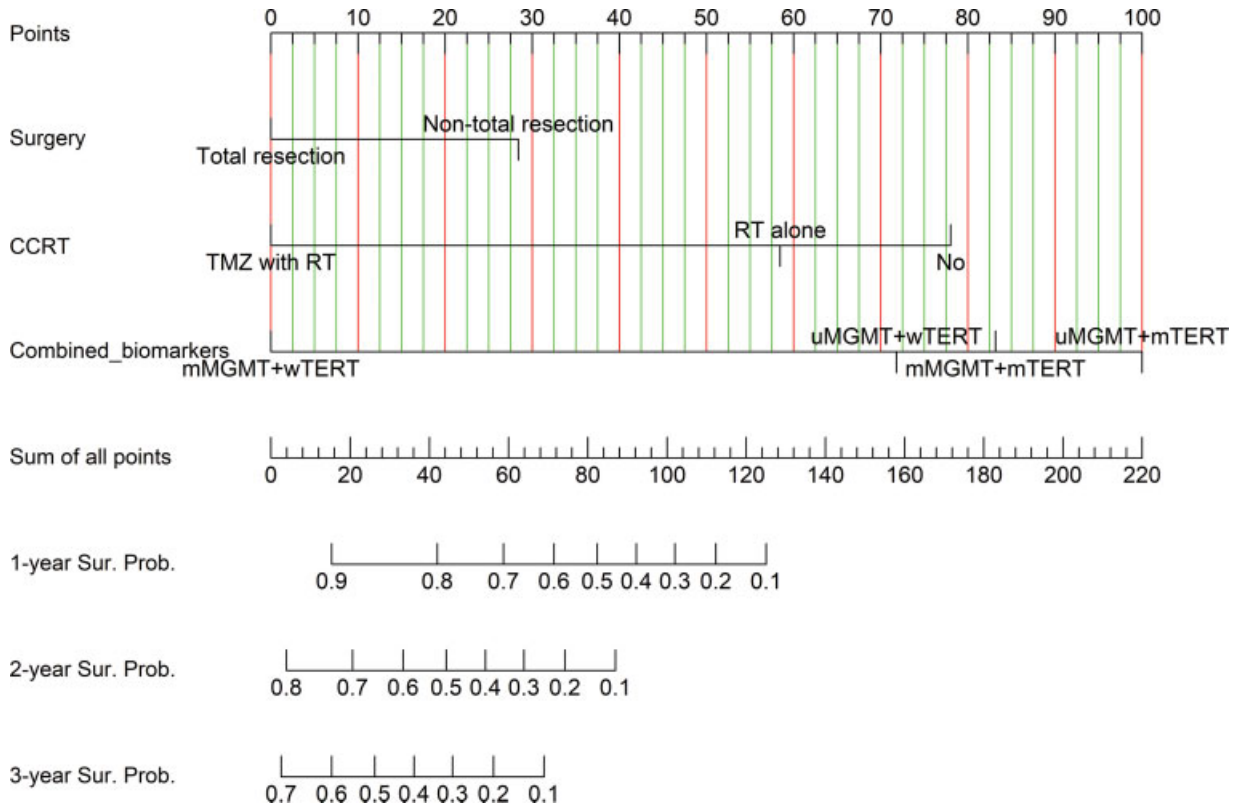


**Fig. 3** Nomogram for predicting the individual survival of glioblastoma patient. CCRT, concurrent chemoradiotherapy; mMGMT, methylated $O^6$-methylguanine-DNA methyltransferase; mTERT, methylated telomerase reverse transcriptase; TMZ, temozolomide; uMGMT, unmethylated $O^6$-methylguanine-DNA methyltransferase; wTERT, wild-type telomerase reverse transcriptase.

with poor prognosis.[1–3] However, *IDH1* mutation was not an independent prognostic factor in the present study. One prior meta-analysis study reported no association of *IDH1* mutation in eastern studies when subgroup analysis was performed based on country region. The effect of *IDH1* mutation on prognosis is likely related to the topographic connection.[21] This biomarker should be explored for its association with prognostication in future studies.

We observed the three key prognostic factors through a multivariable Cox regression analysis: the extent of resection, CCRT, and *MGMT/pTERT* classification. Currently, TMZ with RT is the standard treatment following tumor resection.[22,23] However, the cost of TMZ is high and becomes an economic burden in a limited-resource setting. Therefore, selecting GBM patients by predicted favorable prognosis has been proposed for the likelihood of TMZ cost-effectiveness in real-world situations.[24]

Nomogram is one of the clinical prediction tools (CPT) that have been used for various diseases such as cancer,[25] trauma,[26,27] or degenerative disease.[28] Therefore, we proposed a web-based nomogram for prognostication that will be applied for selecting patients with a favorable prognosis when low- or middle-income settings need to allocate resources.

