The Mechanisms of Action of Tumor Treating Fields

Justin C. Moser[1,2], Ellaine Salvador[3], Karina Deniz[4], Kenneth Swanson[5], Jack Tuszynski[6], Kristen W. Carlson[7], Narasimha Kumar Karanam[8], Chirag B. Patel[9,10], Michael Story[8], Emil Lou[4], and Carsten Hagemann[3]

ABSTRACT

Tumor treating fields (TTFields), a new modality of cancer treatment, are electric fields transmitted transdermally to tumors. The FDA has approved TTFields for the treatment of glioblastoma multiforme and mesothelioma, and they are currently under study in many other cancer types. While antimitotic effects were the first recognized biological anticancer activity of TTFields, data have shown that tumor treating fields achieve their anticancer effects through multiple mechanisms of action. TTFields therefore have the ability to be useful for many cancer types in combination with many different treatment modalities. Here, we review the current understanding of TTFields and their mechanisms of action.

Introduction

Tumor treating fields (TTFields) are a new, divergent treatment modality for cancer. Originally recognized for their antimitotic effects, it is now appreciated that TTFields exert a variety of effects (**Fig. 1**), making it a useful therapy for a variety of cancers. Here, we review the current understanding of its many biological effects and clinical data.

Characteristics of TTFields

TTFields are 100 – 400 kHz alternating current (AC) electric fields transmitted transdermally to tumors using two orthogonal sets of transducer arrays. Transducer arrays are activated sequentially each second, effecting a direction change of the incident field on the target. The optimal AC frequency for different cancers has been determined experimentally and varies by tumor type, for example, 200 kHz for glioblastoma (GBM; ref. 1). TTFields' effects are sensitive to directionality. Early hypotheses that TTFields' mechanism of action involved polarizable intra-cellular structures and mitotic disruption were later confirmed empirically (1–5), which implies that the amplitude of

[1]HonorHealth Research and Innovation Institute, Scottsdale, Arizona. [2]Department of Medicine, University of Arizona College of Medicine- Phoenix, Phoenix, Arizona. [3]Section Experimental Neurosurgery, Department of Neurosurgery, University of Würzburg, Würzburg, Germany. [4]Department of Medicine, Division of Hematology Oncology and Transplant, University of Minnesota, Minneapolis, Minnesota. [5]Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, Massachusetts. [6]Department of Physics, University of Alberta, Edmonton, Alberta, Canada. [7]Department of Neurosurgery, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, Massachusetts. [8]Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, Texas. [9]Department of Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston Texas. [10]Neuroscience and Cancer Biology Graduate Programs, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences.

Corresponding Author: Justin Moser, HonorHealth Research and Innovation Institute, 10510 N 92nd Street Ste 200, Scottsdale, AZ 85258. Phone: 480-323-4638, E-mail: jmoser@honorhealth.com

TTFields' effect seen by subcellular structures, and hence its efficacy, would depend on the direction of the imposed electric field. Thus, Kirson and colleagues predicted that TTFields' efficacy would increase with a periodic change of the direction imposed on the cellular target, which was confirmed for direction-change intervals of 1 and 2 Hz (1, 2). The strongest TTFields effects occurred when the incident field was aligned with the cell axis during early mitosis, although a lesser, secondary effect was seen with an orthogonal field, indicating two mechanisms of action. No effect was seen when the field was oriented 45° to the cell axis, implying a lack of synergy between the two mechanisms of action. Later work showed that more frequent changes of direction, up to 20 Hz, improved TTFields efficacy as well (6).

Dose–response curves for multiple human cancer lines showed a threshold for significant efficacy of ∼1 V/cm. Since then, recommended TTFields' field strength is often cited as 2 V/cm. However, the dose-response curves show that a field strength over 2 V/cm, for instance ∼2.45 V/cm for brain cancer (**Fig. 2A**), is predicted for 100% efficacy, ∼2.6 V/cm for lung cancer (**Fig. 2B**), nearly 3.5 V/cm for breast cancer (**Fig. 2C**), and 1.4 V/cm for melanoma (**Fig. 2D**). The curve-fits resemble those for linear-quadratic theory in radiation therapy. Assuming cells, organelles, and other structures, are randomly oriented in vivo, 2 orthogonal field direction changes will deliver high field strength to all structures in the 2-dimensional plane of field incidence. However, structures oriented orthogonal to that 2D plane may experience very little effective field strength (e.g., the cosine of 90° is 0). Thus, a third direction change may be necessary for full efficacy.

The electric field distribution of TTFields has been tested in various models. Studies using 3D shaped head models show that although electric field distribution is heterogeneous, large portions of the brain receive a dose greater than 1 V/cm (7). Computational models of the abdomen and thorax have also shown > 1 V/cm of electric field intensity with TTFields (8–10). *In vivo* studies have confirmed >1V/cm deliver of electric fields to the thorax (11).

TTFields' Antimitotic Mechanism of Action

Deregulated cellular proliferation is a hallmark of cancer (12, 13). Therefore, many anticancer agents have been developed to target dividing cells (14). Such agents induce the mitotic spindle assembly checkpoint (SAC), leading to a cell-cycle arrest, often followed by mitotic slippage, and subsequent cell death or senescence (15–17). SAC
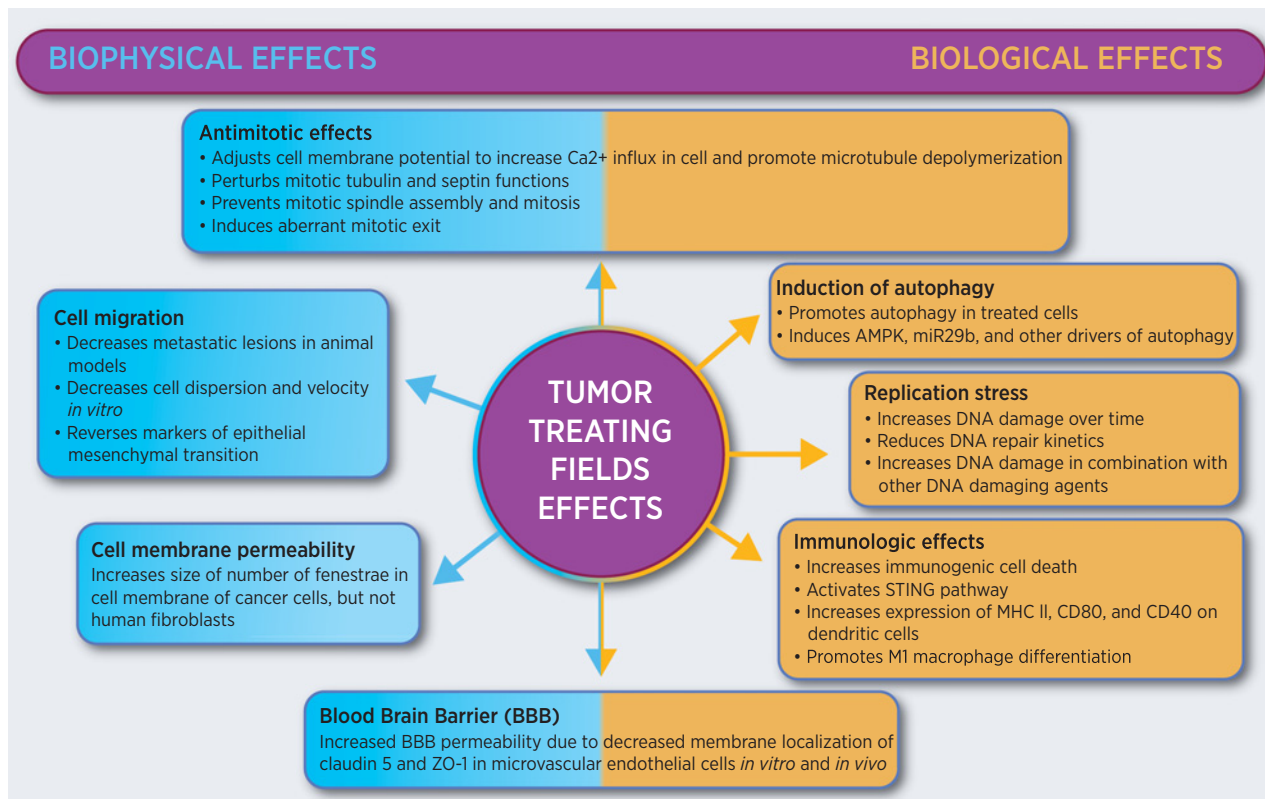
Figure 1.
Biological and biophysical effects of TTFields.

