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Differences in Clinical Outcomes Based on Molecular Markers In Glioblastoma Patients Treated With Concurrent Tumor-Treating Fields and Chemoradiation: Exploratory Analysis of the SPARE Trial

Louis Cappelli
Thomas Jefferson University

Mehak Majid Khan
Thomas Jefferson University

Allison Kayne
Thomas Jefferson University

Spencer Poiset
Thomas Jefferson University

Ryan Miller
Thomas Jefferson University

See next page for additional authors

Original Article

Differences in clinical outcomes based on molecular markers in glioblastoma patients treated with concurrent tumor-treating fields and chemoradiation: exploratory analysis of the SPARE trial

Louis Cappelli[1], Mehak Majid Khan[2], Allison Kayne[1], Spencer Poiset[1], Ryan Miller[1], Ayesha Ali[1], Muneeb Niazi[1], Wenyin Shi[1], Iyad Alnahhas[2]

[1]Department of Radiation Oncology, Thomas Jefferson University Hospital, Philadelphia, PA, USA; [2]Department of Neurology, Thomas Jefferson University Hospital, Philadelphia, PA, USA

Contributions: (I) Conception and design: L Cappelli, W Shi, I Alnahhas; (II) Administrative support: W Shi, I Alnahhas; (III) Provision of study materials or patients: W Shi, I Alnahhas; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Iyad Alnahhas, MD, MSc. Department of Neurology, Thomas Jefferson University Hospital, 901 Walnut St., Room 310G, Philadelphia, PA 19107, USA. Email: iyad.alnahhas@jefferson.edu.

Background: Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. Despite enormous research efforts, GBM remains a deadly disease. The standard-of-care treatment for patients with newly diagnosed with GBM as per the National Cancer Comprehensive Cancer Network (NCCN) is maximal safe surgical resection followed by concurrent chemoradiation and maintenance temozolomide (TMZ) with adjuvant tumor treating fields (TTF). TTF is a non-pharmacological intervention that delivers low-intensity, intermediate frequency alternating electric fields that arrests cell proliferation by disrupting the mitotic spindle. TTF have been shown in a large clinical trial to improve patient outcomes when added to radiation and chemotherapy. The SPARE trail (Scalp-sparing radiation with concurrent temozolomide and tumor treating fields) evaluated adding TTF concomitantly to radiation and chemotherapy.

Methods: This study is an exploratory analysis of the SPARE trial looking at the prognostic significance of common GBM molecular alterations, namely *MGMT*, *EGFR*, *TP53*, *PTEN* and telomerase reverse transcriptase (*TERT*), in this cohort of patients treated with concomitant TTF with radiation and chemotherapy.

Results: As expected, *MGMT* promoter methylation was associated with improved overall survival (OS) and progression-free survival (PFS) in this cohort. In addition, *TERT* promoter mutation was associated with improved OS and PFS in this cohort as well.

Conclusions: Leveraging the molecular characterization of GBM alongside advancing treatments such as chemoradiation with TTF presents a new opportunity to improve precision oncology and outcomes for GBM patients.

Keywords: Glioblastoma (GBM); tumor-treating fields; O6-methylguanine-DNA methyltransferase (*MGMT*); telomerase reverse transcriptase (*TERT*)

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Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults, with a median survival of 14–24 months (1,2). The standard-of-care treatment for patients newly diagnosed with GBM is maximal safe surgical resection followed by concurrent chemoradiation and maintenance temozolomide (TMZ) with adjuvant tumor treating fields (TTF). The combination of concurrent chemoradiation with TMZ followed by maintenance TMZ and TTF in the adjuvant setting has significantly improved overall survival (OS) and progression-free survival (PFS) in this patient population (3).

Tumor-treating fields is a non-pharmacological intervention that delivers low-intensity, intermediate frequency alternating electric fields that arrests cell proliferation by disrupting the mitotic spindle, leading to the disintegration of proliferating cells (4). These effects have been shown to reduce tumor growth *in vitro*, *in vivo* animal models, and in human cancer patients (5). Standard parameters for maximal effective management of GBM with TTF have been established to include 1–3 V/cm intensity and 200 kHz frequency (3). Recent advances in radiation treatment techniques, including scalp sparing methodology and modified computed tomography (CT) simulation workflow, have allowed the use of concurrent TTF and radiation (6,7). The therapeutic benefit of this approach is now an area of active investigation in the TRIDENT trial (NCT04471844).

