Peer Review File

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Reviewer A

- The manuscript by Kutuk et al. summarizes the current literature regarding Tumor Treating fields (TTFields). The manuscript provides the most up to date, comprehensive review of this emerging field while describing both the underlying mechanism of action as well as the clinical outcomes. I find this review to be relevant for researchers and clinicians who would like to be introduced to TTFields and understand both the physical basis of this modality, the biological outcome and clinical landscape.

We sincerely appreciate the reviewer's thoughtful and positive feedback on our manuscript regarding TTFields.

Minor comments:

- The authors defer to TTFields in the abstract as "electrical fields that disrupt cell division in cancer cells" yet, in the body of text they mention a plurality of effects induced by TTFields. It would be beneficial for the reader to understand already early in the manuscript that TTFields affects many processes in cancer cells.

We thank the reviewer for this comment. We have revised the sentence in the abstract as follows:

Abstract introduction: Tumor Treating Fields (TTFields) therapy has emerged as a potentially effective treatment for various malignancies by delivering low-intensity, intermediate-frequency electrical fields that disrupt many processes inside cells, resulting in the interruption of cell division in cancer cells. Additionally, TTFields therapy has been found to be synergistic with existing therapeutic approaches.

- Title - Suggest using only one of "emerging potential" or "promising treatment."

Thank you for the valuable feedback. Based on the suggestion, we have revised the title as follows:

Title: Tumor Treating Fields: Narrative Review of a Promising Treatment Modality for Cancer

- The authors need to distinguish through the text between “TTFields” as a modality and the therapy which is the treatment provide to the patients referred to as “TTFields therapy.”

We appreciate the reviewer's insightful comment regarding the need for a clear distinction between "TTFields" as a modality and the actual therapeutic intervention known as "TTFields therapy." In response, we have carefully revised the text to consistently use "TTFields" when referring to the modality and "TTFields therapy" when discussing the practical application of this treatment for patients, ensuring that readers can easily differentiate between the two terms throughout the manuscript.

- In the introduction, the authors mention multiple effects of TTFields “Extensive evidence demonstrates that TTFields impede mitosis, disrupt the cell cycle, induce autophagy and apoptosis in cancer cells, hinder DNA repair mechanisms, increase membrane permeability and impair cell migration, thereby effectively suppressing tumor growth and invasion” yet they seem to skip a major downstream effect of TTFields which is the immune activation.

We appreciate the reviewer's feedback, and we have revised the sentence as follows:

Introduction: Extensive evidence demonstrates that TTFields impede mitosis, disrupt the cell cycle, induce autophagy and apoptosis in cancer cells, hinder DNA repair mechanisms, augment anti-tumor immune response, increase membrane permeability, and impair cell migration, thereby effectively suppressing tumor growth and invasion.

- The manuscript would benefit if the following topics will be discussed:

- 5 years survival rate (Stupp et al., 2017, Toms et al., 2018)
- progression patterns (Glas et al., 2022)

We thank the reviewer for the comment. We have incorporated the results of these studies into our manuscript.

TTFields Therapy for GBM, 3rd paragraph: The updated findings of the EF-14 trial further revealed a significant improvement in 5-year survival rate with the addition of TTFields to TMZ, with consistent benefits observed across all patient subgroups (13% vs 5%, $p = 0.004$). In the TTFields + TMZ arm, the median PFS was 6.7 months compared to 4.0 months in the TMZ-alone arm ($p < 0.001$), and the median OS was 20.9 months compared to 16.0 months ($p < 0.001$) (Stupp et al., 2017). This study also included a survey to evaluate the quality of life, conducted every 3 months, and the results revealed no worsening in both the short and long term (Zhu et al., 2017). Similar to the EF-11 trial, subsequent analysis of the EF-14 trial revealed that compliance levels of 50% or higher led to a notable enhancement in both PFS and OS. Even more, when the compliance rate exceeds 90% the median OS reaches to 24.9 months, accompanied by an encouraging 5-year survival rate of 29.3% (Toms et al., 2019). The degree of treatment adherence and higher electric field intensity applied to the tumor bed were identified as predictive factors for treatment outcome (Ballo et al., 2019). Furthermore, Kesari et al. published the post-hoc analysis of the EF-14 trial, including 204 patients experiencing recurrence. Findings demonstrated that combining TTFields with chemotherapy after the first recurrence significantly extended the median OS to 11.8 months, compared to 9.2 months with chemotherapy alone ($p = 0.049$, HR: 0.70; 95% CI) (Kesari & Ram, 2017).