activation may also limit the efficacy of such drugs, by allowing time for spindle repair (18, 19). Indeed, it has been shown that GBM cells can be sensitized to antimitotic drugs by SAC inhibition (20).

Prolonged mitotic phase has been observed in TTField-treated cells, in addition to abnormal chromosome segregation and cellular multinucleation (2–5, 21, 22). In addition, GBM cells can be sensitized to TTFields by SAC inhibition (23). Blocking of MPS1, the key regulator kinase of the SAC, led to earlier and prolonged TTFields effects. The concomitant treatment significantly decreased cell proliferation and reduced the number of viable cells compared to the single treatments (23).

TTFields' effect on telophase/cytokinesis is intensity- and frequency-dependent (24). Attachment of sister chromatids to spindle fibers (9) and stability of tubulin heterodimers are both modulated by TTFields field strength (25). The stability of microtubules is affected by electrical field-induced conformational changes that promote depolymerization (25). Hence, TTFields reduce the ratio between polymerized and total tubulin, preventing proper mitotic spindle assembly (4).

TTFields also disrupt mitotic function (3) and confound cell transition from metaphase to anaphase by dielectrophoretic effects (5). TTFields interact with proteins possessing high dipole moments such as heterotrimeric Septin protein complex, a key protein in positioning the cytokinetic cleavage furrow (26, 27). At anaphase, TTFields inhibit Septin localization to the anaphase spindle midline and cytokinetic furrow. They also hinder Septin association with microtubules during cell attachment and spreading to fibronectin. Altogether, these lead to disordered membrane contraction, failed cytokinesis, mitotic catastrophe and p53-dependent apoptotic cell death after a G_0–G_1 cell cycle block (5). The result of aberrant mitotic division for cells that survive is aneuploid daughter cells due to abnormal and unbalanced chromosome segregation (2–5).

However, these effects are challenged by the biophysical state of the intracellular environment and tubulin properties. It is alternatively suggested that TTFields affect cell membrane potential and ion channels (28), leading to an influx of Ca^{2+} into the cell, reducing microtubule polymerization and thus the observed TTFields-effects during mitosis (29, 30).

Downstream Antimitotic Effects: ER Stress and Autophagy

TTFields exposure can stimulate autophagy in cancer cells. RNA analysis was performed on TTField-treated and control cells; TTFields resulted in >2 fold increase in multiple genes related to autophagy (31). Cellular changes consistent with autophagy have also been noted in TTFields-treated cells (31). In addition, electron microscopy of TTFields treated cells have also shown autophagy type changes such as increased vacuoles, autophagosomes, and mitochondria with swollen matrices and dilated endoplasmic reticulum (32–34). Using GFP tagged LC3 (LC3-GFP) cell lines, it was shown that the induction of autophagy by TTFields is due to replication stress (35). Treatment with TTFields reduced the number of mitotically active cells and increased the expression of LC3-GFP. In addition, daughter cells of TTFields-treated cells showed marked increase in LC3-GFP expression after completion of mitosis, suggesting that TTFields induced autophagy is due to abnormal mitosis of the treated parent cell, as well as the genomic instability in daughter cells.
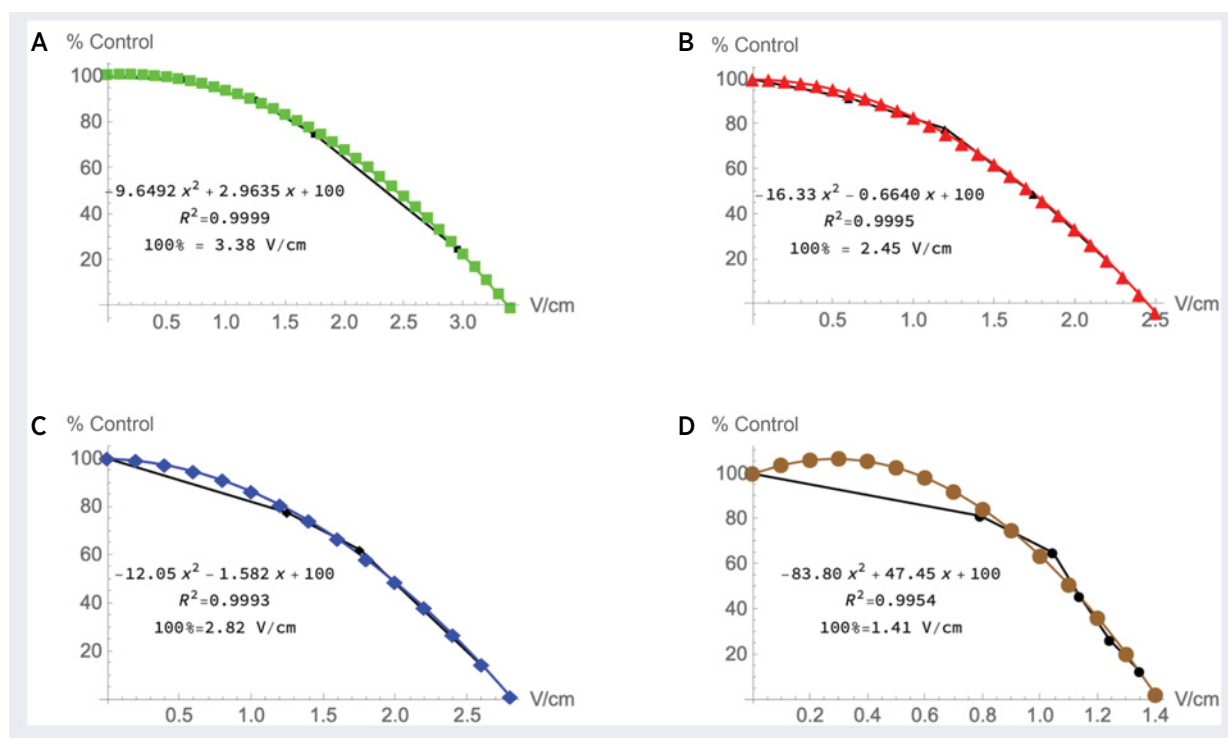
Figure 2.
Dose–response curves (electric field strength vs. % of control) for TTFields applied to four tumor cell types, replicated from Kirson and colleagues (1), curve-fit and extrapolated to show electric field amplitudes predicted to kill 100% of target cells. Quadratic curve-fits show a possible correspondence with the linear-quadratic theory of radiotherapy and similarly, imply two mechanisms of action at low vs. high amplitudes, which may relate to cell cycle phase at which TTFields coincide in asynchronous populations. **A,** Rat glioma. **B,** Human non–small lung cell carcinoma. **C,** Human breast cancer. **D,** Mouse melanoma (poor fit at low amplitudes due to lack of data points compared with the other curves).

