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An overview of potential novel mechanisms of action underlying Tumor Treating Fields-induced cancer cell death and their clinical implications

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**ABSTRACT**

Traditional cancer therapy choices for clinicians are surgery, chemotherapy, radiation and immune therapy which are used either standalone therapies or in various combinations. Other physical modalities beyond ionizing radiation include photodynamic therapy and heating and the more recent approach referred to as Tumor Treating Fields (TTFields). TTFields are intermediate frequency, low-intensity, alternating electric fields that are applied to tumor regions and cells using noninvasive arrays. TTFields have revolutionized the treatment of newly diagnosed and recurrent glioblastoma (GBM) and unresectable and locally advanced malignant pleural mesothelioma (MPM). TTFields are thought to kill tumor cells predominantly by disrupting mitosis; however it has been shown that TTFields increase efficacy of different classes of drugs, which directly target mitosis, replication stress and DNA damage pathways. Hence, a detailed understanding of TTFields’ mechanisms of action is needed to use this therapy effectively in the clinic. Recent findings implicate TTFields’ role in different important pathways such as DNA damage response and replication stress, ER stress, membrane permeability, autophagy, and immune response. This review focuses on potentially novel mechanisms of TTFields anti-tumor action and their implications in completed and ongoing clinical trials and pre-clinical studies. Moreover, the review discusses advantages and strategies using chemotherapy agents and radiation therapy in combination with TTFields for future clinical use.

**Introduction**

Cancer is one of the deadliest diseases as it caused 8.8 million deaths in 2015 according to the World Health Organization statistics. Standard cancer treatment options, such as surgery, chemotherapy, radiation therapy, and immunotherapy (Morgensztern and Govindan 2010; Morgensztern et al. 2010), are commonly used in the clinic, either as standalone therapies or in various combinations. However, despite this multitude of options, survival rates for patients with advanced stage cancers are very low (www.cancer.net). The dual specter of poor prognosis and an unfavorable therapeutic index calls for novel therapeutic interventions and combined therapy modality options to improve overall survival rates in patients. Hence, the cancer research field remains dynamic and is ever evolving to improve existing therapies and discover new modalities of cancer therapy.

**Emergence of Tumor Treating Fields (TTFields) as a new physical modality of cancer therapy**

The scientific community has shown an increasing interest in the biological effect of external electrical fields on cells. Grosse and Schwan (1992) showed that steady state transmembrane voltage can be induced in spherical cells by an external alternating field. Polarization induced by alternating current (AC) may affect cells in a frequency-dependent manner by orienting, deforming, and moving them. Low frequencies below 1 kHz can stimulate nerve, muscle, heart, and other tissues through membrane depolarization. Stimulatory effects gradually decrease when the frequency of the alternating electric fields increases above 1 kHz, because the response time of the cells’ excitable processes is too slow to follow the higher frequency. Higher frequency fields above 1 MHz generate heat due to dielectric loss to disrupted membranes and can cause electroporation and cell death, depending on the field strength (Markx 2008). Consequently, frequencies commonly used in medical treatments for radio frequency tumor ablation are in the high MHz or GHz range (Figure 1).

Intermediate frequency electric fields alternate too quickly to cause tissue stimulation, and they generate minimal heat. Initially, intermediate frequency AC electric fields (KHz to MHz range) were thought to have no meaningful biological effects. The composition of biological molecules that contain positive and negative charges renders them dipolar, the moment that alternating electric fields are applied. Because
of this, it was hypothesized that, with precise spatial and temporal alignment, alternating electric fields at intermediate frequencies can disrupt cells undergoing mitosis. A decade ago, it was shown that electric fields in the intermediate frequency range of 100–500 KHz have an anti-mitotic effect (Kirson et al. 2004, 2007). This finding led to developing TTFields to selectively destroy cancer cells, which have a higher mitotic index than normal cells (Wenger et al. 2015). The advent of TTFields has revolutionized the treatment of solid, therapy-resistant primary and recurrent tumors (Giladi, Schneiderman, et al. 2014; Vymazal and Wong 2013). TTFields neither stimulate nerves/muscle, nor generate heat because of their relatively high frequency range and low intensity (Davies et al. 2013).