According to the concept of translational medicine, T0 research defines the phase of basic science research studied to explore the novel biomarkers or candidate genes through -omic technologies. Further, T1 research defines research translated to humans that involves the tools for screening or diagnostic testing.[29,30] Sam et al studied genetic polymorphism and the risk factors for screening gastrointestinal tract carcinoma in Indian people.[31] Hence, T2 research translates information to patients as an evidence-based guideline that commonly studies cancer staging, prognosis, and treatment response prediction. In detail, the development of CPT corresponds with development, calibration, discrimination, and internal validation in the derivation of CPT.[32] The T3 research translates to general practice via dissemination studies. Therefore, this is in accordance with the external validation phase in the CPT development, while the impact study of the tools achieved T4 research impacts to the society and community as well as changing the health policy or economics.[33]

The present study developed and proposed a nomogram for predicting prognosis by integrating clinical variables and biomarkers. Therefore, the prediction performance of the nomogram was estimated in steps as the T2 research of translational medicine. Harrell's C-index and AUC of bootstrap validation in the present study were acceptable.[34,35] Therefore, the line of the calibration plot in the present study was close the 45-degree line that accepted the performance. The nomogram developed from the GBM patients will be evidence to conduct external validation or dissemination study to apply in health practice in the future.

Nevertheless, certain limitations should also be recognized. First, we considered a small sample of GBM patients. Multicenter research or systematic review and meta-analysis

 

