Tumor treating fields: An emerging treatment modality for thoracic and abdominal cavity cancers

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ABSTRACT

Tumor treating fields (TTFields)-an intermediate-frequency, electric field therapy-has emerged as a promising alternative therapy for the treatment of solid cancers. Since the first publication describing the anticancer effects of TTFields in 2004 there have been numerous follow-up studies by other groups, either to confirm the efficacy of TTFields or to study the primary mechanism of interaction. The overwhelming conclusion from these *in vitro* studies is that TTFields reduce the viability of aggressively replicating cell lines. However, there is still speculation as to the primary mechanism for this effect; moreover, observations both *in vitro* and *in vivo* of inhibited migration and metastases have been made, which may be unrelated to the originally proposed hypothesis of replication stress. Adding to this, the *in vivo* environment is much more complex spatially, structurally, and involves intricate networks of cell signaling, all of which could change the efficacy of TTFields in the same way pharmaceutical interventions often struggle transitioning *in vivo*. Despite this, TTFields have shown promise in clinical practice on multiple cancer types, which begs the question: has the primary mechanism carried over from *in vitro* to *in vivo* or are there new mechanisms at play? The goal of this review is to highlight the current proposed mechanism of action of TTFields based primarily on *in vitro* experiments and animal models, provide a summary of the clinical efficacy of TTFields, and finally, propose future directions of research to identify all possible mechanisms *in vivo* utilizing novel tumor-on-a-chip platforms.

Introduction

Cancer is the second leading cause of death in the US and worldwide [1]. The majority of cancers originate within the abdominal or thoracic cavities and a significant number of them have a poor prognosis. Lung cancer is the leading cause of death in both males and females, and four of the top five leading cancers in cancer mortality originates in these cavities [2]. Moreover, while overall survival of certain cancers has significantly improved over the last four decades (an absolute increase of ~17% 5-year survival for all cancers), cancers such as pancreatic and lung have not seen as dramatic an increase in survival (~6% increase) [2]. Additionally, current treatment modalities such as chemotherapy, immunotherapy, and radiation come along with unwanted toxicities, so using them in combination is challenging. As such, new treatment regimens that are more effective and less toxic are necessary to improve patient survival and outcomes. One potential method is the use of

electromagnetic fields (EMFs) as a primary or adjunctive treatment to one of the standard treatments listed above. A new, intermediate-frequency, electric field-based therapy, termed Tumor Treating Fields (TTFields), has emerged in the last decade as a promising therapeutic option [3]. This interest stems from the fact that the proposed anticancer effect is disease-agnostic, which could deliver benefit to several malignancies within thoracic or abdominal cavities, coupled with the relatively low toxicity profile of these interventions. The relative safety of TTFields stems from limited overlap with traditional treatment toxicities, allowing concurrent use of this modality with traditional therapeutics.

TTFields work by generating an alternating electric field between parallel electrodes. Unlike tumor ablation, which uses high frequency and high field strength EMFs, TTFields are non-thermal, utilizing a lower field strength in an intermediate frequency range. It is important to note that the term EMF implies an existence of both an electric and magnetic

field, which is true in any case of alternating fields. However, a treatment can be described as either an electric or magnetic field treatment based on which of the fields is dominant. In the case of TTFields, since they involve generation of an electric field with a negligible magnetic field component, they are classified as an electric field therapy. TTFields show their greatest efficacy on highly replicating cells, implicating a disruption of the mitosis process. However, the primary mechanism of cell death remains unclear. Regardless, TTFields have shown their efficacy in glioblastoma multiforme (GBM) *in vivo* in several clinical trials. As a result of the promising findings, TTField application has been extended to several cancers in the abdominal and thoracic cavities for clinical trial, including lung, liver, ovarian, and pancreatic cancers. In this review, we will discuss the various proposed mechanisms of action for TTFields based on *in vitro* and animal model experiments, summarize the status of clinical trials for abdominal and thoracic cancers, and discuss the future direction of experiments to ascertain the therapeutic role of TTFields *in vivo*.

Discussion

Background of electromagnetic field therapy in biology

It has been known for decades that endogenous electric fields exist within the human body down to the individual cellular level. These physiological electric fields have been shown to affect intracellular protein guidance [4], embryogenesis [5], cellular differentiation and growth [6], and wound healing [7,8]. The discovery of these interactions led to therapeutic applications of EMFs to intervene or stimulate certain natural processes. These include the use of electric pulses to open cellular membranes to deliver pharmacological molecules [9,10], magnetic field pulses to stimulate healing of bone fractures [11–13], and electric field generating bandages for wound healing [14]. In addition, several methods have been devised for cancer intervention including tumor ablation, and more recently, TTFields.

