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**REVIEW PAPER**

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# An overview of the preclinical and clinical studies of the effects of tumor treating fields on malignant glioma cells

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**ABSTRACT**

Anaplastic astrocytoma (AA, WHO grade III) and glioblastoma multiforme (GBM, WHO grade IV) are malignant tumors of the brain. The average survival time of patients with GMB is approximately one year and two years in the case of anaplastic astrocytoma with standard therapy based on surgical tumor resection followed by chemotherapy or radiotherapy. High invasiveness of gliomas, the ability of rapid division and so-called diffusive infiltration of tumor cells into normal brain tissue, which prevents complete surgical removal, are hallmarks of theses tumors. Therefore, new specific therapies for eliminating cancer cells are needed to treat this tumors. Recently, it has been demonstrated that alternating electric field, also known as tumor treating fields (TTFields) has a unique mechanism of destroying glioma cells. TTFields applies electromagnetic energy frequency-dependent and intensity-dependent and disrupts cancer cell replication as they undergo mitosis. Futhermore, TTFields turn out to act comparably to conventional chemotherapeutics, lacking numerous side adverse associated with chemotherapy. The authors provide an up-to-date review of the mechanism of action as well as preclinical and clinical data on TTFields.

**Keywords**. anaplastic astrocytoma, glioblastoma multiforme, brain tumor, tumor treating fields, tumor therapy

## Introduction

The term malignant gliomas comprises WHO grade III tumors (e.g. anaplastic forms of astrocytomas, oligodendroglioma, and oligoastrocytoma) and WHO grade IV tumors, such as glioblastoma multiforme (GBM). Glioblastoma multiforme is the most frequent and the most devastating primary malignant glioma. Most patients diagnosed with a GBM survive less than a year despite intensive treatment, which may include maximal safe surgical resection, radiation and chemotherapy. The prognosis for patients with anaplastic astrocytoma (grade III) is somewhat better. Due to the slower growth of cancer, the average survival time is about 2 years. The hallmarks of gliomas include diffusive invasion into normal brain tissues, high proliferation rate, aggressive growth pattern and microvascular proliferation.[1] From the molecular stand-

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point, several signaling pathways responsible for regulation, proliferation, differentiation and survival have been found to be differentially activated or silenced, hence gliomas typically do not respond to currently available therapies and what is worse is most of the therapeutic options have been exhausted.[2-4] Therefore, development of new methods for gliomas treatment are particulary important. Recently much attention in this regard is paid to alternating electric fields referred to here as Tumor Treating Fields (TTFields), currently widely discussed in terms of cell biology effects, physical properties and clinical trial data. Compared with historic cancer treatment modalities, TTFields have an innovative mechanism of action, and more importantly do not have sufficient energy to induce mutagenic damage to DNA and cannot cause the cellular damage usually associated with cancer initiation.[5] A series of publications present experimental studies conducted for potential genotoxicity of electric and magnetic fields which have shown negative results provided strong support for this view.[6-7] The biological effects of TTFields were first observed during in vitro experiments by analyzing the values of the electric field that affect proliferation and viability of cancer cells in culture. These experiments revealed a tight range of cytotoxic effect, inducing prolonged or completely arrested mitotic phase of treated cells leading to cell death, generated by TTFields at a frequency range between 150 and 200 kHz, and was not observed at frequencies <50 kHz and >500 kHz. Furthermore, these analysis allowed also to observed that the effect of TTFields is also dose-dependent and the inhibitory effect of TTFields starts at 1 V/cm and increases with increasing intensity of the field. According to the ability of electric field to kill cancer cells mentioned above, a pioneering technology has been developed, described and referred as Tumor Treating Fields.[8-10]

## Molecular Targets of TTFields

In order to analyze a mechanism of action of TTFields, a systematic review of the literature data was performed. Exposure of multiple cancer cell lines, e.g. glioma, lung, prostate, breast, to TTFields reveals exertion of a strong growth inhibitory effect by inducing cell cycle arrest and in consequence, apoptosis, while no effect was induced on non-dividing cells.[8-13] Disruption of cells by TTFields during mitosis suggest that they exert forces or movement on definable molecular targets, the functions of which are critical to a mitotic process or processes.[14] Cells treated with TTFields exhibited a variety of abnormalities indicative of mitotic catastrophe and aberrant mitotic exit, including cells in polyploidy prophase, rosettes, multi-spindled metaphase, single-spindled metaphase, and asymmetric anaphase.[8] Indeed, cells exhibit violent membrane blebbing as they enter anaphase and attempt to divide which results in aberrant mitotic exit and subsequent cell death *in vitro*.[15] The inhibitory effect of TTFields of proliferation inhibition is largely manifested in malfunction in the mitotic spindle apparatus. That is why molecular targets of TTFields includes proteins characterized by high dipole moments such mitotic septin complex and the α/β-tubulin monomeric subunit of microtubules.[8,14-16] The dipole moments of such proteins will align within an electric field to orient towards the oppositely charged pole of the fields. Therefore, the re-polarization of the alternating field will induce a re-alignment of the protein dipoles within the field. Thus, such proteins would be expected to experience rotational forces within TTFields. α/β-Tubulin form the building blocks for microtubules. The functional subunit of microtubules is a heterodimer consisting of α- and β-tubulin, which possesses a high predicted dipole moment of 1660 Debyes (D).[17-19] Therefore, it is possible that TTFields interfere with a critical mitotic function performed by microtubules, including the formation of the metaphase and anaphase spindles and their respective mechanical functions, or the astral microtubules that help regulate the cytokinetic cleavage furrow (CCF).[8,9,15,20,21] Septins, in particularly septin 2, 6 and 7, characterized by an extremely large dipole moment of 2711 Debyes, oligomerize into a heterotrimer and is active in mitosis.[8,19] The main function of this complex is to regulate contractile function within the cytokinetic furrow, and it is likely to provide tensile strength needed within the submembranosus cortical cytoskeleton to restrain the hydrostatic pressure within the cytoplasm during cell division. Once it is recruited, it then oligomerizes and organizes contractile elements within the cytokinetic furrow above the equatorial cleavage plane by binding to F-actin filaments and spatially regulates myosin activation. The perturbation of the septin complex is particularly enticing because of its known roles in the regulation of CCF function and actin bundle cross-linking and organization of structures such as the cellular submembranous actin cytoskeleton that is required for its rigidity.[22,23] Short hairpin RNA-driven depletion of septin 7 of the heterotrimer results in mitotic bleebing similar to that seen when cells attempt to divide in the presence of TTFields.[8,23] Therefore, perturbation of either α/β-Tubulin or septins may perturb microtubule function.[15,24] These observations strongly suggest a mechanism of action were TTFields perturb mitosis by interfering with normal septin localization and function during mitosis, leading to membrane blebbing and aberrant mitotic exit.