Generation of TTFields

Clinical generation of TTFields using the NovoTTF system

The FDA approved Optune (NovoCure), a TTFields-generating transducer array, for treating recurrent and newly diagnosed GBM in combination with temozolomide, and unresectable and advanced malignant pleural mesothelioma (MPM) in combination with platinum-based chemotherapy. Novocure Inc. developed a TTFields-generating first generation Optune device called the NovoTTF 100 A system, which is portable, can be used at home or work and which only minimally impacts normal daily activities. The second generation NovoTTF 200 A system, which is lighter and more compact than the first generation system, was approved by the FDA for clinical use in 2016. The NovoTTF 200 A system mainly consists of two components: (1) the electric field generator and (2) insulated transducer arrays. The transducer arrays are directly applied to bare skin to produce two perpendicular electric fields that alternate 200,000 times per second between positive and negative polarity (a frequency of 200 kHz) when treating glioblastoma (GBM) and 150,000 times per second (a frequency of 150 kHz) when treating malignant pleural mesothelioma (MPM). Continuous daily use of TTFields therapy for more than 18 hours (>75% of the time) and optimal placement of the transducer arrays properly are critical for good clinical benefit. The NovoTAL software program derives the optimal orientation of transducer arrays to deliver the highest intensity of TTFields to the site of the tumor. Mild to moderate scalp irritation and headache are the most common adverse effects related to using the system (Benson 2018).

TTFields induced mechanisms of action

TTFields exposure leads to mitotic aberrations

Although several hypotheses have been proposed to explain the mechanistic basis of TTFields’ anti-cancer effects, interfering with mitosis was the first mechanism of action identified. TTFields treatment generates intracellular heterogeneity that induces a dielectrophoretic movement of polar molecules such as tubulin and septin toward the region of higher field intensity, thereby affecting tubulin polymerization, septin localization and cytokinesis (Gonzalez and Remcho 2005). Due to their high mitotic index TTFields specifically target cancer cells, thus effectively sparing their normal counterparts. Dividing hematopoietic cells are unaffected because the surrounding muscle and bone create interference (Stupp et al. 2015).

TTFields inhibit human and rodent tumor cell proliferation and induce cell death (Giladi, Schneiderman, et al. 2015) by preventing the proper formation of the mitotic spindle apparatus and activating the mitotic spindle checkpoint (Kirson et al. 2004, 2007). This leads to instability of plasma membrane and blebbing that disrupts cytokinesis, eventually result in abnormal chromosome segregation, cell
cycle arrest, and injured cell production; these cells subsequently undergo cell death/apoptosis (Gera et al. 2015). Earlier it was shown that sensitivity to TTFields treatment is p53 status dependent (Gera et al. 2015); but recent results suggest that TTFields treatment induced biological effects are independent of p53 status (Giladi, Schneiderman, et al.

Figure 2. Schematic drawing of the TTFields influence on key events of mitosis and DNA damage, replication stress pathways in cancer cells. TTFields exposure affects mitosis process by increasing mislocalization of septins, mitotic spindle disruption and interfering with tubulin polymerization, which results abnormal cell division and chromosome segregation thereby leading to mitotic catastrophe and cell death. FA pathway genes expression decreases under TTFields treatment which are implicated in DNA damage repair and replication fork stabilization processes. Because of improper response to ongoing high replication stress and DNA damage it eventually lead to cell death. Surviving cells undergo prolonged TTFields exposure. UV: Ultra Violet; DSBs: Double Strand Breaks; SSBs: Single Strand Breaks; BRCA: BReast CAncer; FANC: Fanconi Anemia Complementation Group.
TTFIELDS inhibits DNA damage repair and induces replication stress
The increased efficacy of drugs affecting mitosis and spindle assembly checkpoint in combination with TTFIELDS, identified in pre-clinical studies and clinical studies, can be explained by the established role of TTFIELDS in mitosis, as mentioned earlier. However, TTFIELDS in combination with other major drug classes such as pemetrexed, doxorubicin, temozolomide (TMZ), gemcitabine, and platinum-based compounds (Schneiderman et al. 2010; Giladi, Schneiderman, et al. 2014; Giladi, Weinberg, et al. 2014; Giladi, Lee, et al. 2015; Voloshin et al. 2016; Kessler et al. 2018), which primarily affect DNA damage and replication stress pathways, also showed improved efficacy. These results suggest that TTFIELDS not only intervene in the mitosis process but also affects other major pathways, which cumulatively contribute to the TTFIELDS anti-tumor effect. Kim et al. (2016) showed increased γ-H2AX foci which is a marker of DNA damage upon TTFIELDS treatment but did not provide a mechanistic explanation for their observation. Karanam et al. (2017) examined TTFIELDS treatment-induced gene expression changes in a set of NSCLC cells, and a provided mechanistic reasoning behind TTFIELDS-induced DNA damage. TTFIELDS treatment decreases Fanconi Anemia (FA) pathway gene expression, which plays an important role in DNA damage and repair, may contribute to TTFIELDS-induced cell death. TTFIELDS exposure results in increased DNA damage and delay DNA repair kinetics over time after ionizing radiation (IR) exposure. TTFIELDS treatment alone increased the frequency of chromatid type aberrations and number of γ-H2AX foci, besides slowing the repair kinetics of double-strand breaks (DSBs) induced by IR. Karanam et al. proposed that TTFIELDS treatment generates a conditional vulnerability, BRCA1/2 (Turner et al. 2004), due to the downregulation of the BRCA1/2 genes. Giladi et al. (2017) also described that TTFIELDS exposure slowed the repair kinetics of radiation- or chemo agents-induced DNA damage.