- In the introduction (lines 78-79), the authors write “The optimal frequency of TTFields depends on the specific cancer type and is influenced by the doubling time of malignant cells (Porat et al., 2017).”. This claim is not supported by the literature but rather that rapidly dividing cells are more sensitive to treatment (Giladi et al., 2015).

We appreciate this recommendation from the reviewer. Following the suggestion, we have removed the "and is influenced by the doubling time of malignant cells" part to avoid any potential misunderstanding.

- Lines 102-104: the authors state that “Notably, in non-uniform electric fields characterized by converging lines of force, the intensity of the electric field becomes amplified at smaller electrodes. This phenomenon, known as dielectrophoresis”. While dielectrophoresis is indeed an important out of TTFields application to dividing cells, the claim that this effect will be amplified through the use of smaller electrodes is not supported by literature.

Thank you for this important comment from the reviewer. Based on the recommendation, we have removed the reference to “smaller electrodes” from the text.

- Lines 105-107 the authors state that “During cell division, the non-uniform electric field produced by dividing cells can influence the localization of polar components towards the cleavage furrow between the two daughter cells”. This sentence is misleading as it gives the impression the electric fields are produced by dividing cells while in fact they are produced inside the cells.

We thank the reviewer for this comment. In response to the recommendation, we have revised the sentence as follows:

TTFields mechanism of action 1st paragraph: This phenomenon, known as dielectrophoresis, drives the movement of polar cellular components towards regions of higher field intensity. During cell division, the non-uniform electric field produced inside the cells can influence the localization of polar components towards the cleavage furrow between the two daughter cells.

- Lines 142-142: there seems to be a switch in the order of the in vitro and in vivo. Suggest verifying.

Thank you very much for this feedback. Based on the feedback, we have switched the order and revised the text as follows:

Changes in membrane permeability (nuclear membrane, cell membrane, blood–brain barrier) 1st paragraph: Moreover, TTFields have been shown to reversibly weaken the blood-brain barrier (BBB) in both in vivo (rat models) and in vitro (murine cerebellar microvascular endothelial cells, cerebEND) as well as in a 3D co-culture model of the BBB.

- Chapter 3. “Immune regulation” fails to mention the activation of cGAS-STING and Aim2 and the inflammasome. This effect is discussed under chapter 5 “DNA damage signaling pathways” which is less relevant. Consider revising.

We appreciate the reviewer's comment. We have replaced cGAS-STING and Aim2 under the immune regulation chapter.

- Chapter 5 “DNA damage signaling pathways” would benefit if the 2 papers by Karanam describing the induction of a state of BRCAness, will be mentioned (Karanam et al., 2017, Karanam et al., 2020).

We thank the reviewer for this recommendation. We have incorporated the results of the advised papers and revised the text as follows:

DNA damage signaling pathways 1st paragraph: Karanam et al. revealed that exposure to TTFields leads to the downregulation of the BRCA1 signaling pathway, which plays a key role in repairing DNA double-strand breaks. In addition to slowing down the pace of DNA damage repair, the observed accumulation of γ-H2AX foci, colocalized γ-H2AX/53BP1 foci, and increased occurrence of chromatid-type aberrations supported the idea that TTFields also induce replication stress (Karanam et al., 2017). Moreover, TTFields potentially lead to DNA damage through a reduction in the expression of crucial replication genes (MCM10 and MCM6). The study also highlights the impact of TTFields on R-loops, which are unique nucleic acid structures formed during transcription, playing a role in gene expression regulation. TTFields exposure amplifies R-loop formation, and the persistence of R-loops leads to DNA damage. BRCA1 and BRCA2 play vital roles in resolving these R-loops, and their depletion amplifies DNA damage (Karanam et al., 2020).

- Line 194: references to EF-11, PRIDE, EF-14 studies are missing.

Thank you for the comment. We have added the missing references.

- When discussing EF-14, it would be good to mention QoL in this study.

We thank the reviewer for this recommendation. We have incorporated the sentence as follows:

TTFields Therapy for GBM, 3rd paragraph: This study also included a survey to evaluate the quality of life, conducted every 3 months, and the results revealed no worsening in both the short and long term.