Mechanistically, TTFields may induce autophagy via multiple pathways. AMPK, a known positive regulator of autophagy, was significantly upregulated in TTFields-treated cell lines, and silencing of AMPK with siRNA prevented the induction of autophagy in treated cells (35). Studies have also shown that TTFields treatment increases the interaction of Beclin1 with Vps34 or Atg14L, while reducing its interaction with Bcl2 (31). Treatment with 3-methyladenine (3MA) prevented autophagy in TTFields-treated cells. RNA analysis of TTFields-treated cells has shown reduced levels of AKT2, another known regulator of autophagy (31). Further studies revealed that this reduction may be due to the TTFields-induced expression of miR-29b, which acts to inhibit expression of AKT2.

It is currently unknown if autophagy is part of the cytotoxic effects of, or a resistance mechanism to TTFields. Knockdown of Beclin1 or ATG5 improve cell survival during treatment with TTFields (31). Treatment of cells with 3MA has also been shown to attenuate the cytotoxic effects of TTFields (32). However, other studies have shown that preventing autophagy using shATG-7 or chloroquine increased the cytotoxic effects of TTFields (35).

Other Biological Effects: DNA Replication Stress

TTFields have been shown to improve efficacy for medications that cause DNA damage and replication stress. On the basis of this, it was evident that TTFields may play a mechanistic role in DNA damage. TTFields have been shown to decrease the expression of multiple

Fanconi anemia pathway genes (36) in cancer cell lines. TTFields exposure also resulted in increased DNA damage over time and slowed DNA repair kinetics after ionizing radiation (IR). In addition, TTFields alone caused an increase in chromatid type aberrations and the number of γH2AX foci, as well as slowed the repair of IR-induced double-strand breaks. Amassing of γH2AX foci, a marker of DNA damage, has been seen following treatment with TTFields (22). It was proposed that the downregulation of the BRCA1/2 genes expression upon TTFields exposure caused conditional vulnerability, that is, BRCAness (37). It was shown that TTFields delay the repair of DNA damage caused by radiation or chemicals (38). More recently these effects were confirmed in *in vivo* models (39). Based upon IHC results, cisplatin combined with TTFields induced elevated γH2AX foci, suggesting TTFields amplified DNA damage induced by cisplatin (39). Elevated γH2AX foci is a marker of stalled replication forks and may be brought about by TTFields due to reduction in MCM6 and MCM10 (40, 41).

TTFields have also been shown to induce the formation of DNA R-loops, a marker of replication stress (42, 43). This replication stress may contribute to the anticancer effects of TTFields. However, it could also lead to resistance via genomic instability. Prolonged treatment with TTFields has been reported to induce activating mutations in mTOR and/or deep loss of CDKN2A raising the possibility of resistance to TTFields treatment (44).

Because of the absence of DNA replication in noncancerous cells, TTFields have little effect on DNA in normal tissue. This is supported by the lack of toxicity seen in the many clinical trials of TTFields, reviewed below.

Downstream Antimitotic Effects: Immunologic Effects

TTFields may stimulate antitumor immune effects. This was first suggested by a leporine model of abdominally implanted VX -2 tumors. TTFields treatment of abdominal tumors, with limited exposure to the lungs, reduced burden of subsequent metastatic lung disease as compared with controls (45). Metastatic tumors from treated animals also exhibited a higher number of total CD45^{+} cells and increased CD4^{+} and CD8^{+} populations. In addition, the histologic distribution of infiltrating lymphocytes differed between treated and control tumors (45) and was reminiscent of immunologically "hot" (TTField-treated) versus "cold" (control) tumors (46).

TTFields may induce antitumor immunity via multiple mechanisms. TTFields treatment has been shown to increase expression of calreticulin, and the secretion of ATP and high mobility group protein 1 (HMGB1; ref. 33); markers of immunogenic cell death (ICD; ref. 47). One possible driver of this ICD mitotic catastrophe that results in aneuploidy (2, 5, 32). Aneuploid cells exhibit the hallmarks of ICD including the ability to vaccinate mice against tumor formation in syngeneic tumor models (48). ICD can also be stimulated by DNA damage, cell stress responses, and increased reactive oxygen species (47), which have been documented in response to TTFields exposure in cell culture (34, 36, 49). Autophagy may also play a significant role in improving immune recognition and elimination of TTFields-treated tumors (35). Importantly, none of these effects on cells necessarily excluded the other's ability to produce cellular stress that may culminate in stimulating antitumor immunity.

In vivo studies showed that TTFields-treated Lewis lung carcinoma cells activated bone marrow-derived DCs, which exhibited stimulated expression of MCH IIB, CD80, CD40, and increased phagocytosis of the TTFields-treated cells, indicative of DC maturation (33). Similarly, coculturing of murine bone marrow-derived macrophages (BMDM) with previously exposed TTFields-treated CT26 cells resulted in increased expression of proinflammatory activation markers (50). TTFields treatment of *in vitro* M1 and M2 macrophages has also resulted in increased production of proinflammatory cytokines (CXCL1, IL18, IL23, IL12p70, TNFα, IL12p40, CCL22, G-CSF, CCL17, and IL1β) as well as skewing of M2 macrophages to M1 phenotype (51). Together, these data suggest the intriguing possibility that TTFields-damaged cells stimulate immune responses and possibly promote adaptive immune response against tumor neoantigens.

TTFields-exposed T cells retain interferon gamma secretion, cytotoxic degranulation, and antigen-directed cell cytotoxicity (52); however, exposure to TTFields reduces viability of activated but not nonproliferating, naïve T-cell cultures. Interestingly, there were significant increases in the numbers of infiltrating lymphocytes with signs of activation in gliomas from newly diagnosed patients following TTFields + standard chemoradiation (52).

A recent study revealed that TTFields-induced mitotic catastrophe results in the formation of micronuclei that possess weak nuclear envelopes, resulting in DNA release into the cytoplasm thereby activating the cGAS/STING and AIM2 DNA detection pathways (53). TTFields were shown to drive the expression of inflammatory mediators including IL1, IL6, IL8, type I interferons, and CXLC10, but this effect was absent when both STING and AIM2 were knocked down. Importantly, when TTFields-treated murine glioma cells were orthotopically implanted into syngeneic mice they provoked antitumor immunity that was also STING/AIM2-dependent. This immunity further protected mice from re-challenge with the same cell line. Tumor immune phenotyping revealed shifts in leukocyte phenotypes

toward activated states in both the lymphoid and myeloid compartments including the production of durable central memory T cells. Analysis of glioma patient PBMCs also showed increased T-cell numbers and clonal expansion as well as increases in memory T cells and activation of macrophages and dendritic cells following TTFields treatment, similar to the previous analysis (52). However, T cells also showed increased PD-1 expression, suggesting that TTFields stimulation may also drive T-cell exhaustion, and possible need for combination with checkpoint inhibitor therapy.