The molecular classification of gliomas is rapidly evolving. The 2016 World Health Organization (WHO) classification of brain tumors, and more recently, the 2021 WHO classification incorporated molecular information into the diagnosis and subtyping of gliomas (8). This molecular information is relevant not only for diagnosis, but also for prognostication. It is becoming increasingly essential to take the molecular information into account when designing and interpreting the results of clinical trials in GBM (9). An example to pathology advances that may have future impact on personalized medicine approaches and clinical trial design includes the use of whole genome methylation testing and classification as a predictor of response to radiation and chemotherapy as well as novel treatment options (10).

In adults diagnosed with GBM, methylation of the O6-methylguanine-DNA methyltransferase (*MGMT*) gene promoter is appreciated in about 45% of patients. In patients receiving TMZ chemotherapy, *MGMT* methylation is associated with more favorable clinical outcomes. Lack of *MGMT* promoter methylation is associated with resistance to TMZ chemotherapy (11). Epidermal growth factor receptor (*EGFR*) is also frequently altered in GBM. *EGFR* alternations are represented as an amplification in approximately 40% of patients, an overexpression in about 60%, and mutated in 24–67% of patients diagnosed with GBM (12).

Additionally, mutations in the telomerase reverse transcriptase (*TERT*) gene occur in the majority of GBMs (13) and have been associated with poor survival (14-16). Moreover, based on the updated WHO guidelines for the diagnosis of brain tumors (8), *TERT* promoter mutations upgrade IDH-wild-type astrocytoma to grade IV. Other genes commonly mutated in cancer, such as *TP53* and *PTEN*, are also frequently mutated in GBM (12,17), but their prognostic interpretation is less clear.

Leveraging the molecular identities of GBMs alongside advancing treatments such as chemoradiation with TTF presents a new opportunity to improve the precision and, ideally, the efficacy of GBM treatment. It is unknown how the cellular effects caused by chemoradiation and TTF interact with the already complex genomic alterations occurring in GBM. If GBMs with specific mutations are better targeted with specific treatment protocols, identifying these associations could inform future therapeutic decisions and improve outcomes. This is the first study aimed to identify if any known genomic alterations are associated with improved clinical outcomes in GBM patients when treated with concurrent chemoradiation and TTF. This was performed via a secondary analysis of the SPARE trial (Scalp-sparing radiation with concurrent temozolomide and tumor treating fields; NCT03477110).

Highlight box

Key findings

- *TERT* promoter mutation is associated with favorable overall-survival and progression-free survival in a cohort of glioblastoma patients who received concurrent tumor-treating fields with radiation and chemotherapy.

What is known and what is new?

- *MGMT* promoter methylation is a known favorable prognostic marker in glioblastoma.
- Little is known about molecular prognostic markers associated with tumor-treating fields.

What is the implication, and what should change now?

- Further studies and validation are needed to solidify the role of *TERT* promoter mutation in the tumor-treating fields' mechanism of action.

Methods

This study was designed as a secondary analysis of the SPARE trial, in which 30 enrolled patients aged ≥18 years old and with Karnofsky performance status (KPS) ≥60 received chemoradiation and concurrent TTF for newly diagnosed GBM. Each patient had histologically-confirmed IDH-wildtype GBM and received concurrent chemoradiation and TTF followed maintenance TMZ and TTF. One patient with IDH-mutant GBM was excluded from analysis. We looked at mutations in *PTEN*, *TP53*, *EGFR*, and *TERT*, along with *MGMT* hypermethylation status were evaluated and analyzed for any association with favorable or unfavorable clinical outcomes, including OS and PFS.

Molecular data from the enrolled patients was collected from genomic profiles following surgical resection or biopsy of GBM tumor specimens at Thomas Jefferson University (TJU) Hospital between 5/2018–10/2020. Molecular profile data was assessed with next-generation sequencing. The brain tumor gene sequencing panel at TJU provides comprehensive detection of somatic mutations, including single nucleotide variants and small insertion/deletions up to 30 bp, in 38 genes that have been linked to gliomas and other central nervous system tumors. It is performed on DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue. DNA sequencing is performed using the custom VariantPlex TJH Brain panel from Archer Dx, which uses anchored multiplex polymerase chain reaction (PCR) to amplify regions of interest in 38 genes. Amplicons are sequenced on an Illumina NextSeq next-generation sequencer. The panel does not cover complete coding regions of all 38 genes but is intended to include mutation-prone (hotspot) regions. *MGMT* methylation status was determined by high-resolution melting.

The medical records of each patient on trial were reviewed. Information on tumor pathology, treatment, and survival outcomes was compiled and de-identified. OS was defined as the time from diagnosis until death from any cause. PFS was defined as the time from diagnosis until confirmed disease progression on imaging.