Cancer, which involves the dysregulation of normal cell function, is likely to have aberrant electrical regulation as well [15]. Indeed, the abnormal maintenance membrane potential and surface charge of cancer cells may be a driver of metastasis [16,17]. These properties of malignant cells make cancer an attractive candidate for EMF intervention. Over the last several decades, numerous studies across the spectrum of EMF frequencies and intensities have identified various phenomena and functional responses of cells to EMFs [18,19]. The primary parameters for EMFs are the field strength and frequency. Low frequency fields (< 1 kHz) provide enough time for cells to respond and polarize to the incident field. High frequency fields (> 10 MHz) oscillate too rapidly for polarization or biological response, instead leading to generation of thermal energy due to dielectric heating. The dielectric heating phenomena can be seen in tumor ablation treatment, where high frequency electric fields superheat the tumor [20]. Lower frequency fields can cause thermal effects as well due to joule heating but require much higher field strengths depending on the conductivity of the medium. TTFields are considered a non-thermal therapy due to their intermediate frequency (~100 kHz) and low field strength (~1 V/cm) (**Fig. 1**).

Tumor treating field in vitro studies

One of the first studies which showed potential therapeutic benefit of an intermediate frequency electric field was that of Kirson et al. which showed the ability of a 100 kHz electric field to severely reduce the proliferative rate of multiple cancer cell types and consequently termed Tumor Treating Fields (TTFields) [3]. Since then, there have been many *in vitro* and preclinical studies replicating and expanding on this original manuscript (**Table 1**). Important to note is that the optimal frequency which elicits the greatest anti-proliferative effect varies between cancer cells type.
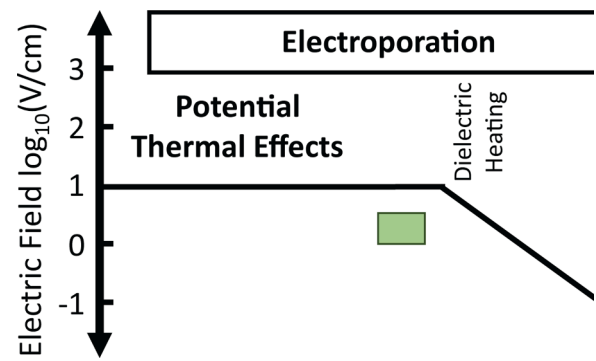

Fig. 1. Electric field parameter space for cellular interactions. The boundary for potential thermal effects due to joule heating is based on thermal generation of 500 mW/cm^3 for a media of 1 S/m conductivity ($E^2\sigma/2$). Dielectric heating boundary is based on a relative permittivty of 1000 ($2\pi f\varepsilon_0\varepsilon_r\ E^2$). Dielectric heating becomes dominant once freq $> \sigma/(2\pi\varepsilon_0\varepsilon_r)$.

The original proposed mechanism was the disruption of mitotic replication by way of interfering with the alignment of tubulin dimers [3]. A key part to this theory is the focusing of the electric field in the interior of the cell during the telophase and cytokinesis stage of cell replication. During telophase and cytokinesis stage of cell replication the cleavage furrow separates the two daughter cells. This cleaving results in a momentary narrow bridge between the two daughter cells that causes a focusing of the electric field. It is estimated that the electric field is magnified up to 10 to 20 times the exterior field strength (from 1 V/cm to 10 to 20 V/cm) [21–24]. In addition to the increase in the field strength there is also a significant gradient to the electric field near the bridge and therefore dielectrophoretic forces. These two phenomena are implicated in several mechanisms that involve the disruption of proper cell separation by preventing proper orientation of critical molecules such as tubulin and septin [25]. Tuszynski et al. provide an overview of the different possible interactions of microtubules with externally applied electric fields and the field strengths and frequencies that are required to impart significant forces [21]. It is possible that the variation in optimal frequency is due to the variation in cell size and electrical characteristics among different types of cancer cells. Further support to the cell separation being a key variable is the observation that utilizing perpendicular electrode arrays increased the efficacy of TTFields. Moreover, the increase in efficacy was dependent on the rate of switching between the two sets of electrodes [24].

While the observations on cancer cell replication impairment has been consistently reproduced *in vitro*, several new mechanisms of action have been proposed as well (**Fig. 2**), including inhibition of DNA repair [26,27]. Karanam et al. showed evidence of a reduction in gene expression within the BRCA1 pathway which led to an increase in DNA damage in non-small cell lung cancer cells [26]. This could be advantageous after radiation treatment which typically results in breakages in DNA. Inhibiting the ability of cells to repair DNA could improve the outcome of radiation therapy. The authors also point out that the variation in cell sensitivity to TTFields may implicate multiple mechanisms. It is not known how or when the DNA-repair inhibition takes place, but regardless, it is clear that there is more at play in the anti-proliferative effects than just microtubule disruption. Other proposed mechanisms from *in vitro* studies include localized heating, however this is based primarily on computer models [24].