Interestingly, TTFIELDS exposure in and of itself was shown to produce γ-H2AX foci, which is a marker of DNA damage as well as a marker for stalled replication forks, suggesting that TTFIELDS not only delay DNA damage repair, but also induces replication stress. TTFIELDS treatment downregulates the expression of MCM6 and MCM10 genes, essential components of the DNA replication complex and members of the FA pathway genes, leading to an elevated number of chromatid type aberrations. Furthermore, as part of the induction of replication stress, there is a decrease in the length of newly synthesized DNA and an increase in R-loop formation (Karanam et al. 2018, 2019). Mitosis and DNA damage pathways are tightly regulated through feedback mechanisms. By monitoring temporal gene expression changes associated with regulators of mitosis and DNA damage pathways, Karanam et al. showed that mitotic aberrations and DNA damage events while certainly linked to one another likely also occur independent of each other. These results established the role of TTFIELDS in DNA damage repair and replication stress pathways. Key events in mitosis and DNA damage and replication stress pathways that are affected by TTFIELDS are shown schematically in Figure 2.

TTFIELDS upregulate autophagy and induce immunogenic cell death
TTFIELDS-treated C57BL/6 mice inoculated with malignant melanoma cells and New Zealand rabbits implanted with VX-2 kidney tumors developed a lower number of lung metastases per tumor cross-section than controls (Kirson, Giladi, et al. 2009). A mononuclear cell infiltration was observed around and within metastases, and the extent of this cell infiltration was more profound in TTFIELDS-treated animals. Immunohistochemical staining for lymphocyte subsets revealed that TTFIELDS treatment induced a significantly higher CD4, CD8, and CD45 T cell count than controls, suggesting a T cell-mediated immune response in rabbits. Interestingly, an abundant intra-tumoral cell infiltration was observed though most of the immune cell infiltration was seen in the peri-tumoral location (Kirson, Giladi, et al. 2009). Post-hoc analysis of a phase III clinical trial comparing TTFIELDS vs best physician’s choice (BPC; Stupp et al. 2012) provided an opportunity to study the effect of dexamethasone, an anti-inflammatory and immunosuppressive drug. Patients who received a lower dose of dexamethasone (<4.1 mg/day) in combination with TTFIELDS exhibited better overall survival (OS) than patients who received a higher dose of dexamethasone (>4.1 mg/day) in combination with TTFIELDS. These results support the role of immune competence in the effectiveness of TTFIELDS treatment. In addition, a significant correlation between overall survival and T-lymphocyte counts was observed in patients treated with TTFIELDS in combination with dexamethasone (Wong et al. 2015). In support of a potential enhanced immune response in tumors, TTFIELDS-treated cells showed sign of endoplasmic reticulum (ER) stress leading to calreticulin translocation to the cell surface, and to the release of chromatin binding protein HMGB1 and ATP. TTFIELDS treatment stimulates phagocytosis by dendritic cells (DCs) and maturation of DCs under co-culture conditions (Voloshin et al. 2018). All of these results together suggest that TTFIELDS treatment induces a T-cell mediated anti-tumor immune response.