TTFields may also affect the tumor microenvironment (TME) through metabolic changes. Analysis of glioma cell lines following treatment with TTFields demonstrated decreased uptake of [18F]DASA-23, a probe that detects the presence of PKM2, an isoform of pyruvate kinase that promotes anaerobic glycolysis and lactate production (54). Western blot analysis showed a decrease in PKM2 expression (55). This may have direct and indirect effects on the tumor, as dimeric PKM2 is important in driving anaerobic glycolysis required for tumor growth (56) as well as driving the production of lactic acid, which is immunosuppressive in the TME (57). In addition, dimeric PKM2 also acts as a HIF1α coactivator and controls the expression of genes that play cell autonomous roles in immune evasion and tumor progression (58).

Dexamethasone is a commonly used supportive medication in patients with GBM. Retrospective analysis of patients in the phase III EF-11 trial testing TTFields in recurrent GBM revealed that a strong predictor of response to TTFields was low dexamethasone usage (59). Further analysis revealed a cutoff of less than 4.1 mg dexamethasone/day, which correlated with better outcomes in TTFields-treated patients (60). Interestingly, the effects of high dexamethasone were also seen in the control arm (60). While dexamethasone usage and dose could be related to disease severity, when dexamethasone dose was aggressively reduced to below 4.1 mg/day, mOS was similar to that found in the low dexamethasone population (60). Corticosteroids such as dexamethasone are potent anti-inflammatory agents that have been shown to reduce antitumor immunity in patients with glioma (61–63). Therefore, it is likely that the inferior outcomes associated with dexamethasone use are due to its immunosuppressive effects.

Other Biological Effects: Cell Migration and Invasion

Cell migration and invasion are processes central to the metastatic spread of solid tumors. These aspects require dynamic changes in biophysical properties and are targetable by TTFields. TTFields have been shown to suppress the migration and invasion of LN-18 glioma cells in Boyden chamber and wound-healing assays (22, 32, 64). Confocal microscopy showed abnormal changes in cellular shape and accumulation of actin within the nucleus of TTFields-treated cells, indicating cell stress (32). Similar findings of reduced migration and invasion have been seen *in vitro* (32). TTFields treatment also led to an upregulation of the epithelial marker E-cadherin and downregulation of vimentin expression, a mesenchymal marker, suggesting reversal of epithelial–mesenchymal transition, a known marker of malignant transformation. TTFields also showed inhibition of p38, ERK, JNK, and AKT phosphorylation. It was later hypothesized that this inhibition of phosphorylation could indicate a mechanism of how cell migration and invasion are inhibited (64).

To date there have been limited published studies evaluating TTFields on cell motility in animal models (32, 45, 64). Two studies in the lungs of mice and New Zealand White rabbits revealed that

TTFields-treated animals had a reduced number and size of lung melanoma and VX2 pancreatic cancer metastases, respectively, compared with control animals (45, 65), which could be the result of reduced cell migration.

Impacted cell migration and invasion properties can also be shown by reduced cell dispersal and reduced cell velocity in vitro. TTFields-treated human GBM cell lines showed decreased cell dispersal compared with control cells (65). TTFields also led to a reduction in cell migration velocity compared with control cells (65), because slower cell motility can impact the intensity of cell invasion.

The majority of studies of TTFields at the cellular level have focused on microtubules, however TTFields likely have similar effects on filamentous actin. TTFields have been shown to reduce tunneling nanotubes formation, ultrafine thread-like protrusions of F-actin that connect cells and mediate transfer of intracellular content, shown in Supplementary Fig. S1A and S1B, in MPM cells. This effect is independent of cell proliferation (66). Further studies are necessary to understand this topic of ell communication and invasion properties.

Other Biological Effects: Cell Membrane Permeability

One of the most recently discovered mechanisms of action of TTFields is its permeabilization of cancer cell membranes (67). It has been demonstrated that the application of TTFields to cancer and noncancer cell lines facilitated the ingress of reagents up to 20 kDa in size, by increasing the size and number of fenestrae in the cancer cell membrane (67). This effect was not observed in human fibroblast cells exposed to the same TTFields conditions. In a separate series of experiments, a 24-hour cessation period during which TTFields exposure was halted demonstrated a reversal of the increased size and number of membrane pores (67). 5-aminolevulinic acid (5-ALA) was also studied in human GBM cells after they were exposed to TTFields for 6 or 24 hours, and in both cases, there was increased fluorescence in the TTFields-exposed cells (67). However, no increased fluorescence was seen in cocultured noncancer fibroblasts. These findings suggest that TTFields could be used to help increase penetration of diagnostic (e.g., 5-ALA used in the operating room during neurosurgical resection of brain cancers) and therapeutic (e.g., chemotherapy) agents into cancer cells.

Three biophysical models of cancer cells were reviewed to explain the observed phenomenon of TTFields-induced cancer cell membrane permeabilization (68). The possibility of TTFields influencing ion channel activation was considered (68). However, the size of voltage-gated calcium channels is much smaller than the fenestrate visualized on scanning electron microscopy. Second, the bioelectrorheological model (69) of the cell unites the relationships between TTFields, cell membrane destabilization, and shape deformations, which could ultimately lead to the third proposed model, electroporation (68). Electroporation has long been used in the biomedical sciences for cell transfection (70). Although aqueous pore formation causes increased plasma membrane permeability, structural and chemical alterations in the lipid and protein components of the membrane itself also contribute to the permeabilization effect (71).

Other Biological Effects: Blood–Brain Barrier

The restrictiveness of the blood–brain barrier (BBB) remains to be the greatest challenge for drug delivery into the brain. Majorly composed of endothelial cells, with developmental and functional support from astrocytes, pericytes, and neurons, the BBB acts as a gateway sealed together by junctional proteins (72–74).

As stated above, the ability of TTFields to increase cancer cell membrane permeability was recently identified. Healthy human fibroblasts, however, did not demonstrate similar changes, pointing to cancer-specificity of the phenomenon (67). Although the effects of TTFields on tumor cells such as human skin, brain, lung, prostate, breast (2) and ovarian (9), as well as hamster pancreatic (21), rat brain, mouse skin (2), and small lung (75) cancer have been demonstrated, little is known about their effects on normal cells. Preliminary experiments with murine cerebellar microvascular endothelial cells cerebEND (76), a cell line used for drug transport studies as well as an in vitro model of stroke and the senescent BBB (77–81), revealed the ability of TTFields to induce alterations in the cellular morphology.