Besides the anti-proliferative effect of TTFields, there have also been observations of inhibition of cell migration *in vitro* as well as a reduction in metastases in animal models [28–30]. As these processes are separate from replication, new mechanisms of TTField interaction have been proposed including a reduction in EMT markers and a downregulation of the PI3K/AKT signaling in the case of glioblastoma cancer cells [29]. It is important to point out that it was *in vivo* data which guided discovery of

Table 1
In Vitro TTFields Studies.

Primary Study	Refs.	Cell Type(s)	Key Observation(s)
Inhibition of Cell Replication	Kirson et al. [3]	Human melanoma (Patricia), glioma (U-118, U-87), lung (H-1299), prostate (PC3), and breast (MDA-MB-231). Mouse melanoma (B16F1) and adenocarcinoma (CT-26). Rat glioma (F-98, C-6, and RG2).	Reduction of cell proliferation rates due to field treatment. Effect is frequency and strength dependent. Cell destruction during cytokinesis. Tumor growth inhibition in mice using implanted wires.
	Giladi et al. [80]	Human ovarian carcinoma (A2780), lung adenocarcinoma (H-1299 and A549), pancreatic adenocarcinoma (AsPC-1), mesothelioma (NCI-H2052 and MSTO-211H), glioblastoma (U-118MG and U-87MG), cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF7 and MDA-MB-231), and rat glioblastoma (F98).	Treated cells display microtubule disruption across multiple cell types.
	Gera et al. [25]	Human cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7 and MDA-MB-231), colorectal carcinoma (HCT-116)	Reduced septin at midline during anaphase.
	Jo et al. [81]	Human melanoma (A375SM), mouse melanoma (B16F10), mouse embryo (NIH3T3)	Observed increase in DNA double strand breaks
	Huang et al. [82]	Human hepatoma (Huh7)	Reduced cell viability in spheroids
Combination Therapy	Schneiderman et al. [83]	Clonal derivative of Chinese hamster ovary cells (AA8). Human breast adenocarcinoma (MCF-7 and MDA-MB-231)	TTFields show efficacy on multi-drug resistant cells. Additive effect when combined with **doxorubicin** and **paclitaxel**, even on drug resistant cells.
	Giladi et al. [49]	Human NSCLC (H1299, A549, HTB-182, and HCC827). Mouse lung carcinoma (LLC-1) and squamous cell carcinoma (KLN205).	Additive effect when combined with **pemetrexed, cisplatin, paclitaxel**, or **erlotinib**.
	Giladi et al. [56]	Hamster pancreatic adenocarcinoma (PC-1.0). Human pancreas adenocarcinoma (AsPC-1 and BxPC-3).	Additive effect when combined with **gemcitabine, irinotecan, 5FU**, or **paclitaxel**.
	Voloshin et al. [60]	Human ovarian carcinoma (A2780), adenocarcinoma (OVCAR-3 and Caov-3). Mouse ovarian surface epithelium (MOSE).	Additive effect when combined with **paclitaxel.**
	Kim et al. [84]	Human glioblastoma (U-87 and U373)	Synergistic effect when combined with **radiation** therapy

Table 1 (*continued*)

Primary Study	Refs.	Cell Type(s)	Key Observation(s)
	Giladi et al. [27]	Human glioblastoma (U-118 and LN-18)	Synergistic effect when combined with **radiation** therapy
	Karanam et al. [26]	Human NSCLC (H157, H4006, A549, H1299, and H1650)	BRCA1 pathway down regulated. Additive effect when combined with **radiation**.
	Huang et al. [85]	Human hepatocarcinoma (Huh7)	Reduced viability of cells in spheroids. Additive effect when combined with **doxorubicin** due to spheroid dissociation.
	Kessler et al. [86]	Human glioblastoma (U-87 and GaMG)	Additive effect when combined with spindle assembly checkpoint inhibitor.
	Shteingauz et al. [87]	Human biphasic mesothelioma (MSTO-211H), pancreatic adenocarcinoma (AsPC-1), and gliomas (A172, U-87, LN229). Mouse squamous cell carcinoma (KLN-205), lung carcinoma (LLC-1). Rat glioma (F98).	TTFields upregulate autophagy. Additive effect when combined with autophagy inhibitor, **chloroquine**.
	Karanam et al. [88]	Human NSCLC (H157, H4006, A549, H1299, and H1650)	Additive effect when combined with **cisplatin** or **olaparib**. Further increase when combined with **olaparib** and **radiation** together.
	Voloshin et al. [78]	Mouse lung carcinoma (LLC-1), colon carcinoma (CT-26), and transformed ovarian epithelial (MOSE-L). Human hepatocellular carcinoma (HEPG2) and lung squamous cell carcinoma (H520)	TTFields induces autophagy and immune cell recruitment. Additive effect when combined with anti-PD-1 therapy.
Permeability	Chang et al. [89]	Patient derived glioblastoma (GBM2 and GBM39). Human glioblastoma (U-87). Mouse astrocytoma (KR158B). Human fibroblasts (PCS201)	TTFields increased permeability of cell membrane to small molecules (< 50 kDa)
Migration /Metastases	Kirson et al. [28]	Mouse malignant melanoma (B16F10). Rabbit squamous cell carcinoma (VX2)	Reduced lung metastases in mice melanoma and rabbit squamous carcinoma models treated with TTFields
	Kim et al. [29]	Human glioblastoma (U87 and U373)	Reduced invasion and migration. Reduction in EMT marker expression. Downregulation of PI3K/AKT/NF-kB signaling. Reduced HIF1alpha, VEGF, MMP9, and MMP2.
	Voloshin et al. [30]	Human lung adenocarcinomas (H1299 and A549)	Disruption of microtubule and actin cytoskeleton