Cells exposed to TTFIELDS undergo autophagy and necroptosis-mediated cell death associated with increased numbers of autophagosomes, dilated ER, and abnormal mitochondrial structures (Silgine et al. 2017). TTFIELDS treatment was shown to increase cellular granularity by accumulating larger acidic lysosomal pools. TTFIELDS exposure increases the number of autophagosomes and
TTFields increase cancer cell membrane permeability and activates calcium channels

Chang et al. (2018) recently showed that exposure to TTFields increase the number and size of holes on GBM cancer cell membranes. Exposure to TTFields not only makes GBM cells more permeable to small substances, as small as 4 kDa, but also more permeable to substances as large as 20 kDa, but not greater than 50 kDa. Interestingly, this phenomenon was not observed in normal human primary dermal fibroblasts (PCS-201). Moreover, this effect can be modulated with the duration of cell membrane permeability dependent upon the length of TTFields exposure. Increased cancer cell permeability may have clinical implications such as increased uptake of chemotherapeutic agents which would be especially important when considering the potential to open up the blood–brain barrier in the treatment of GBM (Salvador et al. 2020).

TTFields exposure was also shown to induce calcium signals in a dose-dependent manner by activating L-type calcium channels (CACNA1C) in GBM cells (Neuhaus et al. 2019). Those results suggest that the pharmacological blockade of calcium channels with agents like benidipine and nifedipine may augment the effects of TTFields exposure. Summary of important findings are listed in Table 1.

Clinical trials

Completed clinical trials in GBM

Two clinical trials for GBM, EF-11 and EF-14, have been completed to date. The EF-11 trial was conducted in patients with recurrent GBM with OS as a primary end point (Stupp et al. 2012). The efficacy of TTFields as a monotherapy (median OS 6.6 months) was similar to that of the best physician’s choice (BPC) arm (median OS 6.0 months). However, TTFields therapy exhibited less frequent systemic toxicities and much better quality of life compared to BPC therapy. Post-hoc analysis revealed that patients whose compliance was ≥75%, that is a minimum of 18 hours per day, achieved a median OS of 7.7 months. For patients whose compliance was less than 75%, that is less than 18 hours per day, the median OS was only 4.5 months (Kanner et al. 2014; Vymazal and Wong 2014).

The EF-14 clinical trial compared TTFields with adjuvant TMZ and TMZ monotherapies in patients with newly diagnosed GBM with progression free survival (PFS) and median OS as primary end points (Stupp et al. 2015). The median OS for patients treated with TTFields plus TMZ was significantly higher (19.6 months in intent to treat population and 20.5 months in as per-protocol population) than that for patients treated with TMZ monotherapy (16.6 months in intent to treat population and 15.5 months in as per-protocol population). Although TTFields treatment did not cause any systemic toxicities relative to chemotherapy alone, mild to moderate skin irritation was observed in 43% and severe skin reactions in 2% of patients. These TTFields-associated dermatological toxicities may be managed prophylactically (Lukas et al. 2017). Recent mature data from the EF-14 clinical trial showed a significantly higher median OS in patients treated with TTFields plus TMZ (median OS 20.9 months) than in patients treated with TMZ alone (median OS 16.0 months; Stupp et al. 2017).

STELLAR clinical trial in MPM

TTFields were recently approved for treatment of unresectable locally advanced or metastatic malignant pleural mesothelioma (MPM). 80 patients were recruited to the STELLAR phase 2 clinical trial to assess the efficacy of TTFields in combination with standard of care chemotherapy, pemetrexed plus cisplatin or carboplatin (Ceresoli et al. 2018). In this trial, patients treated with TTFields plus pemetrexed and either cisplatin or carboplatin responded better (median OS of 18.2 months and median PFS of 7.6 months) when compared to historical control data from patients treated with pemetrexed and either cisplatin or carboplatin (median OS of 12.1 months and median PFS of 5.7 months). No serious adverse effects were reported besides mild to moderate skin irritation in 46% of patients and grade 3 skin irritations in 5% of patients.

Other ongoing clinical trials

Several clinical trials are currently being conducted in different anatomic settings (Wang et al. 2019), including the advanced stage trials described below.

The LUNAR phase II clinical trial was conducted in 41 patients with inoperable stage IIIB and IV NSCLC who had tumor progression after at least one line of chemotherapy (pemetrexed). An overall median survival of 13.4 months was reported in patients treated with TTFields plus chemotherapy, with only device-related adverse events such as mild to moderate contact dermatitis (Pless et al. 2011). The LUNAR pivotal phase III clinical trial with an expected enrollment of 534 patients is ongoing to assess the efficacy of TTFields in combination with the immune checkpoint inhibitor anti-PD-1 or docetaxel in patients with advanced inoperable stage IV NSCLC (Weinberg et al. 2019).