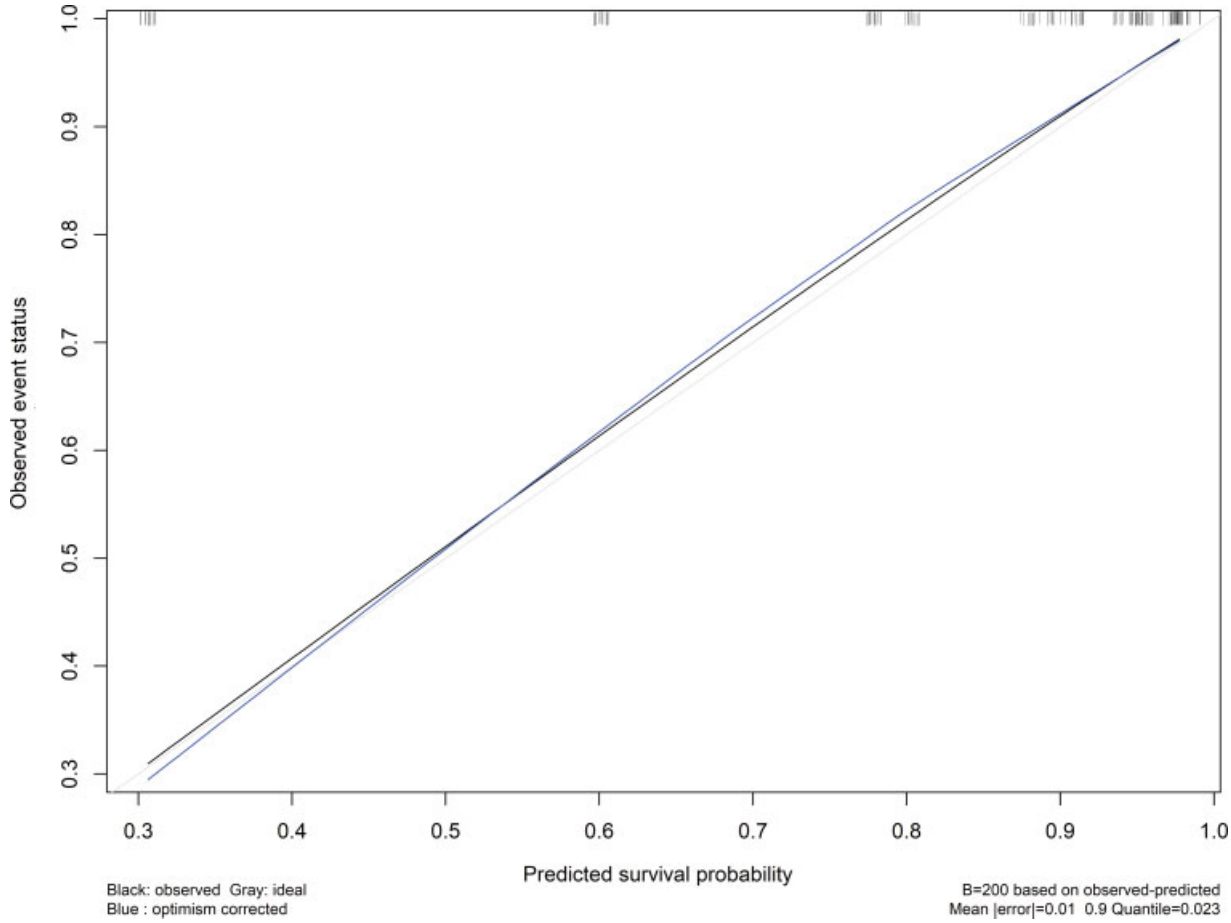


**Fig. 4** Calibration plot of the nomogram closing the 45-degree line.

will potentially confirm this association by cumulative study populations. Second, the investigation of *pTERT* mutation was performed only at positions -124 (C228T); *pTERT* mutation at another position may have been missed. However, we used droplet digital polymerase chain reaction assays for detecting the mutations, a technique that has the potential to detect *pTERT* mutations[15,36] specifically. For future research, a multicenter study should be conducted to increase the sample size as well as to confirm the association of *MGMT/pTERT* classification and prognosis in GBMs. Moreover, comparison between nomograms and other prediction tools such as clinical prediction rules[37] or machine learning algorithms[17] should be performed to evaluate their predictive performance.

## Conclusion

The interaction of *MGMT* methylation and *pTERT* mutation was confirmed for predicting prognosis. The results from the present study could help physicians create treatment strategies for GBM patients in real-world situations.

### Note

This research was a part of a retrospective cohort study that will be published elsewhere, whereas this study focused on survival analysis of *MGMT/TERT* GBM classification.

### Ethical Approval

All procedures performed in the study that involved studies involving human participants were following the ethical standards of the institutional and/or national research committee or both and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

### Authors' Contributions

T.T. and S.S. conceived the study and designed the method. S.S., P.T., and K.K. supervised the conduct of the data collection. T.T. undertook recruitment of participating centers and patients and managed the data, including quality control. S.S. and P.T. provided statistical advice on the study design and analyzed the data and T.T. drafted the manuscript, and all authors contributed substantially to its revision. T.T. takes responsibility for the article as a whole.

### Funding

The study was supported by the Faculty of Medicine, Prince of Songkla University (Kho Hong, Thailand; grant no. 61-065-10-1).

 

Conflict of Interest
None declared.

Acknowledgment
The authors would like to offer their special thanks to Associate Professor Paramee Thongsuksai and Dr. Pasuree Sangsupawanich for their advice about manuscript preparation. Furthermore, they also thank Mrs. Wanwisa Maneechay and Ms. Kanda Tongmit for administration about direct sequencing and immunohistochemistry investigation, respectively.