Table 1 (*continued*)

Primary Study	Refs.	Cell Type(s)	Key Observation(s)
		and glioblastomas (U-87MG, A-172, LN-229, and LN-18)	resulted in decreased cancer cell motility.

alternate disrupted cell functions and pathways.

Tumor treating fields: clinical evidence

Following the original study by Kirson et al., several reports have documented the *in vitro* and *in vivo* efficacy of TTFields regardless of the cancer type, while bearing no significant effects on non-neoplastic cells and tissues (Table 1). These reports suggest that TTFields could be a cancer-agnostic therapeutic modality, especially since the proposed mechanisms of actions are not specific to a particular tumor. As a result, several clinical trials targeting various cancers were initiated. As TTFields is a spatio-anatomic therapeutic modality, the design of the apparatus involved the development of region-specific transducers arrays designed for the cranial, thoracic, and abdominopelvic cavities. Computational simulations have been published showing the feasibility and safety of delivering therapeutic intensities in target organs in these cavities [31–34]. Below is a summary of the published and ongoing clinical trials targeting these anatomical areas (Table 2).

Cranial cavity directed therapy

TTFields in glioblastoma multiforme

The initial evaluation of TTFields therapy in clinical trials was in treating Glioblastoma Multiforme (GBM) with a frequency of 200 kHz based on optimal frequency noted on glial tumor cell lines *in vitro* [35]. The position and orientation of the transducer arrays on the scalp were determined using the NovoTAL system [36,37]. In a pilot trial, 10 patients with recurrent GBM were treated with TTField monotherapy. The median time to disease progression was 6 months, and median overall survival (OS) was more than 14 months. Both values are more than double the reported historical controls and presented no safety concerns [38]. A subsequent phase III trial of TTFields in recurrent GBM (EF-11 trial) randomized 237 patients to receive either the chemotherapy-free arm with TTFields or physicians' choice, best active chemotherapy [39]. Median survival and progression-free survival (PFS) were analogous between the TTFields arm and chemotherapy. The 1-year survival rate was 20% in both arms while the PFS rate at 6 months was 21.4 and 15.1% ($P = 0.13$) in TTFields and active control patients respectively. No significant toxicities were reported in the TTFields arm, with only 2% of patients developing moderate rash at the transducer array site. The results of the EF-11 trial confirmed the safety and efficacy of TTFields therapy and led to US FDA approval of TTFields for recurrent GBM on April 8th, 2011. Since EF-11, a phase IV clinical study (EF-19) has confirmed the safety and efficacy of TTFields as a monotherapy for recurrent GBM [40].