The PANNOVA phase II clinical trial was conducted in 40 patients with newly diagnosed locally advanced or metastatic PDAC either with TTFields + gemcitabine or TTFields + gemcitabine and nab-paclitaxel. The median PFS was 8 months and the median OS was 14.9 months in patients who were treated with TTFields plus gemcitabine. The median PFS was 12.7 months and the median OS was not reached in patients who had received TTFields + gemcitabine and nab-paclitaxel (Rivera et al. 2019).
<table>
<thead>
<tr>
<th>Study</th>
<th>Journal and year</th>
<th>Cancer type</th>
<th>Outcome of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuhas, E. et al.</td>
<td>Cancers 2019</td>
<td>Human GBM cell lines</td>
<td>TTFields activate L-type calcium channels (CACNA1C); calcium channels inhibition augments TTFields effects.</td>
</tr>
<tr>
<td>Shteingauz, A. et al.</td>
<td>Cell Death and Disease 2018</td>
<td>Human GBM, rat glioma cell lines</td>
<td>TTFields treatment upregulates AMPK-dependent autophagy, which serves as a cancer cell survival mechanism.</td>
</tr>
<tr>
<td>Chang, J. et al.</td>
<td>Cell Death Discovery 2018</td>
<td>Prostate cancer cell lines</td>
<td>TTFields slow down IR-induced DNA damage; the effect of TTFields combined with IR is synergistic.</td>
</tr>
<tr>
<td>Jo, Y. et al.</td>
<td>Cell Death Discovery 2018</td>
<td>Melanoma cell lines</td>
<td>TTFields cause selective damage to cancer cells, but spare normal cells. TTFields treatment decreases tumor volume and decreases the spread of solid tumors to the surrounding normal tissue.</td>
</tr>
<tr>
<td>Giladi, M. et al.</td>
<td>Scientific Rep 2015</td>
<td>NSCLC cell lines and mouse model</td>
<td>TTFields increase the efficacy and sensitivity of chemotherapy agents, i.e. paclitaxel, doxorubicin, and cyclophosphamide, and DTIC in breast and glioma cancer cell lines as well as in VX2 kidney tumor model in rabbits. TTFields in combination with temozolomide significantly increase OS and PFS in GBM patients.</td>
</tr>
<tr>
<td>Kirson, E. et al.</td>
<td>Cancer Res 2004</td>
<td>Human (melanoma, glioma, lung, prostate, breast and cervical cancer) and patient data</td>
<td>TTFields reduce proliferation of various cancer cell lines growth rate through induction of apoptosis and significantly decrease tumor volume and metastatic spread of solid tumors to the surrounding normal tissue. TTFields in combination with chemotherapy agents show an additive effect in vitro and in vivo.</td>
</tr>
<tr>
<td>Kirson, E. et al.</td>
<td>Anticancer Res 2007</td>
<td>NSCLC cell lines and mouse model</td>
<td>TTFields significantly increase OS and PFS in 20 GBM patients.</td>
</tr>
<tr>
<td>Kirson, E. et al.</td>
<td>Cancer Res 2004</td>
<td>Human melanoma cell lines</td>
<td>TTFields increase efficacy and sensitivity of chemotherapy agents, i.e. paclitaxel, doxorubicin, and cyclophosphamide, and DTIC in breast and glioma cancer cell lines as well as in VX2 kidney tumor model in rabbits. TTFields in combination with temozolomide significantly increase OS and PFS in GBM patients.</td>
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<td>Kirson, E. et al.</td>
<td>Clin Exp Metastasis 2009</td>
<td>NSCLC cell lines and mouse model</td>
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<td>BMC Med Phys 2009</td>
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</tbody>
</table>
The in vitro and in vivo data described above provide the rationale for the combination therapies being tested in the INNOVATE-3, PANOVA, and LUNAR clinical trials where MTAs such as paclitaxel, docetaxel, and nab-paclitaxel are being used.

