The mechanisms of action of Tumor Treating Fields

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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 anti-mitotic effects were the first recognized biological anti-cancer activity of TTFields, data have shown that tumor treating fields achieve their anti-cancer 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 anti-mitotic effects, it is now appreciated that TTFields exert a variety of effects (Figure 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, e.g. 200 kHz for glioblastoma (GBM).(1) TTFields' effect 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 TTFields' effect seen by sub-cellular structures, and hence its efficacy, would depend on the direction of the imposed electric field. Thus, Kirson et al. 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 (Figure 2A) , is predicted for 100% efficacy, ~2.6 V/cm for lung cancer (Figure 2B) , nearly 3.5 V/cm for breast cancer (Figure 2C), and 1.4 V/cm for melanoma (Figure 2D) . The curve-fits resemble those for linear-quadratic (LQ) 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 Anti-Mitotic Mechanism of Action:

Deregulated cellular proliferation is a hallmark of cancer.(12, 13) Therefore, many anti-cancer 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 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 anti-mitotic 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 concominant 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 G0/G1 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 Anti-Mitotic 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) Additionally, electron microscopy of TTFields treated cells have also shown autophagy type changes such as increased vacuoles, autophagosomes, 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. Additionally, 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.

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 Bcl-2.(31) Treatment with 3-methyladenine (3MA) prevented autophagy in TTFields treated cells. RNA analysis of TTFields-treated cells have 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. Based on 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 (FA) 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 γ-H2AX foci, as well as slowed the repair of IR-induced double-strand breaks (DSBs). Amassing of γ-H2AX 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, i.e., 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 immunohistochemical results, cisplatin combined with TTFields induced elevated γ-H2AX foci, suggesting TTFields amplified DNA damage induced by cisplatin.(39) Elevated γ-H2AX 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)

Due to the absence of DNA replication in non-cancerous 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 Anti-Mitotic Effects; Immunological Effects:

TTFields may stimulate anti-tumor 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 to controls. (45)

Metastatic tumors from treated animals also exhibited a higher number of total $CD45^+$ cells and increased $CD4^+$ and $CD8^+$ populations. Additionally, the histological 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 anti-tumor 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)(33); markers of immunogenic cell death (ICD)(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 anti-tumor 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, co-culturing of murine bone marrow-derived macrophages (BMDM) with previously exposed TTFields-treated CT26 cells resulted in increased expression of pro-inflammatory activation markers.(50) TTFields treatment of *in vitro* M1 and M2 macrophages has also resulted in increased production of proinflammatory cytokines (CXCL1, IL-18, IL-23, IL-12p70, TNF-α, IL-12p40, CCL22, G-CSF, CCL17 and IL-1β) 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 neo-antigens.

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 non-proliferating, naive 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 IL-1, IL-6, IL-8, 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 anti-tumor 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 towards 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 which 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 co-activator and controls the expression of genes that play cell autonomous roles in immune evasion and tumor progression.(58)

Dexamethasone (dex) 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 dex usage.(59) Further analysis revealed a cutoff of less than 4.1 mg dex /day which correlated with better outcomes in TTFields-treated patients.(60) Interestingly, the effects of high dex were also seen in the control arm.(60) While dex usage and dose could be related to disease severity, when dex dose was aggressively reduced to below 4.1 mg/day, mOS was similar to that found in the low dex population.(60) Corticosteroids such as dex are potent anti-inflammatory agents that have been shown to reduce anti-tumor immunity in glioma patients.(61-63) Therefore, it is likely that the inferior outcomes associated with dex use is 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 to 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 to control cells.(65) TTFields also led to a reduction in cell migration velocity compared with control cells(65), since 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 nanotubules formation, ultrafine thread-like protrusions of F-actin that connect cells and mediate transfer of intracellular content, shown in Supplemental Figure 1A and B, 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 non-cancer 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 co-cultured non-cancer 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 transendothelial 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 1 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% vs. 9.6% and median overall survival (mOS) 6.6 months (mo) vs 6 mo, these were not statistically significant. Post Hoc non-inferiority 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 (TMZ) for patients with newly diagnosed GBM who had previously been treated with surgery, when feasible, and chemoradiation was initiated. Patients were randomized 2:1 to maintenance TTFields and TMZ or TMZ 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 mo vs. 16.6 mo, 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 mo confirmed benefit for maintenance TTFields.(87) Median progression free survival (mPFS) and mOS were significantly longer for patients who received TTFields (6.7 mo vs 4.0 mo p=<0.001 and 20.9 mo vs. 16.0 mo p=<0.001). In patients who received TTFields, increased use correlated with improved outcomes.(88) Although patients only received TTFields therapy for up to 24 mo, a persistent survival benefit was noted with an increase in the 5-year OS rate of 13% vs. 5%.