Building on the favorable safety profile of TTFields, combinatorial therapy with chemotherapy was the next step. In the same pilot trial, 10
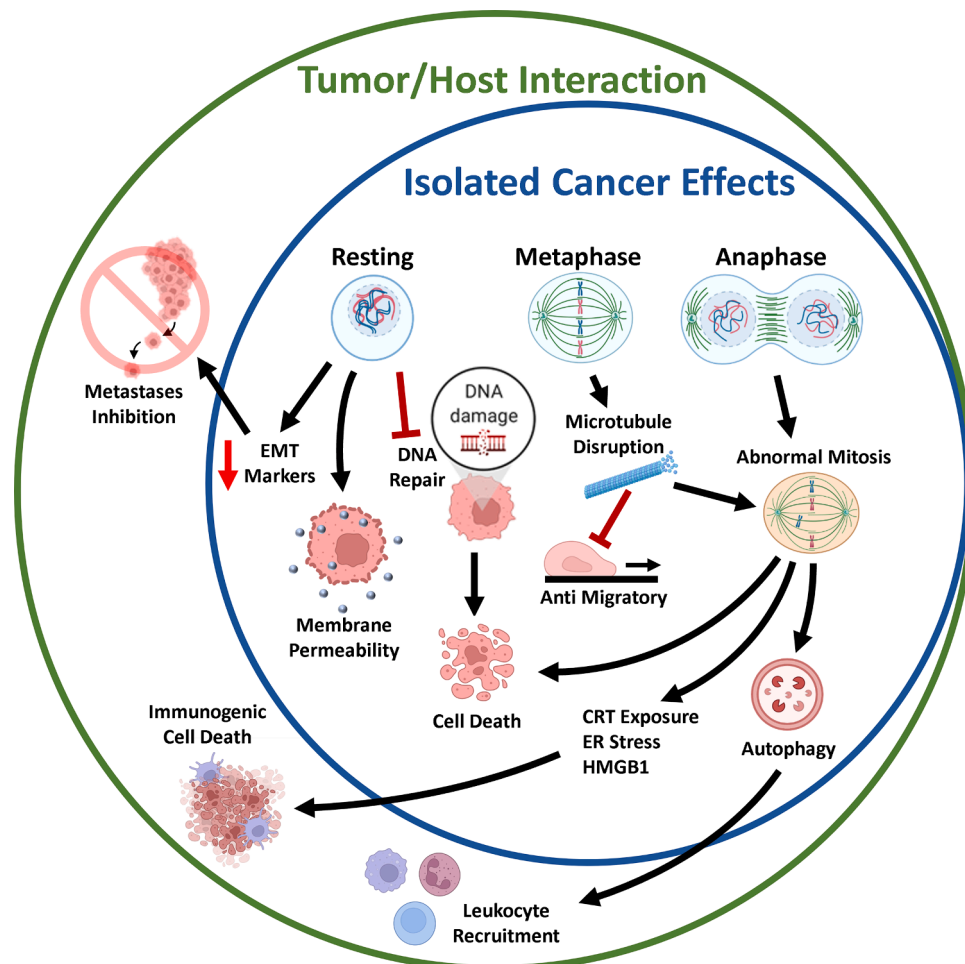

Fig. 2. Proposed mechanisms of action of Tumor Treating Fields. Isolated cancer effects are independent of the tumor/host interaction. Created with BioRender.com.

Table 2

TTField clinical trials.

	Ref.	Identifier	Phase	Study Name	Cancer Type	Arms	N	Outcomes
Cranial	[39]	NCT00379470	III	EF-11	Recurrent GBM	Chemo	117	6-mo PFS 15.1% *vs* 21.4% ($p = 0.13$); Median Survival 6.0 vs 6.6 mo ($p = 0.27$); 1-y Survival 20% *vs* 20%
						TTFields	120	
	[41]	NCT00916409	III	EF-14	GBM	TMZ	229	Median PFS 4.0 vs 6.7 mo ($p < 0.001$); Median OS 16.0 vs 20.9 mo ($p < 0.001$)
						TMZ + TTFields	466	
	[40]	NCT01756729	IV	EF-19	Recurrent GBM	TTFields	192	Median OS 7.4 vs 6.4 mo ($p = 0.053$)
						EF-11 BSC	117	
	[47]	NCT01755624	II	EF-21@@@@@ (COMET)	Brain Mets from NSCLC	BSC	18	No safety concerns
	[48]	NCT02831959	III	EF-25@@@@@ (METIS)	Brain Mets from NSCLC	TTFields	270	Ongoing
						BSC		
Thoracic	[51]	NCT00749346	I/II	EF-15	NSCLC	pemetrexed + TTFields	42	Median PFS 28 wks; Median Overall PFS 22 wks; OS 13.8 mo
	[52]	NCT02973789	III	EF-24@@@@@ (LUNAR)	NSCLC	ICI/Doc	534	Ongoing
						ICI/Doc + TTFields		
	[54]	NCT02397928	II	EF-23@@@@@ (STELLAR)	Mesothelioma	pemetrexed + carboplatin/cisplatin + TTFields	80	Median OS 18.2 mo; 1-y OS 62.2%; 2-y OS 41.9%; Median PFS 7.6 mo
Abdominal	[57]	NCT01971281	I/II	EF-20@@@@@ (PANOVA)	PDAC	gemcitabine + TTFields	20	Median PFS 8.3 vs 12.7 mo; Median OS 14.9 mo *vs* NR
						gemcitabine + nab-paclitaxel + TTFields	20	
	[59]	NCT03377491	III	EF-27@@@@@ (PANOVA-3)	PDAC	gemcitabine + nab-paclitaxel + TTFields	556	Ongoing
	[61]	NCT02244502	I/II	EF-22@@@@@ (INNOVATE)	Recurrent Ovarian	paclitaxel + TTFields	31	Median PFS 8.9 mo; Median OS NR
	[90]	NCT03940196	III	EF-28@@@@@ (INNOVATE-3)	Recurrent Ovarian	paclitaxel + TTFields	540	Ongoing
	[63]	NCT03606590	II	EF-30@@@@@ (HEPANOVA)	HCC	sorafenib + TTFields	25	Ongoing
		NCT04281576	II	EF-31	Gastric Cancer	XELOX + TTFields	28	Ongoing

