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The effects of tumor treating fields and temozolomide in MGMT expressing and non-expressing patient-derived glioblastoma cells

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ABSTRACT

A recent Phase 3 study of newly diagnosed glioblastoma (GBM) demonstrated the addition of tumor treating fields (TTFields) to temozolomide (TMZ) after combined radiation/TMZ significantly increased survival and progression free survival. Preliminary data suggested benefit with both methylated and unmethylated O-6-methylguanine-DNA methyl-transferase (MGMT) promoter status. To date, however, there have been no studies to address the potential interactions of TTFields and TMZ. Thus, the effects of TTFields and TMZ were studied *in vitro* using patient-derived GBM stem-like cells (GSCs) including MGMT expressing (TMZ resistant: 12.1 and 22 GSC) and non-MGMT expressing (TMZ sensitive: 33 and 114 GSC) lines. Dose-response curves were constructed using cell proliferation and sphere-forming assays. Results demonstrated a $\geqslant$10-fold increase in TMZ resistance of MGMT-expressing (12.1 GSCs: IC_{50} = 160 μM; 22 GSCs: IC_{50} = 44 μM) compared to MGMT non-expressing (33 GSCs: IC_{50} = 1.5 μM; 114 GSCs: IC_{50} = 5.2 μM) lines. TTFields inhibited 12.1 GSC proliferation at all tested doses (50–500 kHz) with an optimal frequency of 200 kHz. At 200 kHz, TTFields inhibited proliferation and tumor sphere formation of both MGMT GSC subtypes at comparable levels (12.1 GSC: 74 ± 2.9% and 38 ± 3.2%, respectively; 22 GSC: 61 ± 11% and 38 ± 2.6%, respectively; 33 GSC: 56 ± 9.5% and 60 ± 7.1%, respectively; 114 GSC: 79 ± 3.5% and 41 ± 4.3%, respectively). In combination, TTFields (200 kHz) and TMZ showed an additive anti-neoplastic effect with equal efficacy for TTFields in both cell types (i.e., ± MGMT expression) with no effect on TMZ resistance. This is the first demonstration of the effects of TTFields on cancer stem cells. The expansion of such studies may have clinical implications.

1. Introduction

In 2005, Stupp et al. defined the standard of care for newly diagnosed glioblastoma (GBM) after maximal surgery as temozolomide (TMZ) combined with radiation, followed by a post-radiation 'adjuvant' phase of temozolomide administration [1]. First reported by Hegi et al. MGMT promoter methylation in GBM patients is now well established as a positive prognostic factor, and a marker for sensitivity and benefit for TMZ administration [2]. Methylation of MGMT promoters inhibits MGMT expression, thereby enhancing tumor sensitivity to TMZ; whereas unmethylated MGMT promoters permit MGMT expression and lead to relative tumor resistance to TMZ [2,3]. What is now less defined is the benefit of TMZ to patients with unmethylated MGMT tumors. Until recently, it was common clinical practice to offer TMZ to unmethylated MGMT promoter status GBM patients because a reported ~8% of this patient cohort survived long term at 5 years [4]. In the past two years, however, the value of TMZ in GBM patients with unmethylated MGMT promoter has come into question due to the recognition of false negative MGMT evaluation (e.g., GBM falsely classified as unmethylated MGMT status are actually MGMT methylated and TMZ sensitive) [5,6]. Indeed, since there is low perceived TMZ benefit and an overall poor prognosis for the vast majority of the

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MGMT-unmethylated cohort, it is now acceptable to omit TMZ in clinical trials of therapies for MGMT-unmethylated GBM patients [7,8].

Since that advance, a new technology which delivers alternating low intensity, intermediate-frequency, tumor-treating electric fields (TTFields) to the patients' brain via noninvasive transducer arrays attached to the scalp has been developed and introduced into GBM clinical practice [9]. The preclinical rationale is TTFields' ability to inhibit cell growth and induce cell death in a wide range of tumor models [10,11]. Mechanistically, TTFields disrupt mitotic spindle formation during metaphase-to-anaphase transition and cause dielectrophoretic movement of charged or polar molecules and organelles during anaphase and telophase, resulting in mitotic arrest and apoptosis [12–15]. Other laboratory studies have started to address the potential interaction between TTFields and chemotherapy [16,17].