- Line 246: latest NCCN guidelines (v1.0 2023) it is indicated as preferred regimen in combination with TMZ and radiotherapy in ndGBM

We appreciate the reviewer's feedback. We have updated the sentence as follows:

TTFields Therapy for GBM 3rd paragraph: As a result, the National Comprehensive Cancer Network (NCCN) guidelines (v1.2023) currently recommend the use of TTFields therapy in combination with TMZ and radiotherapy as a preferred regimen for postoperative adjuvant treatment option for patients with newly diagnosed GBM as a category 1 recommendation (Network, 2023) (Horbinski et al., 2023), yet its utilization is variable across institutions and geographic regions (Andrew B Lassman et al., 2020).

- Line 250: the manuscript would benefit from mentioning also the effect demonstrated when TTFields were applied beyond first progression (Kesari et al., 2017).

We appreciate the reviewer's feedback. We have incorporated the results of the advised paper and revised the text as follows:

TTFields Therapy for GBM 3rd paragraph: Furthermore, Kesari et al. published the post-hoc analysis of the EF-14 trial, including 204 patients experiencing recurrence. Findings demonstrated that combining TTFields with chemotherapy after the first recurrence significantly extended the median OS to 11.8 months, compared to 9.2 months with chemotherapy alone ($p = 0.049$, HR: 0.70; 95% CI) (Kesari & Ram, 2017).

- The manuscript would benefit from citing the recent Ballo paper (2023) providing meta-analysis with systematic review on RWE.

We appreciate the reviewer's feedback. We have incorporated the results of the advised paper and revised the text as follows:

TTFields Therapy for GBM 3rd paragraph: Recent data from a meta-analysis, drawing from nine different studies involving 1430 ndGBM patients, provides real-world data demonstrating improved OS with TTFields alongside standard care compared to standard care alone. Moreover, consistent device usage of more than 75% is associated with prolonged survival, highlighting the therapy's effectiveness when used diligently (Ballo et al., 2023).

- Line 301: the final outcomes are now published: https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(23)00344-3/fulltext

We thank the reviewer for this comment. We have edited the text as follows based on the full text of the LUNAR study.

TTFields Therapy for Non-Small Cell Lung Cancer 1st paragraph: The study included 276 patients, and its recently published findings revealed a significant improvement in median OS when TTFields were added to the SOC (13.2 months vs. 9.9 months, p = 0.035, HR (95% CI): 0.74). Moreover, in the subgroup of patients who received a combination therapy of ICI and TTFields, a more pronounced increase in OS was observed when compared to those receiving ICI alone (18.5 months vs. 10.8 months, p = 0.030, HR (95% CI): 0.63). In the subgroup of patients who received DTX, the addition of TTFields to the treatment regimen resulted in a slight increase in OS (11.1 months vs. 8.7 months, p = 0.28, HR (95% CI): 0.81). These results suggested that the addition of TTFields to the standard treatment regimen significantly improves OS, especially when combined with ICI. This trial provided valuable information on the efficacy of combining TTFields with standard therapies in stage IV NSCLC patient population (Leal et al., 2023).

- Line 329: when discussing MPM, consider mentioning the approval route through which TTFields were approved (humanitarian device exemption).

We thank the reviewer for this comment. We have added the following section to our manuscript.

TTFields Therapy for Malignant Pleural Mesothelioma 1st paragraph: After the STELLAR study, the FDA approved TTFields in combination with pemetrexed and a platinum-based

chemotherapy via the Human Device Exemption (HDE) pathway for patients with unresectable, locally-advanced or metastatic MPM in 2019.

- The manuscript would benefit from providing some reference to guidance on how to manage TTFields skin AE (thorax: https://pubmed.ncbi.nlm.nih.gov/36703794/; scalp: https://pubmed.ncbi.nlm.nih.gov/32850308/)

We appreciate the reviewer's feedback. We have added the following section to our paper.

Dermatological Adverse Events

The main adverse events associated with TTFields predominantly manifest as dermatologic issues, particularly in areas where the skin directly interfaces with the arrays. These events encompass a broad spectrum, ranging from mild dermatitis to skin ulcers and secondary soft tissue infections (Lacouture et al., 2020). Extra caution should be exercised when employing combination therapies. For instance, bevacizumab may delay wound healing, while neutropenia and thrombocytopenia resulting from TMZ can make the skin more prone to secondary infections and bleeding (Lacouture et al., 2016). The type of adverse event and the severity of its manifestations define the appropriate intervention. On the other hand, maintaining clean and dry skin under the arrays is fundamental as a prophylactic approach (Anadkat et al., 2023).