other patients with newly diagnosed GBM who had undergone surgery and after that received adjuvant Temozolomide (TMZ) concurrent with radiation therapy were treated with the combination of TTFields and maintenance TMZ [38]. Again, there were no serious adverse effects observed. The only device-related toxicity reported was dermatitis, which appeared most often during the second month of treatment. Dermatitis was managed with topical corticosteroids and periodic electrode repositioning. Dermatitis resolved entirely within days to weeks from treatment termination. Notably, there was no increase in TMZ-related adverse events. These results led to the EF-14 trial, a phase III randomized trial evaluating TTFields combined with TMZ in patients with newly diagnosed GBM after completing chemoradiation [41]. A preplanned interim analysis revealed a significant benefit in PFS and OS for the combination [42]. This prominent benefit drove the independent data and safety monitoring committee to recommend early termination of the trial. Based on that, the FDA expanded the approval of TTFields to include the setting of newly diagnosed GBM following maximal debulking surgery and completion of radiation therapy together with standard of care chemotherapy on October 15th, 2015. The final results of this trial were published, confirming earlier results [43]. Based on the above trials, the use of TTFields has been incorporated into clinical practice and is currently recommended by NCCN and ASCO as a front-line treatment or recurrent disease.

TTFields in brain metastasis

Building on the success of TTFields in GBM, investigating its role in solid cancer brain metastasis became an area of significant interest. Brain metastasis is a devastating condition that complicates many solid tumors, with lung cancer as the leading cause [44]. Autopsy series found that brain metastases occur in as many as 64% of patients dying from lung cancer [45]. Moreover, a major clinical challenge is the cellular, molecular, and physical characteristics of the protective blood-brain-barrier (BBB) and blood-tumor barrier (BTB) restricts the penetration of many therapeutic agents into intracranial tumors [46]. With the preclinical evidence of TTFields activity in non-small cell lung cancer (NSCLC) cell lines, a pilot trial was initiated targeting patients with NSCLC who developed brain metastasis. The safety results of the first six patients were reported without any severe toxicities attributed to TTFields [47]. Based on that, a large pivotal randomized controlled trial known as EF-25 (NCT02831959) was started in July 2016 using the NovoTTF-100 M system in patients with 1–10 newly diagnosed brain metastases from NSCLC. TTFields at 150 kHz will be administered to patients concomitantly with the best standard of care treatments that would typically be used to treat lung cancer. The trial is expected to end in September of 2022 and enroll a total of 270 patients [48].

Thoracic cavity directed therapy

TTFields in lung cancer

Preclinical *in vitro* and *in vivo* evidence of TTFields treatment efficacy in lung cancer has been documented [49]. Based upon the reported findings, a pilot clinical trial (NCT00749346) for advanced NSCLC patients who are candidates for second-line therapy was conducted. Forty-two patients received pemetrexed concurrently with TTFields at 150 kHz frequency daily until disease progression [50]. The combination showed no increased toxicities and showed median PFS of 28 weeks and OS of 13.8 months, compared to 12 weeks and 8.2 months in historical controls receiving pemetrexed alone [51]. Since then, a large phase III randomized trial was started (LUNAR, NCT02973789) testing the efficacy of TTFields in combination with standards of care in the second-line therapy space targeting an initial number of 534 patients. Due to changes in the landscape of the standards of care therapy for NSCLC, the trial combined TTFields with either immune checkpoint inhibitors (PD-1 inhibitors nivolumab or pembrolizumab), docetaxel

versus immune checkpoint inhibitors, or docetaxel alone in patients with stage 4 NSCLC who progressed during or after platinum-based therapy [52]. A planned pre-specified interim analysis for the LUNAR trial performed on the first 210 patients led to the independent Data and Monitoring Committee (DMC) concluding that the trial should continue, as there was no evidence of increased systemic toxicity. The independent DMC recommended that continuing randomization to the control arm is unnecessary and possibly unethical. The DMC recommended reducing sample size to 276 patients, which will provide sufficient overall power for both primary and secondary endpoints.

TTFields in mesothelioma

In vitro experiments documented an anti-proliferative effect on mesothelioma cell lines at a frequency of 150 kHz, and finite element mesh simulations revealed that therapeutic-level distribution of field intensities ($\geq$ 1 V/cm) was demonstrated within the pleura and lung parenchyma in animal models [53]. Based on promising preclinical results in mesothelioma models, the STELLAR study (NCT02397928) was conducted in patients with unresectable malignant pleural mesothelioma. STELLAR was a prospective, multicenter, single-arm, phase II trial for treatment-naive patients with histologically confirmed malignant pleural mesothelioma who were not candidates for definitive resection. Patients received 150 kHz TTFields in combination with pemetrexed and cisplatin or carboplatin. Eighty patients were enrolled. Median overall survival was 18.2 months (95% CI 12.1–25.8). The 1-year overall survival was 62.2% (95% CI 50.3–72.0) and 2-year overall survival was 41.9% (28.0–55.2). Median PFS was 7.6 months [54]. Although no control arm was available, these results were considered favorable for several reasons. Compared to contemporary trials such as MAPS [55], the STELLAR trial outcomes numerically outperformed the control arm and were similar to the investigational arm despite including a higher percentage of patients of the poor prognosis non-epithelioid variant. Again, no significant systemic toxicities were attributed to TTFields.