## References

1 Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016;131(06):803–820
2 Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321 (5897):1807–1812
3 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO Classification of Tumors of the Central Nervous System. 4th edition Lyon: IARC Press; 2016
4 Simon M, Hosen I, Gousias K, et al. TERT promoter mutations: a novel independent prognostic factor in primary glioblastomas. Neuro-oncol 2015;17(01):45–52
5 Kim HS, Kwon MJ, Song JH, Kim ES, Kim HY, Min KW. Clinical implications of TERT promoter mutation on IDH mutation and MGMT promoter methylation in diffuse gliomas. Pathol Res Pract 2018;214(06):881–888
6 Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 2000;343(19):1350–1354
7 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
8 Nabors LB, Portnow J, Ahluwalia M, et al. Central nervous system cancers, version 3.2020, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2020;18(11):1537–1570
9 Arita H, Yamasaki K, Matsushita Y, et al. A combination of TERT promoter mutation and MGMT methylation status predicts clinically relevant subgroups of newly diagnosed glioblastomas. Acta Neuropathol Commun 2016;4(01):79
10 Nguyen HN, Lie A, Li T, et al. Human TERT promoter mutation enables survival advantage from MGMT promoter methylation in IDH1 wild-type primary glioblastoma treated by standard chemoradiotherapy. Neuro-oncol 2017;19(03):394–404
11 Purkait S, Mallick S, Sharma V, et al. Prognostic stratification of GBMs using combinatorial assessment of IDH1 mutation, MGMT promoter methylation, and TERT mutation status: experience from a tertiary care center in India. Transl Oncol 2016;9(04):371–376
12 Sasaki T, Fukai J, Kodama Y, et al. Characteristics and outcomes of elderly patients with diffuse gliomas: a multi-institutional cohort study by Kansai molecular diagnosis network for CNS tumors. J Neurooncol 2018;140(02):329–339
13 Tunthanathip T, Sangkhathat S, Tanvejsilp P, Kanjanapradit K. The clinical characteristics and prognostic factors of multiple lesions in glioblastomas. Clin Neurol Neurosurg 2020;195:105891
14 Brigliadori G, Foca F, Dall'Agata M, et al. Defining the cutoff value of MGMT gene promoter methylation and its predictive capacity in glioblastoma. J Neurooncol 2016;128(02):333–339
15 Corless BC, Chang GA, Cooper S, et al. Development of novel mutation-specific droplet digital PCR assays detecting TERT promoter mutations in tumor and plasma samples. J Mol Diagn 2019; 21(02):274–285
16 Tunthanathip T, Udomwitthayaphiban S. Development and validation of a nomogram for predicting the mortality after penetrating traumatic brain injury. Bull Emerg Trauma 2019;7(04): 347–354
17 Tunthanathip T, Sae-Heng S, Oearsakul T, Sakarunchai I, Kaewborisutsakul A, Taweesomboonyat C. Machine learning applications for the prediction of surgical site infection in neurological operations. Neurosurg Focus 2019;47(02):E7
18 Kim Y, Kong L. Estimation of C-index for Cox proportional hazards model with censored biomarker covariate subject to limits of detection. J Biopharm Stat 2015;25(03):459–473
19 Tunthanathip T, Duangsuwan J, Wattanakitrungroj N, Tongman S, Phuenpathom N. Clinical nomogram predicting intracranial injury in pediatric traumatic brain injury. J Pediatr Neurosci 2020;15 (04):409–415
20 Lacroix M, Abi-Said D, Fourney DR, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg 2001;95(02):190–198
21 Tunthanathip T, Mamueang K, Nilbupha N, Maliwan C, Bejrananda T. No association between isocitrate dehydrogenase 1 mutation and increased survival of glioblastoma: a meta-analysis. PNR 2020;11(01):1–8
22 Stupp R, Mason WP, van den Bent MJ, et al; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352(10):987–996
23 Tunthanathip T, Sangkhathat S. Temozolomide for patients with wild-type isocitrate dehydrogenase (IDH) 1 glioblastoma using propensity score matching. Clin Neurol Neurosurg 2020; 191:105712
24 Wu B, Miao Y, Bai Y, et al. Subgroup economic analysis for glioblastoma in a health resource-limited setting. PLoS One 2012;7(04):e34588
25 Tunthanathip T, Ratanalert S, Sae-Heng S, et al. Prognostic factors and nomogram predicting survival in diffuse astrocytoma. J Neurosci Rural Pract 2020;11(01):135–143
26 Mongkornwong A, Sangthong R, Tunthanathip T, Sangkhathat S. Factors associated with in-hospital mortality in severe burn patients in Songklanagarind hospital: a retrospective study. J Health Sci Med Res 2021;x:401–410
27 Taweesomboonyat T, Kaewborisutsakul A, Tunthanathip T, Saeheng S, Oearsakul T. Necessity of in-hospital neurological observation for mild traumatic brain injury patients with negative computed tomography brain scans. J Health Sci Med Res 2020; 38:267–274
28 Devin CJ, Bydon M, Alvi MA, et al. A predictive model and nomogram for predicting return to work at 3 months after cervical spine surgery: an analysis from the quality outcomes database. Neurosurg Focus 2018;45(05):E9
29 Schully SD, Benedicto CB, Gillanders EM, Wang SS, Khoury MJ. Translational research in cancer genetics: the road less traveled. Public Health Genomics 2011;14(01):1–8
30 Institute for Clinical and Translational Research. What are the T0 to T4 Research Classifications? [internet]. 2021. [cited 2021 May 5]. Accessed June 23, 2021 at: https://ictr.wisc.edu/what-are-the-t0-to-t4-research-classifications/
31 Sam SS, Thomas V, Sivagnanam K, Reddy KS, Surianarayanan G, Chandrasekaran A. ABCB1 genetic polymorphism and risk of upper aerodigestive tract cancers among smokers, tobacco chewers and alcoholics in an Indian population. Pharmacogenet Genomics 2007;17(10):861–866
32 Steyerberg EW, Moons KG, van der Windt DA, et al; PROGRESS Group. Prognosis Research Strategy (PROGRESS) 3: prognostic model research. PLoS Med 2013;10(02):e1001381
33 Wallace E, Smith SM, Perera-Salazar R, et al; International Diagnostic and Prognosis Prediction (IDAPP) group. Framework for the

 

impact analysis and implementation of Clinical Prediction Rules (CPRs). BMC Med Inform Decis Mak 2011;11:62

34 Han K, Song K, Choi BW. How to develop, validate, and compare clinical prediction models involving radiological parameters: study design and statistical methods. Korean J Radiol 2016;17(03):339–350

35 Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. Stat Med 2011;30(10):1105–1117

36 Hosen MI, Forey N, Durand G, et al. Development of sensitive droplet digital PCR assays for detecting urinary *TERT* promoter mutations as non-invasive biomarkers for detection of urothelial cancer. Cancers (Basel) 2020;12(12):3541

37 Beattie P, Nelson R. Clinical prediction rules: what are they and what do they tell us? Aust J Physiother 2006;52(03):157–163

 