Microtubule targeting agents (MTAs) disrupt microtubule (MT) dynamics and induce prolonged mitotic arrest that can eventually lead to cell death. There are two classes of MTAs: (1) microtubule stabilizing agents such as paclitaxel and docetaxel; and (2) microtubule destabilizing agents such as vincristine and vinblastine. TTFields exposure increases the depolymerized microtubule fraction, suggesting a disruption of the mitotic spindle assembly apparatus (Giladi, Schneiderman, et al. 2015). TTFields treatment in combination with paclitaxel or doxorubicin increased cell killing in multi-drug resistant (MDR) cancer cells without elevating the intracellular concentration of the drugs (Schneiderman et al. 2019); TTFields decreased cellular proliferation and survival, and increased sensitivity of taxol, in a hamster model of pancreatic cancer (Giladi, Schneiderman, et al. 2010); TTFields decreased cellular proliferation and increased the cell killing potency of pemetrexed, cisplatin, and docetaxel; and (2) microtubule destabilizing agents such as vincristine and vinblastine. TTFields exposure increases the depolymerized microtubule fraction, suggesting a disruption of the mitotic spindle assembly apparatus (Giladi, Schneiderman, et al. 2015). TTFields treatment in combination with paclitaxel or doxorubicin increased cell killing in multi-drug resistant (MDR) cancer cells without elevating the intracellular concentration of the drugs (Schneiderman et al. 2019); TTFields decreased cellular proliferation and survival, and increased sensitivity of taxol, in a hamster model of pancreatic cancer (Giladi, Schneiderman, et al. 2010); TTFields decreased cellular proliferation and increased the cell killing potency of pemetrexed, cisplatin, and paclitaxel in NSCLC cells both in vitro and in vivo (Kirson, Schneiderman, et al. 2009; Giladi, Weinberg, et al. 2014).

Completed clinical trials are marked bold. GBM: Glioblastoma; NSCLC: Non-small cell lung cancer.

### Rational application of TTFields in combination therapies

#### Targeting mitosis

Anti-mitotic agents are highly selective and effective because the loss of cell cycle control is a hallmark of cancer. Mitosis is a complex and elaborate process, but it is also the shortest and most fragile phase of the cell cycle. The whole cell cycle is tightly regulated through several checkpoints to ensure elimination of mitotically defective and severely damaged cells by triggering mitotic catastrophe and apoptotic cell death or senescence processes. Several studies have reported that TTFields exposure results in the accumulation of cells in the G2/M phase of the cell cycle, suggesting that the G2/M checkpoint may be triggered to prevent cells from prematurely entering mitosis. The major cell cycle control mechanism in mitosis is the spindle assembly checkpoint (SAC), which will induce prolonged mitotic arrest to assure that accurate chromosome segregation takes place. Giladi, Schneiderman, et al. (2015) observed such prolonged mitotic arrest in cells exposed to TTFields and Kessler et al. (2018) recently showed that TTFields increase the efficacy of the SAC checkpoint inhibitor MPS1-1N-3 in GBM cells.

#### Table 2. Summary of completed and ongoing important clinical trials incorporating TTFields with different combination therapies.

<table>
<thead>
<tr>
<th>Clinical trial name</th>
<th>Disease</th>
<th>Combination therapy agent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF-11 (NCT00379470)</td>
<td>Recurrent GBM</td>
<td>Best standard of care</td>
<td>Stupp et al. (2012)</td>
</tr>
<tr>
<td>EF-14 (NCT00916409)</td>
<td>Newly diagnosed GBM</td>
<td>Temozolomide</td>
<td>Stupp et al. (2017)</td>
</tr>
<tr>
<td>STELLAR (NCT02397928)</td>
<td>Unresectable locally advanced or metastatic malignant mesothelioma</td>
<td>Pemetrexed and cisplatin or carboplatin</td>
<td>Ceresoli et al. (2018)</td>
</tr>
<tr>
<td>LUNAR phase III (NCT02973789)</td>
<td>Inoperable stage IV NSCLC</td>
<td>Anti-PD1 or docetaxel</td>
<td>Weinberg et al. (2019)</td>
</tr>
<tr>
<td>PANOVA phase III (NCT01971281)</td>
<td>Locally advanced unresectable pancreatic adenocarcinoma</td>
<td>Gemcitabine and nab-paclitaxel</td>
<td>Rivera et al. (2019)</td>
</tr>
<tr>
<td>INNOVATE phase III (NCT03940196)</td>
<td>Platinum resistant ovarian cancer</td>
<td>Paclitaxel</td>
<td>Vergote et al. (2018)</td>
</tr>
<tr>
<td>METIS phase III (NCT02831959)</td>
<td>1–10 newly diagnosed brain metastasis from NSCLC</td>
<td>Best standard of care</td>
<td>Mehta et al. (2019)</td>
</tr>
<tr>
<td>HEPANOVA phase III (NCT 03606590)</td>
<td>Locally advanced liver cancer</td>
<td>Sorafenib</td>
<td>Grosu et al. (2020)</td>
</tr>
<tr>
<td>TRIDENT (NCT03869242)</td>
<td>Newly diagnosed GBM</td>
<td>Concomitant radiation therapy and temozolomide</td>
<td>Shi et al. (2020)</td>
</tr>
<tr>
<td>PriCo TTF Phase I/II</td>
<td>Newly diagnosed GBM</td>
<td>Prior and concomitant radiation therapy and temozolomide</td>
<td>Glas et al. (2018)</td>
</tr>
</tbody>
</table>

The PANOVA-3 trial is a pivotal phase 3 clinical trial with an expected enrollment of 556 patients will assess the efficacy of TTFields in combination with standard of care gemcitabine and nab-paclitaxel in newly diagnosed, locally advanced, unresectable pancreatic adenocarcinoma.