The first approved use of TTFields was for recurrent GBM [18]. More recently, a Phase 3 clinical study demonstrated that addition of 200 kHz tumor treating fields (TTFields) to adjuvant temozolomide (TMZ) for newly diagnosed glioblastoma (GBM) increases both progression free survival (PFS) and overall survival (OS) [19]. On this basis, the U.S. Food and Drug Administration approved TTFields' use in newly diagnosed GBM. Preliminary data (i.e., a Forest Plot) concerning both O-6-methylguanine DNA-methyltransferase (MGMT) promoter methylated and unmethylated GBM patient cohorts (presented at the American Society of Clinical Oncology – 2015) suggested benefit in both patient groups [20].

Testing a positive interaction between TTFields and TMZ is not readily feasible in the clinical setting. However, there are significant clinical implications for the potential interactions between TTFields and TMZ, particularly for MGMT-unmethylated GBM patients (as a subset of the overall large cohort of TMZ resistant GBM). Indeed, Kirson et al. have speculated that TTFields may act as a TMZ sensitizer and by implication serve to overcome resistance [16]. Therefore, we decided to test the hypothesis that TTFields might exhibit a synergistic, or supra-additive interaction with TMZ, particularly in MGMT protein-expressing GBM cells that are resistant to TMZ (modeling MGMT-unmethylated GBM patients). The *in vitro* analysis of the effects of TTFields and/or TMZ on MGMT protein expressing (representing unmethylated MGMT promotor) and non-expressing (representing methylated MGMT promoter) patient-derived glioblastoma stem cell lines are described in this report.

2. Materials and methods

2.1. Cell lines and culture

Glioblastoma stem-like cells (GSCs) were derived directly from patient specimens anonymously obtained from the operating room, under a protocol approved by the University of Wisconsin-Madison Health Sciences Institutional Review Board (IRB), in surgeries for primary GBM (lines 22, 33, 114) or recurrent GBM (line 12.1). Each cell line was authenticated for stem cell-like properties by validating self-renewal (sphere formation), expression of stem cell markers (CD133, nestin), multi-lineage differentiation, and high efficiency orthotopic *in vivo* tumor initiation in immunodeficient NOD-SCID mice. Briefly, GSCs were enriched from patient specimens and cultured under marker neutral conditions as previously described [21,22]. GSCs were propagated as spheres in suspension culture in serum-free stem cell medium termed 'Passaging Medium, 20′ [PM20: 70% DMEM-high glucose, 30% Ham's F12, 1 × B27 supplement, 5 μg/ml heparin, 1% antibiotics and 20 ng/ml each epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF)], and passaged approximately every 10–21 days by tissue chopping 2× at 200 μm [23]. Cells were maintained at 37 °C with 5% CO_2. Patient-derived GSC lines 12.1 (passages 20–35), 22 (passages 25–40), 33 (passages 20–35), and 114 (passages 5–15) were used in this study.

2.2. Cell proliferation assays

GSC spheres were enzymatically dissociated to single cells using Accutase (Millipore) and counted manually using a hemacytometer. Then 50,000 cells in a 100 μl drop of PM20 medium were plated on a laminin coated plastic coverslip (Thermanox, Thermo Fisher Scientific), and placed in a 6-well tissue culture plate. GSCs were allowed to adhere overnight prior to filling wells with PM20 medium. Coverslips were transferred into ceramic petri dishes using sterile forceps, and TTFields were applied using the *Inovitro* system (Novocure Ltd., Haifa, Israel) [13,24]. Briefly, the *Inovitro* system used two pairs of transducer arrays printed perpendicularly on the outer walls of a petri dish composed of high dielectric constant ceramic [lead magnesium niobate–lead titanate (PMN-PT)]. The transducer arrays were connected to a sinusoidal waveform generator that generated treating fields at the desired frequencies ranging from 50 to 500 kHz. The orientation of the TTFields switched 90° every second, thus covering the majority of the orientation axis of cell divisions, as previously described [10]. For all experiments, cells were treated with TTFields at approximately 1 V/cm intensity. TTFields experiments were performed in a refrigerated incubator (170R Galaxy, New Brunswick) set at 18 °C to dissipate heat generated by TTFields administration, while cells were maintained at 37 °C by *Inovitro* system using feedback from 2 thermistors (Omega Engineering, Stamford, CT) attached to the ceramic walls. TTFields were applied for 8 days, determined as optimal duration from preliminary GSC proliferation curve experiments (data not shown). Culture medium was replaced daily. At the end of experiments, GSCs were enzymatically detached using Accutase and manually counted using a hemacytometer.