- Table 3: Panova-2. only values of one arm are shown.

We thank the reviewer for the comment. We have revised the Table 3 as follows:

PANOVA-2	NCT01971281	PDAC	17	II	2	Gemcitabine + TTFields vs Gemcitabine + nab-paclitaxel + TTFields	PFS: 8.3 mo OS: 14.9 mo vs PFS: 12.7 mo OS: not reached yet

- Table 4: TRIDENT- unclear to the reader. the two arms look identical the way it is written.

Thank you very much for the feedback. We have corrected the typo as follows:

TRIDENT (EF-32)	NCT04471844	ndGBM	950	III	2	RT + TMZ + TTFields vs RT + TMZ

- Table 4: TIGER and TIGER Pro-ACtive should be mentioned.

We thank the reviewer for this feedback. We have incorporated the following information into the main text.

TTFields Therapy for GBM 4th paragraph: Two other studies from Germany, the TIGER study (NCT03258021) and the TIGER PRO-Active Study (NCT04717739), have evaluated TTFields with respect to quality of life (QoL). According to the presented results of the TIGER study, TTFields did not negatively impact QoL, with the exception of a higher incidence of skin itchiness. Results from the TIGER PRO-Active study are expected in 2024 (Bähr et al., 2021; Glas et al., 2022).

Reviewer B

- The authors present a narrative review on TTFields for cancer. It is yet another review among many existing in the literature, although it presents updated information over a 23-year period.

We appreciate the reviewer's feedback. Our narrative review aimed to provide a comprehensive and up-to-date perspective by encompassing 23 years of research and clinical developments, consolidating the latest insights into this evolving field. We believe this approach will offer readers a valuable and timely resource for understanding the current landscape of TTFields in cancer therapy.

Major concerns:

1) The article selection criteria have not been defined and the authors should present a flow chart of the article selection process and further elaborate on the exact selection criteria. It is not possible to repeat the search process.

We conducted this narrative literature search by utilizing search engines such as PubMed, Google Scholar, and ClinicalTrials.gov. The selection of articles was a collaborative process involving all authors, with a specific focus on identifying articles with potential clinical practice benefits. We prepared Table 2 in accordance with the journal's writing guide to display our search strategy. We have revised the Table 2 as follows:

Table 2. The search strategy summary	
Date of Search	01.06.2023- 05.06.2023
Databases	PubMed, Google Scholar, Clinical Trials.Gov
Search terms	Tumor treating fields or TTFields or TTF and Glioblastoma, Non-Small Cell Lung Cancer, Malignant Pleural Mesothelioma, Mesothelioma, Brain Metastasis, Brain Metastases, Pancreatic Cancer, Pancreatic Ductal Adenocarcinoma, Hepatocellular Cancer, Gastric Cancer, Ovarian Cancer, Liver Metastasis, Solid Tumors, Malignant Melanoma, Breast Cancer, Colorectal Cancer
Timeframe	01.01.2000 – 01.06.2023
Inclusion and exclusion criteria	Studies that were written in any language other than English excluded
Selection process	All authors conducted the selection together. Consensus obtained in accordance with possible benefits to the clinical practice

2) The scope of the review is very extensive, spanning mechanisms of action to clinical trials across multiple diseases, and it is unclear what the exact aim and motivation of the study is. What gap in the literature do the authors want to cover?

We appreciate the reviewer's thoughtful comment regarding the scope and aim of our narrative review. Our primary motivation was to provide a comprehensive resource that spans the breadth of TTFields research, from mechanisms of action to clinical trials, across various diseases. By doing so, we aimed to bridge the gap in the literature by offering a holistic view of TTFields' evolving landscape. Our intention was to assist both researchers and clinicians in gaining a thorough understanding of this therapy, its mechanisms, and its clinical applications and provide

a comprehensive and up-to-date synthesis of TTFields research, facilitating informed decision-making in clinical practice and inspiring further exploration in this promising field..

3) Although the review is well-written, it is too extensive and long.

We appreciate the reviewer's feedback regarding the length and comprehensiveness of our review. We understand the importance of balancing depth with readability. In response, we have taken the opportunity to carefully streamline and condense the content where possible, aiming to maintain clarity and focus on the most crucial aspects of TTFields research.