The INNOVATE phase II single arm clinical trial tested the safety and efficacy of TTFields in combination with paclitaxel given weekly in 31 patients with recurrent and platinum-resistant ovarian cancer. The median PFS was 8.9 months whereas the median OS was not reached (Vergote et al. 2018). The INNOVATE-III trial is a pivotal randomized phase III clinical trial that tests the efficacy and safety of TTFields in combination with paclitaxel in patients with platinum resistant ovarian cancer.

The METIS trial is a pivotal phase III clinical trial that assesses the efficacy of TTFields in combination with standard of care in patients with 1–10 newly diagnosed brain metastases from NSCLC (Mehta et al. 2019).

HEPANOVA is a prospective phase II clinical trial in which the overall response rate of TTFields is tested along with the standard of care, sorafenib, in patients who were recently diagnosed with locally advanced liver cancer (Grosu et al. 2020).

TRIDENT is an ongoing international phase III clinical trial which is intended to compare the efficacy of standard radiation therapy and temozolomide plus combinatorial radiation therapy and temozolomide plus concomitant TTFields in newly diagnosed GBM patients (Shi et al. 2020).

PriCo TTF trial is a phase I/II clinical trial, which will evaluate the safety and efficacy of TTFields initiated prior and concomitant to combined radiation and temozolomide therapy in newly diagnosed GBM patients (Glas et al. 2018).

A list of completed and ongoing clinical trials in different cancer settings incorporating TTFields with respective combination therapies are provided in Table 2.
DNA Damage

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Defects in DDR and mild to low levels of replication stress with defective DDR and loss of cell cycle blockage that in turn leads to DNA damage. This effect slows DNA synthesis and/or causes replication fork stalling/collapse is called replication stress. Many commonly used cancer chemotherapeutic agents target replication stress, which is thought to be the primary cause of genome instability (Gaillard et al. 2015). Cancer cells maintain unrestrained proliferation by keeping low to mild levels of replication stress. Normal cells maintain genome stability through the coordinated actions of DDR and cell cycle checkpoints. Defects in DDR and mild to low levels of replication stress are unique to cancer cells (Zhang et al. 2016) and, therefore, can be therapeutically exploited.

To target TTFields-induced replication stress, a combination of chemotherapy drugs with TTFields, which can further increase replication stress was tested. Platinum compounds (cisplatin) are known to generate DNA inter- and intra-strand crosslinks between nucleotide residues (Wang and Lippard 2005; Fu et al. 2012). The intra-strand crosslinks occur on same strand cause DNA lesions in the template strand, and the inter-strand crosslinks which occur between opposite strands lead to defects in DNA unwinding, which is the first essential replication step (Deans and West 2011; Sale et al. 2012). TTFields synergistically enhances cisplatin NSCLC cell killing when the treatments are combined, probably because TTFields inhibit the repair of DNA cross-links produced by cisplatin exposure (Karanam et al. 2018). Dysfunction of BRCA genes predispose cells to chemo agents that target single-strand break (SSB) repair pathways, such as PARP inhibitors, result in ‘synthetic lethality’ (Kaelin 2005). TTFields synergistically enhance the efficacy of the PARP inhibitor olaparib and IR individually, and the triple combination further increases the synergy of cell killing (Karanam et al. 2018).