## Implications of TTFields in therapy

A series of publications provided evidence supporting preclinical studies pointing at the applicability range of TTFields in a various of in vitro and in vivo cancer models, either alone or in combination with standard chemotherapy.[9,25] Animal models of various tumors, including i.a. glioblastoma, non–small cell lung cancer, pancreatic cancer, and

malignant melanoma confirmed the inhibition of tumor growth and moreover metastatic seeding when TTFields were delivered externally at the appropriate frequencies.[16] TTFields were administered to the animal organisms by using a noninvasive single electrically insulated transducer array located on the head or torso surrounding the region of the tumor. As an example, an experimental model of rats with intracranially inoculated GBM cells treated with TTFields at a frequency of 200 kHz over 6 days showed smaller tumors in comparison to untreated animals. The inhibitory effect was significantly increased when at least two or three directional fields were delivered.[9,10,25] Importantly, synergistic antitumor activity was discovered when TTFields were applied in combination with commonly used chemotherapeutic agents such paclitaxel, doxorubicin, cyclophosphamide, or dacarbazine; the sensitivity to chemotherapy was increased one-fold to three-fold by adjuvant TTFields. Hence, TTFields may acts as an animitotic agent and a chemotherapy sensitizing agent.[19,26]

In October 2015, the FDA approved TTFields for use in newly diagnosed GBM patients. To date, two crucial randomized clinical trials for the safety and efficiacy of TTFields therapy have been reported. In patients with recurrent glioblastoma, currently indicated by FDA (U.S. Food & Drug Administration) as the only one for the TTFields therapy, the trial has not demonstrated improved outcome compared with best physicians choice chemotherapy (BPC). However, when TTFields were delivered as a part of the initial treatment in newly diagnosed patients a consistent prolongation of progression-free survival (PFS) and overall survival (OS) has been noted. From the other point of view, TTFields applied in early stage of disease allows for prolonged exposure, and moreover synergy with Temozolomide (TMZ), a standard chemotherapeutic agent commonly used in glioblastoma therapy, observed in vitro may further enhance its efficacy. The average treatment time of patients with recurrent disease was only 2.3 months compared with 9 months in the case of patients with newly diagnosed GBM. Still, TTFields alone in recurrent disease have shown objective responses in 14% of patients, consistent or even numerically higher than that observed in other trials using chemotherapeutic agent Lomustine[27,28] or Temozolomide.[29] It is worth noting that the best results with this novel treatment modality have been achieved when TTFields were administered in the early stage of disease in combination with standard maintenance TMZ therapy[30], similar to that shown 10 years ago when TMZ was combined with standard radiotherapy. The very important issue is to ensure an adequate treatment effect; the values of TTFields intensity and frequency must be adapted to the tumor type and cell properties. The optimal frequency to maximize the antitumor effect is inversely correlated with cell size and when the incident angle of the electrical field is perpendicular to the mitotic plate. As the cell division may occur at any time, prolonged exposure to the electrical fields is required for maximal effect. For the precise delivery of TTFields, a special portable and battery-powered device has been constructed. The electric field is applied to the brain through 4 transducer arrays with 9 insulated electrodes each and continuous temperature sensing fixed to the patient's shaved scalp.[8,26]

## Summary

The ability of TTFields to block the mitotic cell cycle results in cell cycle arrest or delays in cell division and interfere with organelle assembly, particularly the spindle apparatus. The consequences are inadequate cell division and unequal chromosome distribution, and ultimately cell death. The possibility of using this action has become promising. TTFields therapy is currently being tested in gliobalstoma patients and provides more evidence supporting the use of TTFs as an efficacious, antimitotic treatment with minimal toxicity in patients with newly diagnosed and recurrent glioblastoma. Nevertheless, additional studies are needed to further optimize patient selection, determine cost-effectiveness, and assess the full impact on quality of life.[30,31] Moreover, there is a need to integrate this novel TTFields treatment approach with the current standard of care. At this moment, TTFields is the one of the most promising therapeutic methods because of its locoregional action, which allow extension to other types of tumors and metastatic diseases such as brain metastase, ovarian carcinoma, mesothelioma or pancreatic tumors, and trials are currently ongoing.[26] If those trials confirm the positive effects observed in GBM patients, a truly new cancer treatment modality will be born and will find multiple useful indications alone or in combination with other established standard of treatment or new therapy methods.

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