Using this cell line, it was shown that the administration of frequency-, intensity-, as well as duration- dependent TTFields results in the delocalization of its major tight junction protein claudin-5, as well as the cytoplasmic accessory protein zonula occludens-1 (ZO-1), from the cell membrane boundaries into the cytoplasm (82). Allowing the cells to recover up to 96h post-TTFields led to the restoration of the original cellular morphology, pointing to the reversibility of the process. Furthermore, the morphological alteration was accompanied by a compromised BBB integrity demonstrated by decreased trans-endothelial electrical resistance and significant increase in the permeation of fluorescein-conjugated dextran. A similar occurrence was observed in vivo, in rats, whereby treatment with TTFields resulted in increased permeability for immunoglobulin (Ig)-G in the microvessel, as well as for dyes and contrast agents in the brain. Combined treatment of brain-tumor bearing rats with TTFields and paclitaxel, a drug not able to cross the BBB, led to significantly reduced tumor proliferation and volume, indicating enhanced drug permeation and efficacy (83). These findings implicate the potential of TTFields as an innovative method of drug delivery for treatment of brain tumors and other related diseases of the CNS. Hence, further validation of these results in clinical studies would be favorable.

Clinical Experience with TTFields

TTFields were first tested clinically in a phase I trial in patients with advanced solid tumors, **Table 1** (84). Six patients with refractory cancer were included and treated with TTFields at 100–200 kHz based on tumor type. TTFields were applied via electrodes placed over shaved skin and held in place with a layer of adhesive hydrogel and strips. Of the 6 patients treated, a partial response was noted in one patient with a cutaneous metastasis from breast cancer and stable disease was noted in 3 others.

TTFields have been most extensively studied in patients with GBM. Patients with recurrent GBM were randomized to receive TTFields at 200 kHz or standard salvage chemotherapy (85). 237 patients were randomized with 120 patients receiving TTFields and 117 receiving standard chemotherapy. Although a trend towards improved outcomes with TTFields was seen, overall response rate (ORR) of 14% versus 9.6% and median overall survival (mOS) 6.6 versus 6 months, these were not statistically significant. *Post hoc* noninferiority analysis of the study showed TTFields to be equivalent to chemotherapy. Grade 1/2 contact dermatitis was seen in 16% of patients treated with TTFields.

Given these results, a randomized trial of TTFields in combination with maintenance temozolomide for patients with newly diagnosed GBM who had previously been treated with surgery, when feasible, and

Table 1. Clinical trials of TTFields.

Study	Study population	Treatments	Results
PANOVA	Locally advanced or metastatic pancreatic cancer	TTFields with gemcitabine or gemcitabine and nab-paclitaxel	mPFS 8.3 and 12.7 mo, respectively
INNOVATE	Recurrent ovarian cancer	TTFields with weekly paclitaxel	mPFS 8.9 mo, one year survival 61%
STELLAR	Mesothelioma	TTFields with platinum chemotherapy and pemetrexed	mOS 12.1 mo
EF-14	Glioblastoma after completion of concurrent chemoradiation	Temozolomide vs. temozolomide with TTFields	mPFS 4.0 vs. 6.7 mo
HEPANOVA	HCC	TTFields with sorafenib	Disease control rate 76%
NCT00379470	Recurrent GBM	TTFields vs. Chemotherapy	mOS 6.6 vs. 6.0 mo
NCT00749346	2nd line NSCLC	TTFields with pemetrexed	mOS 13.8 mo
Pilot Phase 1	Advance Solid Tumors	TTFields	No Serious Adverse Events
METIS	NSCLC with 1–10 newly diagnosed brain metastases	TTFields after standard stereotactic radiosurgery (SRS) vs. SRS alone	Study Ongoing
LUNAR	NSCLC following platinum based therapy	TTFields in combination with docetaxel or immune checkpoint inhibitors (ICI) vs. docetaxel or ICI alone	Study Ongoing
ENGOT-0v50/ INNOVATE-3	Recurrent ovarian cancer	TTFields in combination with paclitaxel vs. paclitaxel alone	Study Ongoing
TRIDENT	Newly diagnosed GBM	TTFields in combination with chemoradiation vs. TTFields following chemoradiation	Study Ongoing
ZL-8301–001	Newly diagnosed GEJ cancer	TTFields in combination with FOLFOX/XELOX base chemo	Study Ongoing
PANOVA-3	Newly diagnosed locally advanced pancreatic cancer	TTFields in combination with gemcitabine and nab-paclitaxel vs. gemcitabine and nab-paclitaxel alone	Study Ongoing
KEYNOTE B36	Newly diagnosed NSCLC	TTFields in combination with pembrolizumab	Study Ongoing
NCT03203525	Refractory liver metastases	TTFields in combination with various chemotherapies	Study Ongoing
NCT05004025	Metastatic uveal melanoma	TTFields in combination with nivolumab and ipilimumab	Study Ongoing
NCT04605913	Newly diagnosed pancreatic cancer	TTFields in combination with gemcitabine, nab-paclitaxel, and cisplatin	Study Ongoing

Abbreviations: GEJ, gastroesophageal junction adenocarcinoma; HCC, hepatocellular carcinoma; mo, months; NSCLC, non–small cell lung cancer.

chemoradiation was initiated. Patients were randomized 2:1 to maintenance TTFields and temozolomide or temozolomide alone. Uninterrupted treatment with TTFields was recommended. In an interim analysis of 315 patients in the intention to treat population, an improvement in mOS was seen for TTField-treated patients (19.6 vs. 16.6 months, $P = 0.03$), resulting in FDA approval (86). An improvement in the 2-year survival rate was also noted (43% vs. 29%, $P = 0.006$). Final analysis with a minimum follow up of 24 months confirmed benefit for maintenance TTFields (87). Median progression-free survival (mPFS) and mOS were significantly longer for patients who received TTFields (6.7 vs. 4.0 months, $P \leq 0.001$ and 20.9 vs. 16.0 mo, $P \leq 0.001$). In patients who received TTFields, increased use correlated with improved outcomes (88). Although patients only received TTFields therapy for up to 24 months, a persistent survival benefit was noted with an increase in the 5-year OS rate of 13% versus 5%.

Patients who had progression on maintenance temozolomide with TTFields and were treated with 2nd line investigators choice therapy could continue TTFields along with second- line therapy. In a post hoc analysis, OS was compared between those who received second-line treatment with and without continuation of TTFields. OS for those who continued on TTFields was longer (11.8 vs. 9.2 mo, $P = 0.049$; ref. 89). As the majority of patients were treated with a bevacizumab-based regimen, OS was also compared for only patients treated with bevacizumab based therapies; the benefit with TTFields in this setting persisted (11.8 vs. 9.0 mo, $P = 0.043$).

TTFields was FDA approved for the treatment of mesothelioma based on the single-arm phase II STELLAR trial. Patients with malignant mesothelioma who were not candidates for surgical resec-

tion were treated with platinum agent in combination with pemetrexed and TTFields at 150 kHz. mOS, compared with the reported mOS, 12.1 months, in prior phase III trial of platinum agent with pemetrexed (90), was the primary endpoint. Eighty patients were enrolled and included in the final analysis. An overall response rate of 40% was seen with an mOS of 18.2 months with mPFS being 7.6 months, meeting its primary endpoint (91).