By retrospectively examining a recently completed phase III clinical trial, Lu et al. (2019) showed that the triple combination therapy of bevacizumab, irinotecan, and temozolomide plus TTFields significantly improved the overall survival of patients with recurrent GBM. Irinotecan and temozolomide were found to increase replication stress in accordance with recent findings that suggested TTFields’ role in DDR and replication stress. Moreover, these recent findings provide a rationale for added synergistic effects observed with chemotherapeutic agents such as irinotecan, doxorubicin, gemcitabine, 5-FU, cyclophosphamide and DTIC via increased replication stress when used in combination with TTFields (Giladi, Schneiderman, et al. 2014; Giladi, Weinberg, et al. 2014; Giladi, Lee, et al. 2015; Voloshin et al. 2016; Kessler et al. 2018). Increased replication stress may also have played a role in the recent STELLAR trial where TTFields, pemetrexed and cisplatin or carboplatin were combined to treat pleural mesothelioma. Here, overall survival was increased from 12.1 months to...
Targeting DNA damage and repair after ionizing radiation (IR)
Therapeutic doses of ionizing radiation elicit complex cellular responses through several signaling pathways including DNA damage, mitotic catastrophe, apoptosis, autophagy, immune response and senescence (Maier et al. 2016). Because IR is known to primarily induce complex DNA damage, Karanam et al. (2017) studied its combinatory effect with TTFields and found that TTFields synergistically increase the cell killing ability of IR in NSCLC cells. Giladi et al. (2017) reported that TTFields treatment delays DNA damage repair caused by IR in glioma cells and in a rat model. Kim et al. (2016) showed that IR given before TTFields treatment also synergistically increases the cell killing effect, and also decreases migration and invasion in GBM cells. However newly identified mechanisms of TTFields’ action led to the hypothesis that applying TTFields would first develop a conditional lethality environment, making cells more susceptible to agents such as IR or, in the case of BRCA1 downregulation, to PARP inhibition or cisplatin. Indeed, by delivering TTFields before IR treatment, Karanam et al. (2019) showed that IR was more effective than IR treatment before TTFields exposure. Moreover, TTFields application may be beneficial in cases where IR treatment cannot be applied due to the risk of local tissue toxicity. These results strongly suggest that using TTFields may be effective when given either before or concomitantly with IR.

Targeting immune modulation
Immunotherapy, one of the latest and rapidly advancing cancer therapy modalities, relies on augmenting tumor immunity using various strategies. Of all the different immunotherapies, the use of antibodies against immune checkpoint inhibitors (e.g. anti-CTLA4 and anti-PD-1) has been successful for some cancer patients and as a result, anti-checkpoint immune therapy has been approved by the FDA in a number of different settings. Commonly used to treat neurological symptoms caused by GBM, dexamethasone has been shown to affect patient antitumor immunity via global immunosuppression and a retrospective analysis of a phase III clinical trial revealed that the clinical efficacy of dexamethasone was increased when combined with TTFields. OS correlates included CD3+, CD4+, and CD8+ T-lymphocyte counts (Wong et al. 2015). These data strongly suggest that TTFields-induced stimulation of antitumor immunity contributes to its therapeutic efficacy. Furthermore, it was recently shown that combining TTFields with the immune checkpoint inhibitor anti-PD-1 notably increased therapeutic efficacy by inducing autophagy and ER stress, resulting in immunogenic cell death (Voloshin et al. 2018) and that inhibiting autophagy using chloroquine was shown to enhance TTFields’ anti-tumoral activity (Shteingauz et al. 2018). However, considering the double-edged sword of autophagy based upon the stage of cancer, autophagy inhibitors in combination with TTFields needs to be fully understood.

A summary of different molecular mechanisms of TTFields biological action and agents tested in preclinical and clinical settings are provided for in Figure 3.

Conclusions and future directions
TTFields are approved for the treatment of GBM and MPM but the fundamental mechanism of TTFields biological action is not known. One could speculate that because of the effect on tubulin due to the dipole moment generated by TTFields on mitotic cells, that the predominantly interphase effects described above could also be generated by altering the properties of key proteins based upon their charge or polarity. This might actually provide for changes in the activity of any number of proteins whose subsequent cascades of signaling are also altered leading to radiation or chemotherapy agent vulnerability and enhanced cell killing. Our current understanding of TTFields’ mechanisms of action suggests that TTFields affect multiple pathways such as cell cycle, karyokinesis, the DNA damage response, DNA replication, and immune response, the identification of which are nearly all from in vitro experiments with little in vivo validation (Figure 3). Moreover, as a physical modality, as described above, TTFields may be comparable to ionizing radiation in that they both induce more systemic effects that might render cancer cells more sensitive to different classes of drugs in combination therapy. TTFields’ limited efficacy as a monotherapy in the clinic should be noted in this context (Stupp et al. 2012), however because of the vulnerabilities generated by TTFields exposure, with minimal adverse effects on normal cells or tissues, the potential for the use of TTFields as a neoadjuvant therapy is of paramount importance. Already, ‘concomitant’ application has revealed vulnerabilities that rationally explain the outcomes seen in combination therapies that can likely be enhanced if TTFields were used in advance and during radiation or chemotherapy treatments.

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