Mutations in *PTEN*, *TP53*, *EGFR*, and *TERT* genes, along with *MGMT* hypermethylation were evaluated for any association with positive or negative clinical outcomes of OS and PFS in newly diagnosed GBM patients receiving concurrent chemoradiation and TTF using a Cox proportional hazards single variable and multivariable backward model that also included age. Event was defined as death from any reason for OS and tumor progression for PFS. Patients who were alive at the end of the trial reporting period were censored for both analyses. Statistics were performed using SPSS version 15.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Review Board of Thomas Jefferson University Hospital (No. FWA 00002109) and informed consent was taken from all individual participants.

Results

A total of 30 patients were enrolled in the SPARE trial, a single-arm pilot study demonstrating the safety and feasibility of concurrent TTF and chemoradiation for newly diagnosed GBM (7). All patients received chemoradiation with concurrent TTF, including 20 (66.7%) men and 10 (33.3%) women. A total of 12 (40%) patients received a gross total resection and 18 (60%) patients had a subtotal resection. A total of 12 (40%) patients had multifocal disease at presentation. The median age was 58 (range, 19–77) years old. The median KPS was 90 (range, 70–100). Nine patients (31.0%) were found to have methylated *MGMT* promoter, 14 patients (48.3%) had a *PTEN* mutation, 9 patients (31.0%) had *EGFR* mutation or amplification, 7 patients (24.1%) had *TP53* mutation, and 23 patients (79.3%) had *TERT* mutation.

Median follow-up was 15.2 (range, 1.7–23.6) months. A total of 27 (90%) patients had progression, with a median PFS of 9.3 [95% confidence interval (CI): 8.5–11.6] months. The 1-year PFS was 23% (95% CI: 12–45%). The OS was 15.8 months (95% CI: 12.5–infinity). The 1-year OS was 66% (95% CI: 51–86%).

No molecular markers showed OS or PFS significance in univariate Cox models. In a multivariable Cox backward model, a statistically significant improvement in OS was associated with *MGMT* promoter methylation as expected [P=0.032; hazard ratio (HR) =7.18] (*Figure 1A*). Additionally, a *TERT* promoter mutation was associated with improved OS (P=0.012; HR =7.60) (*Figure 1B*). *MGMT* promoter methylation and *TERT* promoter methylation also had favorable effects on PFS (P=0.001; HR =13.86 and P=0.003; HR =17.55, respectively). On the other hand, PFS was worse for patients with *TP53* or *EGFR* alterations (P=0.007; HR =0.16 and P=0.001; HR =0.73, respectively).

Discussion

The use of TTF in the concurrent treatment setting

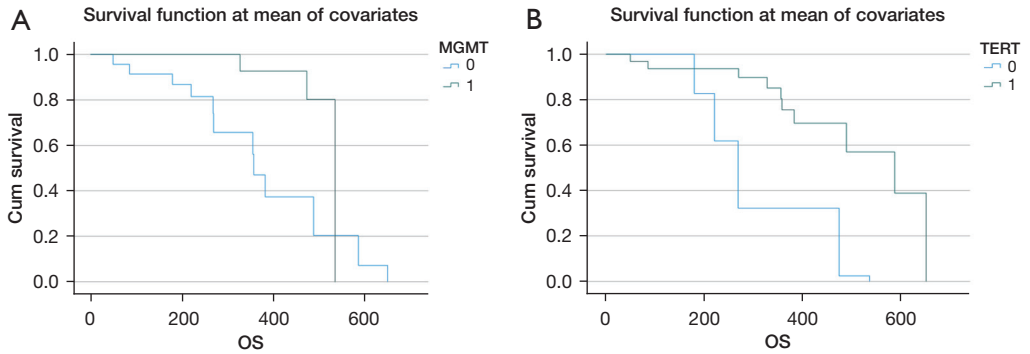

Figure 1 Survival graphs per *MGMT* and *TERT* statuses. (A) Kaplan-Meier survival curve showing better OS with methylated *MGMT* promoter (P=0.032; HR =7.18). (B) Kaplan-Meier survival curve showing better OS with *TERT* promoter mutation (P=0.012; HR =7.60). Cum, cumulative; *MGMT*, O6-methylguanine-DNA methyltransferase; OS, overall survival; *TERT*, telomerase reverse transcriptase; HR, hazard ratio.

in patients newly diagnosed with GBM is still being investigated for clinical benefit. Despite this ongoing investigation, molecular markers in patients diagnosed with GBM have been proven to confer positive and negative clinical outcomes depending on the genetic profile. This study looked to evaluate the prognostic value of the molecular markers *MGMT*, *TERT*, *EGFR*, *TP53*, or *PTEN* in the concurrent TTF treatment setting.