Patients who had progression on maintenance TMZ with TTFields and were treated with 2nd line investigators choice therapy could continue TTFields along with 2nd line therapy. In a post hoc analysis, OS was compared between those who received 2nd line treatment with and without continuation of

TTFields. OS for those who continued on TTFields was longer, 11.8 mo vs 9.2 mo p=0.049.(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 mo vs. 9.0 mo p=0.043.

TTFields was FDA approved for the treatment of mesothelioma based on the single arm phase 2 STELLAR trial. Patients with malignant mesothelioma who were not candidates for surgical resection were treated with platinum agent in combination with pemetrexed and TTFields at 150 kHz. mOS, compared to the reported mOS, 12.1 mo, in prior phase 3 trial of platinum agent with pemetrexed(90), was the primary endpoint. 80 patients were enrolled and included in the final analysis. An overall response rate of 40% was seen with a mOS of 18.2 mo with mPFS being 7.6 mo, 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 2 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) Additionally, there were signals of improved survival for treated patients, particularly in those with locally advanced disease. The phase 3 PANOVA-3 trial (NCT03377491) is currently underway and may confirm these findings. A small single arm phase 2 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 3 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 1/2 study, and is currently under evaluation in phase 3 trial (NCT02973789).(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 1 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 which mitigate this burden.

Summary:

TTFields are a new cancer treatment modality that is currently FDA-approved for two different tumor types. Pre-clinical 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 intratumoral immune responses may spread to non-treated 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 anti-cancer therapy for many tumor types.

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Figures Legends:

Figure 1: Biological and Biophysical effects of Tumor Treating Fields.

Figure 2. Dose-response curves (electric field strength vs. % of control) for TTFields applied to four tumor cell types, replicated from Kirson et al.(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 to the other curves).

Table 1: Clinical Trials of Tumor Treating Fields (TTFields)

Study	Study Population	Treatments	Results
PANOVA	Locally advances for 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 hemotherapy and pemetrexed	mOS 12.1 mo
EF-14	Glioblastoma after completion of concurrent chemoradiation	Temozolomide vs. temozolomide with TTFields	mPFS 6.7 vs 4.0 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**

BIOPHYSICAL EFFECTS

BIOLOGICAL EFFECTS

Antimitotic Effects

- Adjusts cell membrane potential to increase Ca2+ influx in cell and promote microtubule depolymerization
- Perturbs mitotic tubulin and septin functions
- Prevents mitotic spindle assembly and mitosis
- Induces aberrant mitotic exit

Cell Migration

- Decreases metastatic lesions in animal models
- Decreases cell dispersion and velocity in vitro
- Reverses markers of epithelial mesenchymal transition

TUMOR TREATING FIELDS EFFECTS

Induction of Autophagy

- Promotes autophagy in treated cells
- Induces AMPK, miR29b and other drivers of autophagy

Replication Stress

- Increases DNA damage over time
- Reduces DNA repair kinetics
- Increases DNA damage in combination with other DNA damaging agents

Cell Membrane Permeability

Increases size of number of fenestrae in cell membrane of cancer cells, but not human fibroblasts

Immunologic Effects

- Increases immunogenic cell death
- Activates STING pathway
- Increases expression of MHC II, CD80 and CD40 on dendritic cells
- Promotes M1 macrophage differentiation