Abdominal cavity directed therapy

The abdominopelvic cavity encompasses various gastrointestinal cancers, genitourinary cancers, and gynecological cancers. Optimizing TTFields therapy for the abdominopelvic cavity represents a significant challenge due to variations in body habitus and orientations of internal organs compared to the cranium and thoracic cavity. Clinical efforts targeting pancreatic cancer, hepatocellular carcinoma (HCC), and ovarian cancer are ongoing.

TTFields in pancreatic adenocarcinoma

Following extensive preclinical evidence of TTField efficacy in pancreatic cancer models [56], the safety and effectiveness of TTFields at 150 kHz in combination with chemotherapy in advanced pancreatic adenocarcinoma was tested in the PANOVA phase II trial (NCT01971281). The study enrolled 40 patients. Again, no systemic toxicity was attributed to TTFields. The only additional safety concern was a minimal percentage of grade 3 device-related dermatitis. The median PFS for the cohort receiving gemcitabine plus nab-paclitaxel and TTFields was 12.7 months (95%CI: 5.4-NA). The PFS at six months was 65%, the median OS was not reached, and the 1-year survival rate was 72% [57]. These values compare favorably with the historical control of the gemcitabine nab-paclitaxel regimen (PFS: 5.5, OS: 8.5, 1-Y OS of 35%) [58].

The results of the PANOVA trial led to the development of a phase III PANOVA-3 trial (NCT03377491) which is currently enrolling patients. The trial will test the efficacy of adding TTFields to nab-paclitaxel and gemcitabine combination in locally advanced unresectable pancreatic adenocarcinoma and is planned to enroll 556 patients [59].

TTFields in ovarian cancer

Preclinical studies have shown optimal efficacy for TTFields on ovarian cancer cell lines at 200 kHz [60]. A phase II trial was conducted in 31 heavily pretreated recurrent platinum-resistant ovarian cancer patients, where patients received 200 kHz TTFields in combination with weekly paclitaxel [61]. The average number of prior lines of therapy was four, and almost all patients had received prior taxane-containing regimens. No serious adverse events were attributed to TTFields. Some patients developed mild to moderate skin irritation. The median PFS was 8.9 months (95% CI, 4.7–NA). The median OS was not reached. These results were considered encouraging and led to the development of a large pivotal phase III randomized trial where 540 patients with recurrent platinum-resistant ovarian cancer will be randomized to weekly paclitaxel or the same treatment combined with 200 kHz TTFields.

TTFields in hepatocellular carcinoma

TTField efficacy in multiple HCC cell lines and murine models was found to be optimal at 150 kHz combined with sorafenib [62]. A prospective, phase II single-arm study was performed (HEPANOVA trial) that enrolled 25 patients with HCC receiving sorafenib and TTFields at 150 kHz [63]. The primary endpoint was the overall response rate. The interim safety data for the first 9 patients were presented and again showed no unanticipated severe toxicities related to the combinations [64]. It should be noted that most of these patients were again heavily pretreated, including prior use of sorafenib.

Discerning potential in vivo mechanisms in advanced in vitro models

In most solid tumors, malignant cells co-exist with noncancerous host tissue comprised of a variety of extracellular matrix components and cell types, notably fibroblasts, immune cells, and endothelial cells [65]. It is becoming increasingly evident that the non-cancerous host tissue, often referred to as the tumor stroma or the tumor microenvironment, wields tremendous influence in the proliferation, survival, and metastatic ability of cancer cells [66]. It has also recently become clear that electric signals could play a role in the development and metastatic spread of the primary tumor [16]. While TTFields show promise as an alternative or adjuvant therapy for cancer, most of the theories as to the mechanism of interaction between TTFields and cancer cells have been identified *in vitro* in single cell or 2D platforms. The observed efficacy *in vivo* may be a result of more complex interactions. Due to limitations in the design of conventional *in vitro* experiments, it is difficult to predict how the *in vivo* environment will alter the efficacy of treatment. Additionally, the inclusion of other host cells may highlight an alternative mechanism of interaction. There is a clear need to develop more complex platforms to better predict outcomes *in vivo*. This same issue plagues the identification of viable pharmaceutical treatments of cancer and has spurred the development of more complex platforms to better predict treatment efficacy [67].