Tumor-treating fields are approved and included in NCCN treatment guidelines for the management of patients with newly diagnosed and recurrent GBM. Currently, there is level 1 evidence supporting the use of TTF in the adjuvant care setting following maximal safe resection and concurrent chemoradiation. Numerous factors, however, limit the utilization of TTF in patients being treated for GBM, including the burden of device use and the cost of the device. The identification of molecular markers in patients that are candidates for TTF therapy may play a role as a decision-making tool to stress the importance or value of TTF use in patients diagnosed with GBM. Although it should be mentioned, TTF use is still not widely accepted as standard of care for primary treatment of GBM internationally and not a part of the European Association of Neuro-Oncology treatment guidelines.

Treatment with TTF is generally well tolerated in the known adjuvant setting with minimal to no added side effects from the device experienced by patients. The most common side effect associated with TTF use is dermatitis to the scalp secondary to the extended contact with the adhesive used to attached the device. It could be expected that with increased duration of TTF use like in the concurrent chemoradiation setting, patients may be at increased risk of experiencing such side effect.

The data presented demonstrates that in patients with newly diagnosed GBM treated with chemoradiation and concurrent TTF following maximal safe resection or biopsy, the presence of a *TERT* mutation is associated with significantly improved OS. However, mutations in *EGFR*, *TP53*, or *PTEN* were not associated with a change in OS.

An increased OS in the presence of *MGMT* promotor methylation was also observed. This is attributed to the well-known prognostic benefit that TMZ confers to the management of GBM in the setting of *MGMT* methylation.

A recent study by Pandey *et al.* examined how genomic alterations found in resected GBM tumors affected OS (18). In this study, 55 patients underwent standard chemoradiation with adjuvant TTF and 57 patients received standard chemoradiation without TTF. It was found that PFS and OS is improved when combining standard chemoradiation and TTF in the setting of mutations in NF1, wildtype PIK3CA, and wildtype *EGFR*. In our study, we found that *EGFR* alterations were associated with worse PFS in this cohort of patients.

Our study findings need to be validated in larger studies. This study was an exploratory analysis of a small phase I trial and therefore has intrinsic limitations. In this cohort of patients treated with TTF concurrently, *TERT* promoter mutation was associated with improved OS and PFS in this cohort as well. *TERT* encodes telomerase reverse transcriptase, an enzyme responsible for repairing telomeres to retain their length, which assists in the ability of cancer cells to avoid death. Mutations in the promotor region of

TERT have been well-described and result in increased *TERT* expression that promotes cellular immortalization in cancer (19). *TERT* promoter mutations occur in numerous types of cancer and up to 80% of GBM (13).

The prognostic significance of *TERT* promoter mutation in GBM is debatable. *TERT* mutations have been associated with poor prognosis in GBM patients (14,15), whereas other studies found no prognostic relevance when tumor grade, and other genomic alterations are considered (20-22). Two large series looked at the survival significance of *TERT* promoter mutations in *MGMT* methylated and unmethylated IDHwt GBM separately and found conflicting evidence of interaction between the two markers (23,24). One previous study examined the prognostic significance of *TERT* promoter mutation in the setting of rs2853669 polymorphism in the blood and tumors of GBM patients. *TERT* promoters were associated with poor survival but only in patients who did not have the polymorphism (18). These findings highlight the necessity of clarifying how factors from oncogenic mutations, polymorphisms, and treatment regimens interact to influence tumor pathology and patient survival.

This study only examined patients that received standard chemoradiation with concurrent TTF after surgical resection, followed by maintenance TMZ with TTF. Therefore, our observations cannot be applied to patients treated with different regimens. Comparison groups of patients receiving standard chemoradiation without TTF would be needed to draw further conclusions, especially in relation to previous studies. An additional limitation is the relatively small sample size of 30 patients. Given the diverse molecular landscape of GBM, studying large patient populations will be needed to extract prognostically significant interactions.

Conclusions

In this secondary analysis of the SPARE trial, patients with *MGMT* methylation showed statistically significant improved PFS and OS as expected. Patients with a *TERT* promoter mutation demonstrated improved OS in patients who received concurrent TTF with chemoradiation. A *TERT* promoter mutation may therefore be a new molecular biomarker in patients diagnosed with GBM receiving the described treatment approach. Due to the small sample size, further validation studies should be conducted to evaluate the prognostic value of a *TERT* mutation as a molecular biomarker. It should also be understood that this is an exploratory analysis of a phase I trial, and conclusions from this study were extracted outside of the initial endpoint of TTF feasibility.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Review Board of Thomas Jefferson University Hospital (No. FWA 00002109) and informed consent was taken from all individual participants.

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