Parallel TMZ dose–response experiments were performed in 12-well tissue culture dishes (Corning) coated with laminin. Preliminary experiments verified no difference in GSC proliferation rate between coverslips and tissue culture dishes (data not shown).

2.3. GSC sphere-forming assays

Plating densities resulting in visible, near-clonogenic sphere proliferation for 12.1, 22, 33, and 114 GSC lines were first optimized. To establish TMZ dose response curves, GSC spheres were enzymatically dissociated to single cells and re-plated in 96-well plates at optimal density (500–1000 cells) in PM20 medium. After recovery overnight, test TMZ doses or vehicle control (0.1% DMSO) was added to establish a dose–response curve. Upon formation of ≈200 μm diameter spheres in control groups (2–4 weeks), the total number of tumor spheres was manually counted in each culture well. The relative 50% inhibitory concentrations (IC_{50}) of temozolomide were calculated from sphere-forming assays by 4-parameter logistic model [25].

For TTFields experiments, GSCs were assayed for proliferation as described above after exposure to TTFields ± TMZ, enzymatically removed from coverslips, and live cells re-plated at optimal density in PM20 medium for sphere-forming assay. No additional TTFields or TMZ were administered.

2.4. MGMT RT-PCR

Expression of the MGMT DNA repair enzyme was analyzed in GSCs using reverse transcription polymerase chain reaction (RT-PCR). RNA was isolated from stably growing GSCs, per

manufacturer's instructions (RNeasy kit, Qiagen), and cDNA created from 1 μg RNA using reverse transcription for 1 h at 37 °C (Omniscript RT kit, Qiagen) with oligo(dT) primers ($Oligo(dT)_{12-18}$ primer, Invitrogen/Life Technologies). PCR was then performed (Taq PCR Kit, Qiagen) for MGMT, with GAPDH as loading control, using specific primers: MGMT: F, 5′-GGA GGC ACC GCT GTA TTA AA-3′ and R, 5′-GCA GGT AGG AAA CAA AGC TAG A-3′, product size 483 bp; GAPDH: F, 5′-ACC ACAGTC CAT GCC ATC AC-3′ and R, 5′-TCC ACC ACC CTG TTG CTG TA-3′, product size 452 bp. PCR thermocycling parameters included initial denaturation for 3 min at 94 °C; 30 total cycles of denaturation 1 min at 94 °C, annealing for 1 min at 55 °C, extension for 1 min at 72 °C; and a final extension step of 7 min at 72 °C. PCR products were then combined with loading buffer (Promega) and electrophoresed in a 2% agarose gel (Agarose-HR, Ambion) containing 1 μg/ml ethidium bromide. Finally, DNA bands were visualized using an automated imaging system set to image 600 nm wavelength (Odyssey Fc, LI-COR Biosciences). Product sizes were verified using a known molecular weight ladder run in parallel (exACTGene Low Range DNA ladder, Fisher Scientific).

2.5. Statistical analysis

The data were expressed as mean ± standard error mean (SEM). Statistical analysis was performed with Statistical Product and Service Solutions (SPSS version 22, IBM). Two sample comparisons were made using student *T*-tests. Group comparisons were made using analysis of variance (ANOVA) after verification of normal distribution, followed by post hoc Tukey tests. The criterion for statistical significance was chosen as $p < 0.05$.