TTFields has also been evaluated, and is currently undergoing evaluation, in other tumor types as well, **Table 1**. In a small phase II trial of TTFields in combination with gemcitabine or gemcitabine and nab-paclitaxel in patients with pancreatic cancer, no increased safety concerns were noted with the addition of TTFields (92). In addition, there were signals of improved survival for treated patients, particularly in those with locally advanced disease. The phase III PANOVA-3 trial (NCT03377491) is currently underway and may confirm these findings. A small single-arm phase II safety study of TTFields in combination with paclitaxel for patients with recurrent ovarian cancer also showed no concerning safety signals (93). PFS and OS survival of this study was longer than that reported from meta-analyses of other trials in this line of therapy. The phase III INNOVATE-3 study (NCT03940196) is currently underway and may confirm these results. Safety of TTFields in combination with chemotherapy for patients with non–small cell lung cancer has also been confirmed in a phase I/II study, and is currently under evaluation in phase III trial (NCT02973789; ref. 94). TTFields is currently being studied in many other tumor types as well including hepatocellular carcinoma, small cell lung cancer, gastric cancer, astrocytoma, ependymoma, brain metastases, and metastatic cancer to the liver, **Table 1**.

Overall, clinical trials of TTFields have shown them to be safe with little side effects. In the initial phase I study grade 1 skin rash was seen in 3/6 patients; no other adverse events were seen. Skin rash resolved in all patients with repositioning of the electrodes and topical steroid ointments. Other than dermatitis, no other significant safety signals have been noted in subsequent clinical trials. Despite being well tolerated, TTFields do have limitations, mainly patch application and time requirement of use. 2 orthogonal sets of patches can be difficult to place on certain areas of the body, such as the extremities, which add difficulty to studying this therapy in these areas. Additionally, it is generally recommended that TTFields be used 18 hours a day, as previous studies show increased use correlate with improved survival (87). Use of TTFields requires a patient to carry around a power supply; however, newer devices models have smaller easier to carry devices and batteries that mitigate this burden.

Summary

TTFields are a new cancer treatment modality that is currently FDA-approved for two different tumor types. Preclinical studies have shown multiple downstream effects of TTFields on cancer cells, as outlined above. Combinational studies using drugs that target mitosis, ER stress, autophagy, and DNA replication stress in combination with TTFields may be beneficial in this setting. For tumors where drug penetration is a concern, studies combining standard therapies with TTFields should be considered given its effects on the blood brain barrier and cell membrane permeability. It is well known that intra-tumoral immune responses may spread to nontreated tumors. Therefore, studies of TTFields targeting large volume areas of disease, or areas with known low response rates such as the liver, in combination with anti–PD-1 and other emerging immunotherapies should be considered as well. Translational studies accompanying clinical trials are crucial for deeper understanding of TTFields mechanism of action and potential use. Given the many mechanisms of action, TTFields have potential to be effectively combined with many types of anticancer therapy for many tumor types.

Authors' Disclosures

Note

References

1. Kirson ED, Dbalý V, Tovarys F, Vymazal J, Soustiel JF, Itzhaki A, 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.

2. Kirson ED, Gurvich Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y, et al. Disruption of cancer cell replication by alternating electric fields. Cancer Res 2004;64:3288–95.

3. Tuszynski JA, Wenger C, Friesen DE, Preto J. An overview of Sub-cellular mechanisms involved in the action of TTFields. Int J Environ Res Public Health 2016;13:1128.

4. Giladi M, Schneiderman RS, Voloshin T, Porat Y, Munster M, Blat R, 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.

5. Gera N, Yang A, Holtzman TS, Lee SX, Wong ET, Swanson KD. Tumor treating fields perturb the localization of septins and cause aberrant mitotic exit. PLoS One 2015;10:e0125269.

6. Palti Y. Optimizing Characteristic of an Electric Field to Increase the Field's Effect on Proliferating Cells. USPTO, Editor Standen Ltd; 2011.

7. Miranda PC, Mekonnen A, Salvador R, Basser PJ. Predicting the electric field distribution in the brain for the treatment of glioblastoma. Phys Med Biol 2014; 59:4137–47.

8. Bomzon Z, Urman N, Naveh A, Hershkovich HS, Weinberg U, Kirson E. Efficacy and thermal safety of tumor treating fields delivered to the thorax: a simulation-based study. Int J Radiat Oncol Biol Phys 2019;105:E483–E4.

9. Voloshin T, Munster M, Blatt R, Shteingauz A, Roberts PC, Schmelz EM, et al. Alternating electric fields (TTFields) in combination with paclitaxel are

therapeutically effective against ovarian cancer cells in vitro and in vivo. Int J Cancer 2016;139:2850–8.

10. Naveh A, Bomzon Z, Farber O, Urman N, Yesharim O, Kirson E, et al. Transducer array configuration optimization for treatment of pancreatic cancer using Tumor Treating Fields (TTFields). Cancer Res 2018;78(13_Supplement):3204.

11. Blatt R, Davidi S, Munster M, Shteingauz A, Cahal S, Zeidan A, et al. Safety of Tumor Treating Fields (TTFields) Applied to the Torso. Front Oncol 2021;11:670809

12. Hanahan D, Weinberg R. The Hallmarks of cancer. Cell 2000;100:57–70.

13. Hanahan D, Weinberg R. Hallmarks of cancer: The next generation. Cell 2011;144:646–74.

14. Penna LS, Henriques JAP, Bonatto D. Anti-mitotic agents: Are they emerging molecules for cancer treatment? Pharmacol Ther 2017;173:67–82.

15. Matson DR, Stukenberg PT. Spindle poisons and cell fate: a tale of two pathways. Mol Interv 2011;11:141–50.

16. Bates D, Eastman A. Microtubule destabilising agents: far more than just antimitotic anticancer drugs. Br J Clin Pharmacol 2017;83:255–68.

17. Sinha D, Duijf PHG, Khanna KK. Mitotic slippage: an old tale with a new twist. Cell Cycle 2019;18:7–15.

18. Musacchio A, Hardwick KG. The spindle checkpoint: structural insights into dynamic signalling. Nat Rev Mol Cell Biol 2002;3:731–41.

19. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 2007;8:379–93.

20. Tannous BA, Kerami M, Van der Stoop PM, Kwiatkowski N, Wang J, Zhou W, et al. Effects of the selective MPS1 inhibitor MPS1-IN-3 on glioblastoma sensitivity to antimitotic drugs. J Natl Cancer Inst 2013;105:1322–31.

21. Giladi M, Schneiderman RS, Porat Y, Munster M, Itzhaki A, Mordechovich D, et al. Mitotic disruption and reduced clonogenicity of pancreatic cancer cells in vitro and in vivo by tumor treating fields. Pancreatology 2014;14:54–63.

22. Kim EH, Kim YH, Song HS, Jeong YK, Lee JY, Sung J, et al. Biological effect of an alternating electric field on cell proliferation and synergistic antimitotic effect in combination with ionizing radiation. Oncotarget 2016;7:62267–79.

23. Kessler AF, Frömbling GE, Gross F, Hahn M, Dzokou W, Ernestus RI, et al. Effects of tumor treating fields (TTFields) on glioblastoma cells are augmented by mitotic checkpoint inhibition. Cell Death Discov 2018;4:12.

24. Berkelmann L, Bader A, Meshksar S, Dierks A, Majernik GH, Krauss JK, et al. Tumour-treating fields (TTFields): Investigations on the mechanism of action by electromagnetic exposure of cells in telophase/cytokinesis. Sci Rep 2019;9:7362.