Recently, considerable progress has been made in 3D culture technology, including the use of stem cell-derived, self-organizing, and multicellular constructs known as organoids [68]. Organoid technology has been applied to model various human pathologies "in-a-dish," including numerous types of gastrointestinal cancers [69]. Moreover, patient-derived organoids hold much promise to predict therapeutic responses for personalized medicines [70]. However, one challenge with traditional 3D culture of organoids is achieving complete, *in vivo*-like organoid development in a reproducible manner. Proper organoid formation requires sequential addition of growth factors, but conventional 3D culture platforms are highly constrained in their ability to precisely control the local environment of instructive cues for organogenesis [71, 72]. It has been proposed that microfabricated cell culture platforms, commonly referred to as "organs-on-a-chip," can significantly augment organoid cultures by providing improved control of the biochemical and biophysical microenvironment [73]. Moreover, organs-on-a-chip are scalable platforms that are conducive for high throughput screening of candidate drug compounds [74]. In addition, multicompartment organs-on-a-chips can simulate multi-organ interactions, including

metastasis of a primary tumor to distant secondary sites, within a single interconnected microdevice [74]. Finally, while therapeutic applications organs-on-a-chip have focused primarily on drug testing and toxicity screening, there is increasing interest in applying organs-on-a-chip to support the development and testing of medical devices (*i.e.*, "medical-device-on-a-chip") [74]. One such application could be for mechanistic studies that elaborate the biological effects of TTFields. The continued study of TTFields should take advantage of these newly developed platforms to challenge the various hypotheses of TTFields interactions.

Most of the focus of TTField studies *in vitro* has been on their impact on cancer cell viability with evidence that TTFields have no significant impact on non-replicating cell viability [3]. It is now well established that the tumor microenvironment is as unique as the cancerous cells themselves and is heavily involved in the survival, propagation, and dissemination of the primary tumor. For instance, stromal cells have been shown to have an impact on the development and dissemination of tumor cells [75]. However, it is unclear what impact TTFields have on stromal cells, and more importantly tumor associated stromal cells (TASCs). While it has been shown that TTFields do not negatively impact non-cancerous cells in regards to viability [76], there is still the question of whether they may impact other cell functions such as cell-to-cell communication or migration. *In vitro* studies using co-culture of stromal cells and cancer cells to study interaction mechanisms could explore whether TTFields can disrupt this same crosstalk.

Immune cells are an important subgroup of the stromal cells in the microenvironment. Park et al. have shown that TTFields stimulate macrophages to produce cytokines and reactive oxygen species which were able to decrease cancer cell viability [77]. Along those same lines, it was observed *in vivo* that TTFields increased prevalence of CD4, CD8, and CD45 T-cell positive cells around and within the metastases [28]. TTFields have also shown to work synergistically with anti-PD-1 treatment, implicating an immunostimulatory effect [78]. It is still unclear whether the changes observed on immune cells *in vitro* and *in vivo* are responsible for the clinical outcomes. The impact of TTFields on host-immune system and tumor interaction should continue to be studied using novel *in vitro* tumor-on-a-chip platforms [79].

Conclusion

The nonspecific anticancer mechanism of action and the consistently favorable toxicity profile of TTFields represent a solid basis for their utilization as a cancer agnostic modality in various combination regimens. The highly anticipated results of the ongoing phase II & III trials detailed above for thoracic and abdominal cavity cancers have the potential to be practice-changing, as was the case in the GBM studies. Future directions in the study of TTFields will focus on biologically optimized treatment combinations to harness the maximum benefit of the modality. Deeper understanding of intracellular responses to TTFields, such as the induced autophagy as a survival mechanism, could potentially lead to effective combinations with autophagy inhibitors to prevent TTFields resistance. Additionally, better delineation of the tumor-host interactions upon TTFields exposure, such as the immuno-modulatory effect of TTFields, opens the door to exploring immune checkpoint inhibitors combinations with TTFields. Concurrent TTFields investigation in the perioperative setting in the cancers mentioned above represents an area of high interest; specifically, multiple *in vitro* and xenograft models suggest a reduction in migration and metastasis. This apparent inhibition of metastasis is highly attractive for future clinical trials in that space. Moving forward it is critical that experiments are carried out which attempt to characterize these additional mechanisms utilizing *in vitro* platforms which recapitulate the *in vivo* environment. Ultimately, understanding the impact of TTFields across all stages of cancer will aid in optimizing the way in which TTFields are applied clinically for different types of cancers.

CRediT authorship contribution statement

Travis H. Jones: Conceptualization, Writing – original draft, Writing – review & editing. **Jonathan W. Song:** Writing – review & editing, Supervision, Funding acquisition. **Laith Abushahin:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Authors Travis Jones and Jonathan Song have submitted an invention disclosure on technology related to non-contact electric field treatment. The basis of this technology has been published previously by the authors: https://doi.org/10.1089/bioe.2020.0048; https://www.nature.com/articles/s42003–019–0550-z

Laith Abushahin submitted a letter of intent for a clinical trial that will be funded by Novocure. No role for Novocure in conceptualization, data collection, decision to publish, or preparation of the manuscript.

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