3. Results

3.1. Patient-derived GSCs vary in MGMT expression and temozolomide sensitivity

Patient-derived GSC lines were analyzed for MGMT expression using RT-PCR. MGMT RNA expression was detected in 12.1 and 22 GSCs, but was absent/minimal in 33 and 114 GSCs (Fig. 1A). The effect of temozolomide on the multiple patient-derived GSC lines was then tested, and differential GSC responses (sensitivity/resistance) corresponding to MGMT expression were observed (Fig. 1B, $n \geqslant 3$ independent experimental replicates). The IC_{50} for each cell line was derived: 12.1 GSC = 160 μM, 22 GSC = 44 μM, 33 GSC = 1.5 μM, and 114 GSC = 5.2 μM. Thus, as expected, the MGMT-expressing 12.1 and 22 GSC lines (which would clinically correspond to unmethylated MGMT GBM) were relatively resistant to TMZ, and the 33 and 114 GSC lines that do not express MGMT (which would clinically correspond to methylated MGMT GBM) were relatively sensitive to TMZ.

3.2. TTFields inhibition of GSC proliferation

The response of GSCs to TTFields was then analyzed and a frequency response curve was constructed using 12.1 GSCs. This analysis demonstrated maximal GSC proliferation inhibition at 200 kHz TTFields (Fig. 2A, $n \geqslant 3$ independent experimental replicates). TTFields effects on 12.1 and 22 GSC lines (TMZ resistant) and 33 and 114 GSC lines (TMZ sensitive) were determined. Using 200 kHz TTFields, significant GSC proliferation and sphere formation inhibition were measured (Figs. 2B, 2C; $n \geqslant 3$ independent experimental replicates, $p < 0.05$). Cell proliferation inhibition (mean ± S.E.) by TTFields was similar among 12.1 GSCs (74 ± 2.9%), 22 GSCs (61 ± 11%,), 33 GSCs (56 ± 9.5%), and 114 GSCs (79 ± 3.5%). Tumor sphere formation (mean ± S.E.) was also inhibited similarly among 12.1 GSCs (38 ± 3.2%,), 22 GSCs (38 ± 2.6%), 33 GSCs (60 ± 7.1%), and 114 GSCs (41 ± 4.3%).

3.3. TTFields provide additive effect when combined with TMZ

Combination studies to examine interactions between TTFields and TMZ were also performed. The addition of 200 kHz TTFields provided additional inhibitory effects at all doses of TMZ tested for both cell proliferation and sphere-formation of 12.1 and 22 GSCs (MGMT expressing) (Fig. 3A, $n \geqslant 3$ independent experimental replicates) and 33 and 114 GSCs (non-MGMT expressing) (Fig. 3B, $n \geqslant 3$ independent experimental replicates). Equal slopes of TMZ dose response curves with and without 200 kHz TTFields suggested an additive, but not supra-additive or synergistic effect resulting from combining the two treatments.

4. Discussion

The introduction of TTFields to the treatment of newly diagnosed GBM represents the first significant advance since the introduction of TMZ in 2005 [1]. The apparent clinical efficacy in both methylated and unmethylated MGMT promoter GBM [19,26] led to the American Food and Drug Administration approval for the use of TTFields specifically in conjunction with adjuvant TMZ. It is also notable that newly released 2016 National Comprehensive