25. Timmons JJ, Preto J, Tuszynski JA, Wong ET. Tubulin's response to external electric fields by molecular dynamics simulations. PLoS One 2018;13:e02

26. Wong ET, Lok E, Swanson KD. Alternating electric fields therapy for malignant gliomas: from bench observation to clinical reality. Prog Neurol Surg 2018;32:180–95.

27. Spiliotis ET, Kinoshita M, Nelson WJ. A mitotic septin scaffold required for mammalian chromosome congression and segregation. Science 2005;307:1781–5.

28. Li X, Yang F, Rubinsky B. A theoretical study on the biophysical mechanisms by which tumor treating fields affect tumor cells during mitosis. IEEE Trans Biomed Eng 2020;67:2594–602.

29. Gal V, Martin S, Bayley P. Fast disassembly of microtubules induced by Mg2+ or Ca2+. Biochem Biophys Res Commun 1988;155:1464–70.

30. Hepler PK, Callaham DA. Free calcium increases during anaphase in stamen hair cells of Tradescantia. J Cell Biol 1987;105:2137–43.

31. Kim EH, Jo Y, Sai S, Park MJ, Kim JY, Kim JS, et al. Tumor-treating fields induce autophagy by blocking the Akt2/miR29b axis in glioblastoma cells. Oncogene 2019;38:6630–46.

32. Silginer M, Weller M, Stupp R, Roth P. Biological activity of tumor-treating fields in preclinical glioma models. Cell Death Dis 2017;8:e2753.

33. Voloshin T, Kaynan N, Davidi S, Porat Y, Shteingauz A, Schneiderman RS, et al. Tumor-treating fields (TTFields) induce immunogenic cell death resulting in enhanced antitumor efficacy when combined with anti-PD-1 therapy. Cancer Immunol Immunother 2020;69:1191–204.

34. Jo Y, Kim EH, Sai S, Kim JS, Cho JM, Kim H, et al. Functional biological activity of sorafenib as a tumor-treating field sensitizer for glioblastoma therapy. Int J Mol Sci 2018;19:3684.

35. Shteingauz A, Porat Y, Voloshin T, Schneiderman RS, Munster M, Zeevi E, et al. AMPK-dependent autophagy upregulation serves as a survival mechanism in response to Tumor Treating Fields (TTFields). Cell Death Dis 2018;9:1074.

36. Karanam NK, Srinivasan K, Ding L, Sishc B, Saha D, Story MD. Tumor-treating fields elicit a conditional vulnerability to ionizing radiation via the downregulation of BRCA1 signaling and reduced DNA double-strand break repair capacity in non-small cell lung cancer cell lines. Cell Death Dis 2017;8:e2711.

37. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 2004;4:814–9.

38. Giladi M, Munster M, Schneiderman RS, Voloshin T, Porat Y, Blat R, et al. Tumor treating fields (TTFields) delay DNA damage repair following radiation treatment of glioma cells. Radiat Oncol 2017;12:206.

39. Mumblat H, Martinez-Conde A, Braten O, Munster M, Dor-On E, Schneiderman RS, et al. Tumor treating fields (TTFields) downregulate the Fanconi Anemia-BRCA pathway and increase the efficacy of chemotherapy in malignant pleural mesothelioma preclinical models. Lung Cancer 2021;160:99–110.

40. Sirbu BM, Couch FB, Feigerle JT, Bhaskara S, Hiebert SW, Cortez D. Analysis of protein dynamics at active, stalled, and collapsed replication forks. Genes Dev 2011;25:1320–7.

41. Schlacher K, Wu H, Jasin M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer Cell 2012;22:106–16.

42. Karanam NK, Ding L, Aroumougame A, Story MD. Tumor treating fields cause replication stress and interfere with DNA replication fork maintenance: Implications for cancer therapy. Transl Res 2020;217:33–46.

43. Karanam NK, Ding L, Sishc B, Saha D, SMD. Abstract 3217: Newly identified role of tumor treating fields in DNA damage repair and replication stress pathways. Cancer Res 38:17s, 2018 (suppl. abstr. 3217).

44. Robins HI, Nguyen HN, Field A, Howard S, Salamat S, Deming DA. Molecular evolution of a glioblastoma controlled with tumor treating fields and concomitant temozolomide. Front Oncol 2018;8:451.

45. Kirson ED, Giladi M, Gurvich Z, Itzhaki A, Mordechovich D, Schneiderman RS, et al. Alternating electric fields (TTFields) inhibit metastatic spread of solid tumors to the lungs. Clin Exp Metastasis 2009;26:633–40.

46. van der Woude LL, Gorris MAJ, Halilovic A, Figdor CG, de Vries IJM. Migrating into the tumor: a roadmap for T cells. Trends Cancer 2017;3:797–808.

47. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013;31:51–72.

48. Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, et al. An immunosurveillance mechanism controls cancer cell ploidy. Science 2012;337:1678–84.

49. Karanam NK, Story MD. An overview of potential novel mechanisms of action underlying Tumor Treating Fields-induced cancer cell death and their clinical implications. Int J Radiat Biol 2021;97:1044–54.

50. Wong E, Timmons J, SK. Abstract 1707: Tumor treating fields exert cellular and immunologic effects. Cancer Res 78:13s, 2018 (suppl. abstr. 1707).

51. Barsheshet Y, Brant B, Voloshin T, Volodin A, Koren L, Koltun B, et al., editors. Tumor treating fields (TTFields) promote a pro-inflammatory phenotype in macrophages. Cancer Res 82:12s, 2022 (suppl. abstr. 1305).

52. Diamant G, Goldman HS, Plotnitsky LG, Roitman M, Shiloach T, Globerson-Levin A, et al. T Cells retain pivotal antitumoral functions under Tumor-treating electric fields. J Immunol 2021;207:709–19.

53. Chen D, Le SB, Hutchinson TE, Calinescu AA, Sebastian M, Jin D, et al. Tumor treating fields dually activate STING and AIM2 inflammasomes to induce adjuvant immunity in glioblastoma. J Clin Invest 2022;132:e149258.

54. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029–33.

55. Patel CB, Beinat C, Xie Y, Chang E, Gambhir SS. Tumor treating fields (TTFields) impairs aberrant glycolysis in glioblastoma as evaluated by by [^{18}F]DASA-23, a non-invasive probe of pyruvate kinase M2 (PKM2) expression. Neoplasia 2021;23:58–67.

56. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 2008;452:230–3.

57. de la Cruz-López KG, Castro-Muñoz LJ, R-H DO, García-Carrancá A, Manzo-Merino J. Lactate in the regulation of tumor microenvironment and therapeutic approaches. Front Oncol 2019;9:1143.

58. Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell 2011;145:732–44.

59. Wong ET, Lok E, Swanson KD, Gautam S, Engelhard HH, Lieberman F, et al. Response assessment of NovoTTF-100A versus best physician's choice chemotherapy in recurrent glioblastoma. Cancer Med 2014;3:592–602.

60. Wong ET, Lok E, Gautam S, Swanson KD. Dexamethasone exerts profound immunologic interference on treatment efficacy for recurrent glioblastoma. Br J Cancer 2015;113:1642.

