Tumor treating field therapy in non-MGMT-Methylated newly diagnosed glioblastoma: is there a role for temozolomide?

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Since 2005, the standard of care for newly diagnosed glioblastoma (GBM) has been maximal surgery followed by radiation in combination with the alkylating agent temozolomide (TMZ), with an ‘adjuvant’ 6 to 12-month phase of TMZ post radiation [1]. At the time of this paradigm shift in practice, Hegi et al. recognized that O-6-methylguanine-DNA methyl-transferase (MGMT) promoter methylation in GBM patients was a positive prognostic factor, and a biomarker for sensitivity and benefit for TMZ administration [2]. Specifically, methylation of MGMT promoters inhibits MGMT expression, thereby enhancing tumor sensitivity to TMZ; whereas unmethylated MGMT promoters permit MGMT expression and leads to relative tumor resistance to TMZ [2, 3].

The benefit of TMZ in MGMT-methylated GBM is now universally accepted. What is for many less defined is the role of TMZ in treating GBMs with unmethylated MGMT promoters. Until recently it was common clinical practice to offer TMZ to unmethylated MGMT promoter status GBM patients because ~8% of this patient population reportedly experience prolonged survival up to 5 years [4]. Recently, however, the clinical value of TMZ in unmethylated MGMT GBM patients has become more controversial. This relates to a heightened awareness concerning the accuracy of MGMT methylation status, i.e., tumors reported as unmethylated may...
In fact be MGMT methylated and hence TMZ sensitive \([5, 6]\).

In recognition of this false negative phenomena, as well as the overall poor prognosis of the vast majority of unmethylated MGMT GBM patients, it is now acceptable to omit TMZ in clinical trials of therapies for MGMT-unmethylated GBM patient cohorts \([7, 8]\).

Since the TMZ advance over a decade ago, a new technology has been developed and refined: A system of 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 successfully introduced into GBM clinical practice (see Figure 1) \([9]\). The preclinical rationale for TTFields resides in its 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 \([12-15]\). As might be expected, preclinical studies have begun to address the possible interaction between TTFields and chemotherapy \([16, 17]\). TTFields therapy was first approved for recurrent GBM \([18]\). Later, a Phase 3 study in newly diagnosed GBM patients demonstrated that the addition of TTFields (200 kHz) to post-radiation TMZ increased both progression free survival (PFS) and overall survival (OS) \([19]\) which led to U.S. Food and Drug Administration approval. The design of this study required that both methylated and unmethylated MGMT patient populations receive TMZ. A recent update of these data via a Forest Plot at the Society of Neuro-Oncology showed benefit for both MGMT methylation status cohorts \([20]\).

Following the FDA approval of TTFields therapy, National Comprehensive Cancer Network (NCCN) guidelines (Version 1.2016) were updated to include 3 options: (1) TMZ and radiation recommended for MGMT-methylated GBM. (2) Radiation treatment, or radiation with TMZ in unmethylated GBM patients (recognizing the significance of TMZ resistance in unmethylated MGMT patients). (3) Radiation and TMZ followed by adjuvant TMZ plus TTFields for both unmethylated and methylated GBM patients.

The NCCN requirement of TMZ in option 3) for unmethylated GBM (as was the case for the FDA approval) reflects the design of the aforementioned phase 3 study \([19]\) requiring TMZ for both unmethylated and methylated GBM patients. Perhaps implicit in this requirement was the assumption/hope that there might be a positive interaction between TMZ and TTFields. Indeed, Kirson et al. had speculated that TTFields may act as a TMZ sensitizer, and by implication serve to overcome TMZ resistance \([16]\). Relative to this, there is no way to discern from the clinical data accrued to date, whether there is an interaction between TTFields or whether adjuvant TMZ is necessary to elicit the full benefit of TTFields in unmethylated MGMT GBM patients. Unfortunately, testing a positive interaction between TTFields and TMZ is not practical or feasible in the clinical setting, and is very unlikely to be pursued due to financial implications.
Thus, 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 unmethylated MGMT GBM patients), investigators at the University of Wisconsin elected to address this question with an in vitro model (see figure 2).

In view of the clinical relevance of these recently published results [21], the review below summarizes these preclinical experiments:

### Publication Summary [21]

The effects of TTFields and TMZ were studied in vitro using 2 different sets of patient-derived GBM stem-like cells (GSCs) including MGMT expressing (TMZ resistant) GSC and non-MGMT expressing (TMZ sensitive) lines. Dose-response curves were constructed using cell proliferation and sphere-forming assays. Results demonstrated a ≥10-fold increase in TMZ resistance of MGMT-expressing lines. TTFields inhibited GSC proliferation at all tested doses (50-500 kHz) with an optimal frequency of 200 kHz, which is notably the same frequency used clinically. At 200 kHz, TTFields inhibited proliferation and tumor sphere formation of both MGMT GSC subtypes at comparable levels. In combination, TTFields (200 kHz) and TMZ showed an additive anti-neoplastic effect with equal TTFields efficacy in both cell types (i.e., +/- MGMT expression) with no effect on TMZ resistance.

### Commentary

Of note, this report represents the first in vitro demonstration of the effect of TTFields on cancer stem cells, and as such may have clinical implications, and worthy of special consideration. Cancer recurrence is hypothesized due to therapeutically resistant cancer stem cells, and continues to be an area of focus for many investigators.

The results of Clark et al. [21] failing to demonstrate a synergistic effect between the alkylating agent TMZ, although significant, is not surprising. From a mechanistic standpoint, TTFields were shown to interfere with the spindle tubulin polymerization as a consequence of generating non-uniform intracellular fields that exert forces that move organelles and polar macromolecules, resulting in apoptotic cell death [11]. Mechanistically TMZ’s cytotoxicity is mediated via DNA alkylation/methylation rather than mitotic disruption [2, 7]. Alternatively, drugs that interfere with mitosis, e.g., paclitaxel, are predicted to potentially synergize with TTFields. Interestingly, preliminary data regarding TTFields and paclitaxel suggest that possibility [16]. Apropos to future preclinical research, there are taxanes that cross the blood brain barrier, (e.g., carbazitaxel, TPI-287, ANG 1005), and might predictably synergize with TTFields.

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Abbreviations

TTFields: tumor treating field; TMZ: temozolomide; GBM: glioblastoma; GCS, GBM stem-like cells; MGMT: O-6-methylguanine-DNA methyl-transferase; NCCN: National Comprehensive Cancer Network; PFS: progression-free survival; OS: overall survival.

References

17. Schneiderman RS, Shmueli E, Kirson ED, Palti Y. 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.