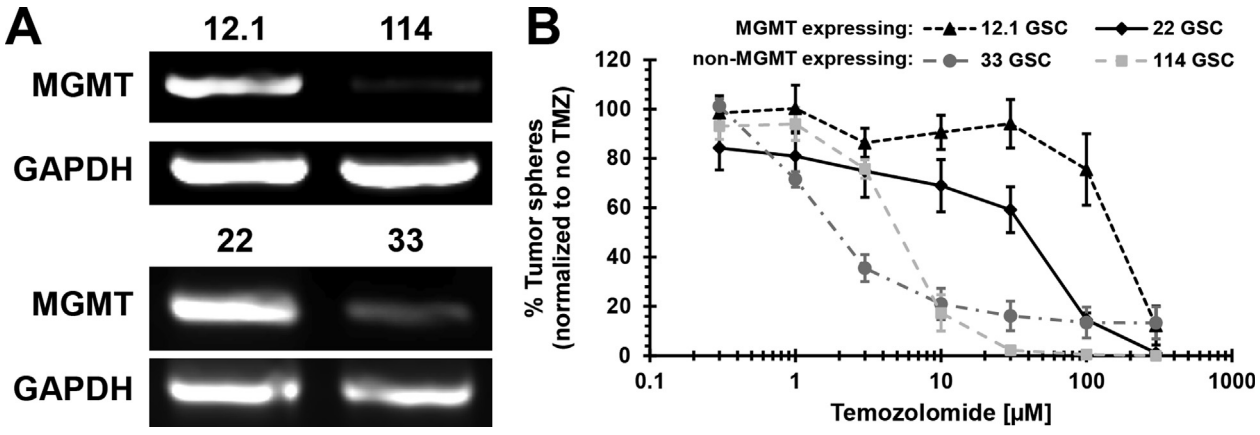

Fig. 1. (A). MGMT expression in patient-derived glioblastoma stem-like cell (GSC) lines determined by RT-PCR. GAPDH RT-PCR serves as RNA loading control. (B) TMZ dose–response curves for GSC lines (mean ± S.E., $n \geqslant 3$ independent experiments). In a tumor sphere forming assay, MGMT expressing 12.1 and 22 GSCs are relatively resistant to TMZ (IC_{50} = 160 μM and 44 μM, respectively) compared to non-MGMT-expressing 33 and 114 GSCs (IC_{50} = 1.5 μM and 5.2 μM, respectively).

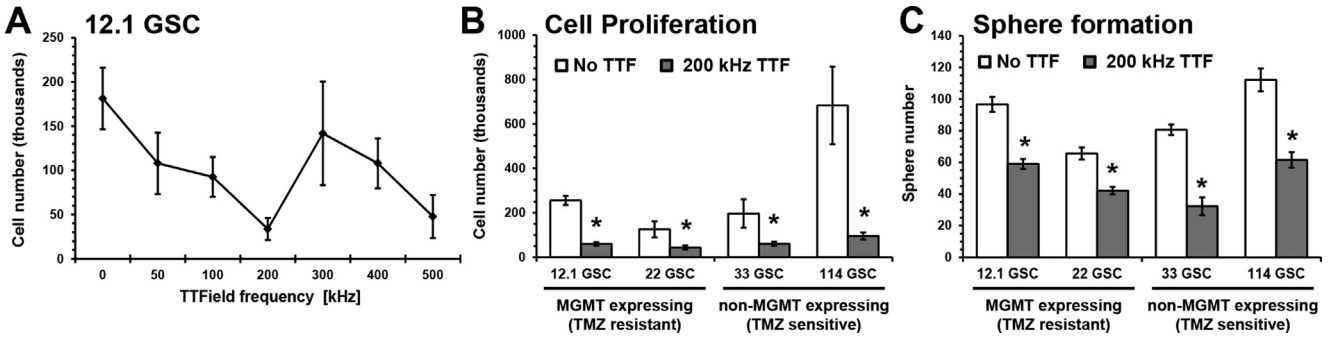

Fig. 2. Tumor-treating Fields (TTFields) inhibit GSC proliferation and tumor sphere formation. Frequency–response plot of GSCs treated with TTFields show optimal inhibition at 200 kHz (A), mean ± S.E., $n \geqslant 3$ independent experimental replicates. TTFields at 200 kHz (gray bars) significantly inhibited cell proliferation (B) and tumor sphere formation (C) compared to untreated controls (white bars) in both MGMT-expressing 12.1 and 22 GSCs (TMZ resistant) and non-MGMT-expressing 33 and 114 GSCs (TMZ sensitive) (mean ± S.E., $n \geqslant 3$ independent experimental replicates, $^{*}p < 0.05$ compared to no TTFields by Student's t-test).