Blood Brain Barrier (BBB)

Increased BBB permeability due to decreased membrane localization of claudin 5 and ZO-1 in microvascular endothelial cells in vitro and in vivo

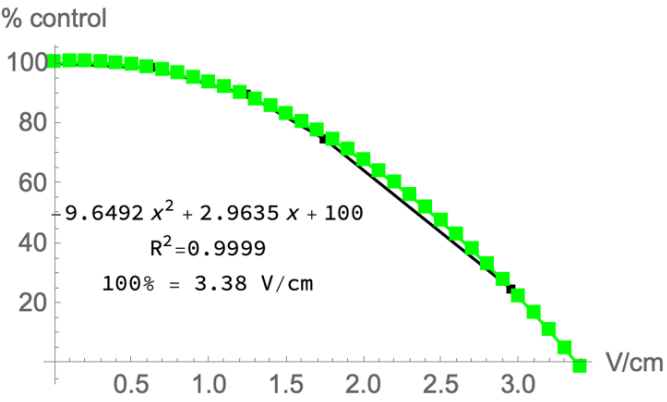

Fig. 2A

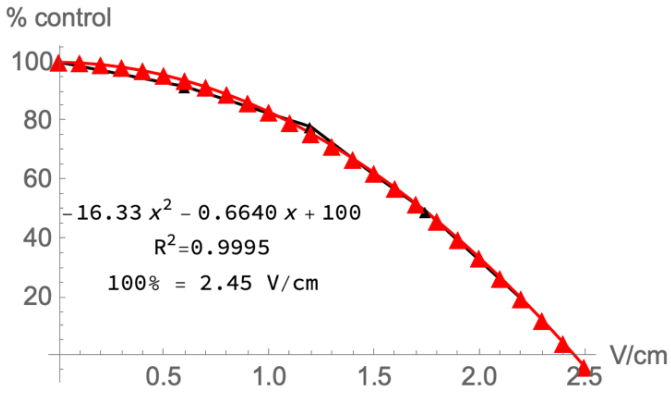

Fig. 2B

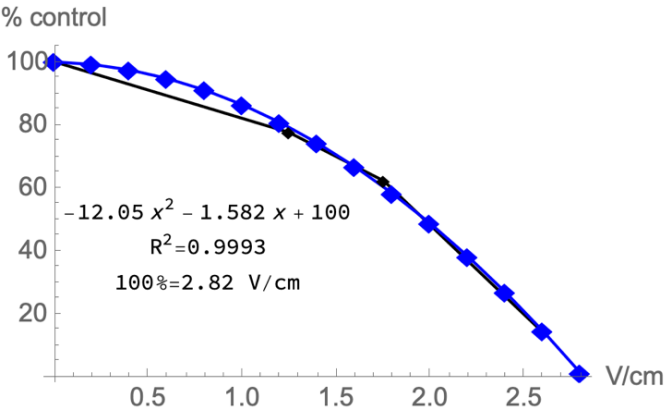

Fig. 2C

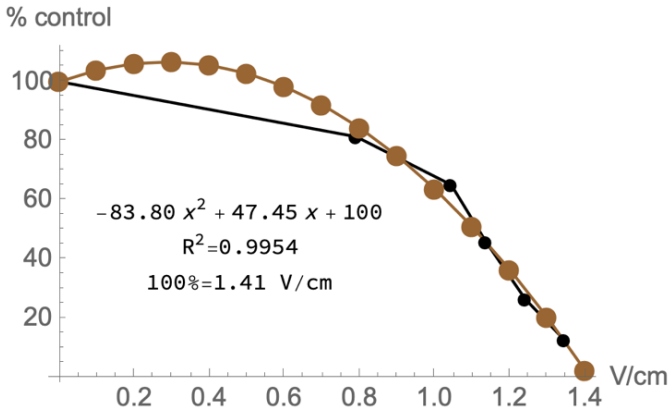

Fig. 2D