61. Giles AJ, Hutchinson MND, Sonnemann HM, Jung J, Fecci PE, Ratnam NM, et al. Dexamethasone-induced immunosuppression: mechanisms and implications for immunotherapy. J Immunother Cancer 2018;6:51.

62. Iorgulescu JB, Gokhale PC, Speranza MC, Eschle BK, Poitras MJ, Wilkens MK, et al. Concurrent dexamethasone limits the clinical benefit of immune checkpoint blockade in glioblastoma. Clin Cancer Res 2021;27:276–87.

63. Pitter KL, Tamagno I, Alikhanyan K, Hosni-Ahmed A, Pattwell SS, Donnola S, et al. Corticosteroids compromise survival in glioblastoma. Brain 2016;139: 1458–71.

64. Kim EH, Song HS, Yoo SH, Yoon M. Tumor treating fields inhibit glioblastoma cell migration, invasion and angiogenesis. Oncotarget 2016;7:65125–36.

65. Voloshin T, Schneiderman RS, Volodin A, Shamir RR, Kaynan N, Zeevi E, et al. Tumor Treating Fields (TTFields) hinder cancer cell motility through regulation of microtubule and acting dynamics. Cancers 2020;12:3016.

66. Sarkari A, Korenfeld S, Ladner K, Wong P, Martinez A, Dor-On E, et al., editors. Abstract 2011: Tumor treating fields induce cellular and morphologic changes including disruption of intercellular communication networks in malignant pleural mesothelioma. AACR Annual Meeting; 2021: Cancer Research.

67. Chang E, Patel CB, Pohling C, Young C, Song J, Flores TA, et al. Tumor treating fields increases membrane permeability in glioblastoma cells. Cell Death Discov 2018;4:113.

68. Aguilar AA, Ho MC, Chang E, Carlson KW, Natarajan A, Marciano T, et al. Permeabilizing cell membranes with electric fields. Cancers 2021;13:2283.

69. Pawlowski P, Fikus M. Bioelectrorheological model of the cell. 1. Analysis of stresses and deformations. J Theor Biol 1989;137:321–37.

70. Shi J, Ma Y, Zhu J, Chen Y, Sun Y, Yao Y, et al. A review on Electroporation-based intracellular delivery. Molecules 2018;23:3044.

71. Kotnik T, Rems L, Tarek M, Miklavčič D. Membrane electroporation and electropermeabilization: mechanisms and models. Annu Rev Biophys 2019; 48:63–91.

72. Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. Blood-brain barrier: From physiology to disease and back. Physiol Rev 2019;99:21–78.

73. Salvador E, Burek M, Förster CY. Tight Junctions and the Tumor Microenvironment. Curr Pathobiol Rep 2016;4:135–45.

74. Rubin LL, Staddon JM. The cell biology of the blood-brain barrier. Annu Rev Neurosci 1999;22:11–28.

75. Giladi M, Weinberg U, Schneiderman RS, Porat Y, Munster M, Voloshin T, 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:S35–41.

76. Silwedel C, Förster C. Differential susceptibility of cerebral and cerebellar murine brain microvascular endothelial cells to loss of barrier properties in response to inflammatory stimuli. J Neuroimmunol 2006;179:37–45.

77. Neuhaus W, Burek M, Djuzenova CS, Thal SC, Koepsell H, Roewer N, et al. Addition of NMDA-receptor antagonist MK801 during oxygen/glucose deprivation moderately attenuates the upregulation of glucose uptake after subsequent reoxygenation in brain endothelial cells. Neurosci Lett 2012;506:44–9.

78. Neuhaus W, Gaiser F, Mahringer A, Franz J, Riethmüller C, Förster C. The pivotal role of astrocytes in an in vitro stroke model of the blood-brain barrier. Front Cell Neurosci 2014;8:352.

79. Burek M, Burmester S, Salvador E, Möller-Ehrlich K, Schneider R, Roewer N, et al. Kidney Ischemia/reperfusion injury induces changes in the drug transporter expression at the Blood-brain barrier. Front Physiol 2020;11:569881.

80. Rösing N, Salvador E, Güntzel P, Kempe C, Burek M, Holzgrabe U, et al. Neuroprotective effects of isosteviol sodium in murine brain capillary cerebellar endothelial cells (cerebEND) After Hypoxia. Front Cell Neurosci 2020;14: 573950.

81. Salvador E, Burek M, Löhr M, Nagai M, Hagemann C, Förster CY. Senescence and associated blood-brain barrier alterations in vitro. Histochem Cell Biol 2021; 156:283–92.

82. Salvador E, Kessler A, Hormann J, Domrose D, Schaeffer C, Burek M, et al. Tumor treating fields effects on the blood-brain barrier in vitro and in vivo. ASCO Annual Meeting: Journal of Clinical Oncology.

83. Salvador E, Kessler A, Burek M, Tempel-Brami C, Voloshin T, Giladi M, et al. Breaching the Blood-Brain Barrier via Tumour Treating Fields. Annual Conference of the German Society for Neurosurgery; 2021.

84. Salzberg M, Kirson E, Palti Y, Rochlitz C. A pilot study with very low-intensity, intermediate-frequency electric fields in patients with locally advanced and/or metastatic solid tumors. Onkologie 2008;31:362–5.

85. Stupp R, Wong ET, Kanner AA, Steinberg D, Engelhard H, Heidecke V, 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.

86. Stupp R, Taillibert S, Kanner AA, Kesari S, Steinberg DM, Toms SA, et al. Maintenance therapy with Tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: A Randomized Clinical Trial. JAMA 2015;314: 2535–43.

87. Stupp R, Taillibert S, Kanner A, Read W, Steinberg D, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA 2017;318:2306–16.

88. Toms SA, Kim CY, Nicholas G, Ram Z. Increased compliance with tumor treating fields therapy is prognostic for improved survival in the treatment of glioblastoma: a subgroup analysis of the EF-14 phase III trial. J Neurooncol 2019; 141:467–73.

89. Kesari S, Ram Z, Investigators E-T. Tumor-treating fields plus chemotherapy versus chemotherapy alone for glioblastoma at first recurrence: a post hoc analysis of the EF-14 trial. CNS Oncol 2017;6:185–93.

90. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21: 2636–44.

91. Ceresoli GL, Aerts J, Madrzak J, Dziadziuszko R, Ramlau R, Cedres S, et al. Final results of Phase II STELLAR trial: TTFields with chemotherapy in unresectable malignant pleural mesothelioma. Cancer Res 79:13s, 2019 (suppl. abstr. CT201).

92. Rivera F, Benavides M, Gallego J, Guillen-Ponce C, Lopez-Martin J, Küng M. Tumor treating fields in combination with gemcitabine or gemcitabine plus nab-paclitaxel in pancreatic cancer: Results of the PANOVA phase 2 study. Pancreatology 2019;19:64–72.

93. Vergote I, von Moos R, Manso L, Van Nieuwenhuysen E, Concin N, Sessa C. Tumor treating fields in combination with paclitaxel in recurrent ovarian carcinoma: Results of the INNOVATE pilot study. Gynecol Oncol 2018;150: 471–7.

94. Pless M, Droege C, von Moos R, Salzberg M, Betticher D. A phase I/II trial of tumor treating fields (TTFields) therapy in combination with pemetrexed for advanced non-small cell lung cancer. Lung Cancer 2013;81:445–50.