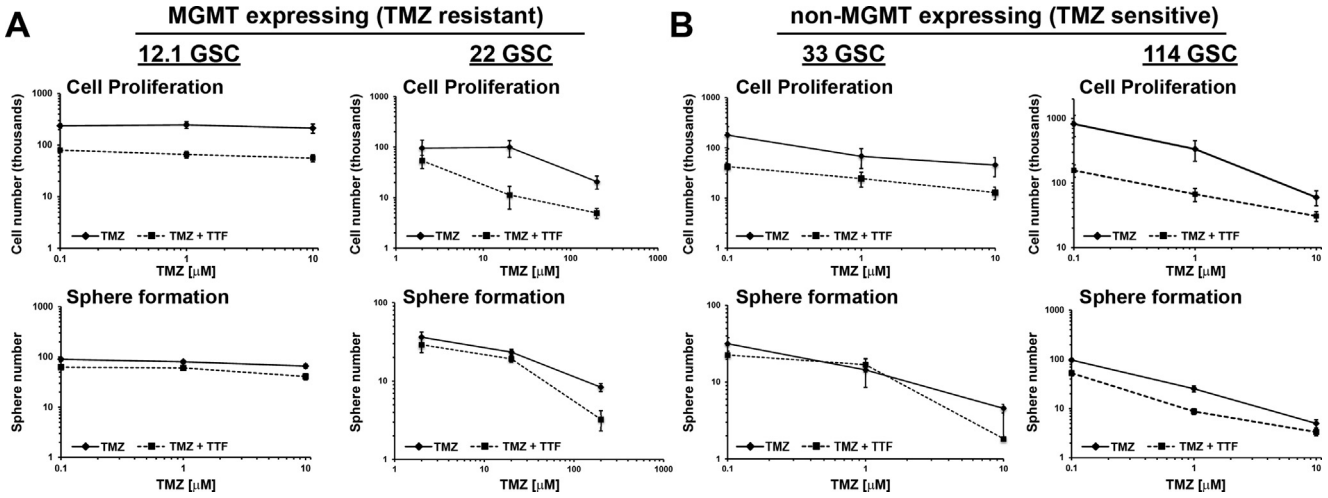

Fig. 3. Anti-neoplastic effects of TTFields and TMZ were additive. In MGMT expressing (TMZ resistant) (A) and non-MGMT expressing (TMZ sensitive) (B) GSCs, additional treatment with 200 kHz TTFields inhibited cell proliferation and sphere formation more than TMZ alone, at all tested doses (mean ± S.E., $n \geqslant 3$ independent experimental replicates).

Cancer Network (NCCN) revised guidelines, recognizing the significance of TMZ resistance in non-methylated GBM patient's, offers the option of radiation treatment only (which is not a stated option for MGMT methylated GBM cohorts) versus radiation with TMZ with or without TTFields. However, radiation alone with TTFields is not an option even in a non-methalyated MGMT patient population. What is not clear from the clinical data now available, however, is whether there is an interaction between TTFields and TMZ. An important corollary question is whether adjuvant TMZ is necessary to elicit the full benefit of TTFields in non-methylated MGMT GBM patients.

To address this question preclinically, patient-derived GSCs were chosen for *in vitro* studies on the basis of their highly efficient tumor initiation capacity and evidence that they are more genetically similar to *in vivo* patient GBM and applicable for clinically useful biomarker discovery [22,27–29]. To the best of our knowledge this is the first study to report the effects of TTFields on cancer stem cells. The results presented here demonstrate equal efficacy for TTFields in both types of GBM cells (both ± MGMT expression), consistent with the reported observation of clinical benefit in both patient populations. There was no indication of an interaction with TMZ. A possible clinical implication of these data is that adjuvant treatment omitting TMZ and only using TTFields therapy post radiation is predicted to be efficacious against GBMs with unmethylated MGMT promoters.

Interestingly, the optimal 200 kHz frequency for TTFields inhibition (hypothesized to partly depend on tumor cell size) that was empirically determined in our study, is also the clinically used frequency for treating GBM patients [19]. It is also notable that our studies demonstrate the existence of two separate frequencies leading to maximal response, i.e., 200 kHz and 500 kHz (Fig. 2). There are two physical phenomena which underlie the effect of TTFields: dielectrophoresis which occurs mainly during cytokinesis, and dipole alignment. Electric field simulations have demonstrated that the frequencies expected to cause maximal dielectrophoretic forces during cytokinesis are within the range of 100–200 kHz, while the maximal effect on dipole alignment is expected to appear at higher frequencies (towards 1 MHz) [30]. It is therefore possible that the biphasic nature of the observed response is the outcome of dielectrophoresis induced at 200 kHz, and dipole alignment occurring at higher frequencies. An alternative explanation could be the result of heterogeneous cancer stem cell plasticity and aberrant differentiation resulting in multiple populations with different characteristics. It is possible that the different GSC populations are susceptible to the effects of two distinct frequencies, and that the biphasic response is the cumulative effects on the different cells.

Our studies demonstrating a lack of supra-additive or synergistic effect in combining TTFields with TMZ against GSC of either MGMT status were not entirely unexpected. Mechanistically,

TTFields have been shown to interfere with the spindle tubulin polymerization by generating non-uniform intracellular fields that exert forces that move polar macromolecules and organelles, resulting in apoptosis driven cell death [11]. In contrast, TMZ's mechanism of action is based on its ability to alkylate/methylate DNA rather than disrupting mitosis [2,7]. However, drugs that interfere with mitosis, e.g., paclitaxel, might be predicted to synergize with TTFields; indeed, preliminary data regarding TTFields and paclitaxel suggest that possibility [16].

The expansion of these studies has clinical implications specifically for patients with unmethylated GBM. Since the efficacy of TMZ in MGMT unmethylated patients is considered by many to be minimal [5–7] (possibly reflecting inadequate MGMT marker testing), clinical researchers may consider testing TTFields in combination with other agents as adjuvant GBM therapy. We are now performing additional preclinical investigations with TTFields that include assessment of TTFields' effects on additional patient-derived tumor lines derived from GBM and other cancers. Analysis of the potential interactions in combining ionizing irradiation and TTFields, and/or blood brain barrier-permeable drugs that might mechanistically synergize with TTFields (e.g., inhibit mitosis) are also in progress. In summary, our *in vitro* data does not provide evidence for combining TTFields therapy with TMZ in unmethylated GBM patients, but does support the use of TTFields alone as adjuvant therapy in this patient subset. This work highlights the need for continued laboratory and clinical research for this unique and emerging therapy.

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Conflict of interest

H.I.R. served on a Novocure Advisory Board in 2012.

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References

[1] Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987–96.
[2] Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997–1003.
[3] Friedman HS, McLendon RE, Kerby T, et al. DNA mismatch repair and O6-alkylguanine-DNA alkyltransferase analysis and response to Temodal in newly diagnosed malignant glioma. J Clin Oncol 1998;16:3851–7.
[4] Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:459–66.
[5] Hegi ME, Stupp R. Withholding temozolomide in glioblastoma patients with unmethylated MGMT promoter–still a dilemma? Neuro Oncol 2015;17:1425–7.
[6] Shah N, Schroeder B, Cobbs C. MGMT methylation in glioblastoma: tale of the tail. Neuro Oncol 2015;17:167–8.
[7] Wick W, Weller M, van den Bent M, et al. MGMT testing–the challenges for biomarker-based glioma treatment. Nat Rev Neurol 2014;10:372–85.
[8] Wick W, Steinbach JP, Platten M, et al. Enzastaurin before and concomitant with radiation therapy, followed by enzastaurin maintenance therapy, in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation. Neuro Oncol 2013;15:1405–12.
[9] Fonkem E, Wong ET. NovoTTF-100A: a new treatment modality for recurrent glioblastoma. Expert Rev Neurother 2012;12:895–9.
[10] Kirson ED, Dbaly V, Tovarys F, et al. Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. Proc Natl Acad Sci U S A 2007;104:10152–7.
[11] Kirson ED, Gurvich Z, Schneiderman R, et al. Disruption of cancer cell replication by alternating electric fields. Cancer Res 2004;64:3288–95.
[12] Gutin PH, Wong ET. Noninvasive application of alternating electric fields in glioblastoma: a fourth cancer treatment modality. Am Soc Clin Oncol Educ Book 2012:126–31.
[13] Giladi M, Schneiderman RS, Voloshin T, et al. Mitotic spindle disruption by alternating electric fields leads to improper chromosome segregation and mitotic catastrophe in cancer cells. Sci Rep 2015;5:18046.
[14] Pless M, Weinberg U. Tumor treating fields: concept, evidence and future. Expert Opin Investig Drugs 2011;20:1099–106.
[15] Gera N, Yang A, Holtzman TS, et al. Tumor treating fields perturb the localization of septins and cause aberrant mitotic exit. PLoS One 2015;10:e0125269.
[16] Kirson ED, Schneiderman RS, Dbaly V, et al. Chemotherapeutic treatment efficacy and sensitivity are increased by adjuvant alternating electric fields (TTFields). BMC Med Phys 2009;9:1.
[17] Schneiderman RS, Shmueli E, Kirson ED, et al. TTFields alone and in combination with chemotherapeutic agents effectively reduce the viability of MDR cell sub-lines that over-express ABC transporters. BMC Cancer 2010;10:229.
[18] Stupp R, Wong ET, Kanner AA, et al. NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modality. Eur J Cancer 2012;48:2192–202.
[19] Stupp R, Taillibert S, Kanner AA, et al. Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: a randomized clinical trial. JAMA 2015;314:2535–43.
[20] Stupp R, Taillibert S, Kanner A, et al. Tumor treating fields (TTFields): A novel treatment modality added to standard chemo- and radiotherapy in newly diagnosed glioblastoma—First report of the full dataset of the EF14 randomized phase III trial. J Clin Oncol 2015;33(suppl). abstr 2000.
[21] Clark PA, Iida M, Treisman DM, et al. Activation of multiple ERBB family receptors mediates glioblastoma cancer stem-like cell resistance to EGFR-targeted inhibition. Neoplasia 2012;14:420–8.
[22] Zorniak M, Clark PA, Leeper HE, et al. Differential expression of 2′,3′-cyclic-nucleotide 3′-phosphodiesterase and neural lineage markers correlate with glioblastoma xenograft infiltration and patient survival. Clin Cancer Res 2012;18:3628–36.
[23] Svendsen CN, ter Borg MG, Armstrong RJ, et al. A new method for the rapid and long term growth of human neural precursor cells. J Neurosci Methods 1998;85:141–52.
[24] Giladi M, Weinberg U, Schneiderman RS, et al. Alternating electric fields (tumor-treating fields therapy) can improve chemotherapy treatment efficacy in non-small cell lung cancer both in vitro and in vivo. Semin Oncol 2014;41 (Suppl 6):S35–41.
[25] Sebaugh JL. Guidelines for accurate EC50/IC50 estimation. Pharm Stat 2011;10:128–34.
[26] Stupp R, Wong E, Scott C. Investigators eaftE-T. Interim analysis of the EF-14 trial: a prospective, multi-center trial of NovoTTF-100A together with temozolomide compared to temozolomide alone in patients with newly diagnosed GBM. Neuro Oncol 2014;16:167.
[27] Pointer KB, Clark PA, Zorniak M, et al. Glioblastoma cancer stem cells: Biomarker and therapeutic advances. Neurochem Int 2014;71:1–7.
[28] Zorniak M, Clark PA, Kuo JS. Myelin-forming cell-specific cadherin-19 is a marker for minimally infiltrative glioblastoma stem-like cells. J Neurosurg 2015;122:69–77.
[29] Lee J, Kotliarova S, Kotliarov Y, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006;9:391–403.
[30] Wenger C, Salvador R, Basser PJ, et al. The electric field distribution in the brain during TTFields therapy and its dependence on tissue dielectric properties and anatomy: a computational study. Phys Med Biol 2015;60:7339–57.