**JoVE58937 Review Comments Eric T Wong, MD, October 16, 2018**

**Editorial comments:**

Changes to be made by the author(s) regarding the written manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Thank you.

2. Please revise lines 80-84, 128-130, 433-435, and 501-503 to avoid previously published text.

The sentence in lines 80-84 has been referenced.

The sentence in lines 128-130 has been changed to “Since TTFields represent an additional anti-cancer treatment modality with few toxicities, neuro-oncologists should consider incorporating this therapy into current treatment regimens for both newly diagnosed and recurrent glioblastoma.”

The lines 433-435, “For proper treatment planning, the MRI images of the patient's brain must include the margins of the scalp. To ensure adequate contact between electrode and scalp, the hair stubbles must be shaved down to surface of the scalp until no hair remains.”, are original texts.

For lines 501-503, the wording in that paragraph is changed to “Furthermore, the side effects are unknown when the patient is using TTFields concurrentlyContraindications include use in patients with an active implanted device, such as deep brain, spinal cord, or vagus nerve stimulators, defibrillators, and cardiac pacemakers, or patients with a metallic fragment (i.e. bullet) or apparatus (i.e. aneurysm clip) in the brain.” The rest of the paragraph consists of original text.

3. Please revise the title to avoid punctuation.

The title is changed to “Clinical Application of Tumor Treating Fields Therapy”, which is more consistent with the subject matter in the submission.

4. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution’s human research ethics committee.

The application of TTFields therapy in the protocol steps is part of medical practice. Written consent was obtained from the patient using our institution’s authorization form and this is enclosed.

5. Please revise the protocol to be a continuously numbered list. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

This is done.

6. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

1.1.2 This is changed to “Incomplete delineation of the full thickness of the scalp interferes with the electric field calculations”

7. Please revise the protocol (lines 292-310, 316-325, etc.) to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

The following changes are made in the protocol sections:

1.1.2 “The MRI scan includes the margins of the scalp for treatment planning.”

1.3.6 “To achieve an optimal response, TTFields therapy is used on a continuous basis for a minimum compliance of 75%, or 18 hours a day”

1.4.3 “Treatment is discontinued until ulcer heals or infection clears”

2.1.1 “Dexamethasone is weaned in a stepwise fashion due to its hysteresis effect”

2.1.3 “The dose is cut in half quickly every 7-10 days to achieve a daily dose of to 4 mg/day”

2.1.4 “If signs of unacceptable neurologic deficits and/or adrenal suppression appear, the previous dose of dexamethasone is re-applied”

2.1.5 “Other means for reduction of dexamethasone is sought (see concurrent bevacizumab administration)”

2.2.2 “Before treatment, make sure the patient has acceptable blood counts, kidney function, normal blood pressure and urine dipstick protein <100 mg/dL”

2.2.3 “Once the patient is deemed to be an acceptable candidate, bevacizumab is administered at a dose of 2.5, 5.0 or 10 mg/kg”

“TTFields treatment is started either before or after initiation of bevacizumab”

2.2.4 “If there is no adverse event, subsequent doses can beis administered over 30 minutes”

8. Line 175: What are the inclusion and exclusion criteria for participating patients?

This patient is being treated in routine clinical practice and is not under a research protocol.

9. Line 177: Please do not number “Note”.

This is done.

10. Lines 180-189: How to make these measurements, by software? Please specify.

This is specified in 1.1.3 as “Using axial T1 sequence MRI scans and the tools on the DICOM image viewer, take baseline measurements of the front to back, right to left, and right to midline based on axial view head size (mm).”

11. Line 262: Please number the steps continuously; i.e., start with 5.

We numbered the two big sections in the protocol:

1. Application of the Second Generation TTFields Device
2. Removal of Systemic Agents that May Interfere with Anti-Tumor Immunity

Line 262 should be the second section.

12. Line 267: Please describe how this is actually done. Probably include here the information in lines 272-274.

The description of the dexamethasone weaning procedure is outlined in section 2.1.3.

13. Line 269: What dose of trimethopreme-sulfamethaxazole is applied?

This is clarified in 2.1.2 as “(400 mg-80 mg single-strength tablet daily or 800 mg-160 mg double-strength tablets 3 times per week)”

14. Are the videos of blebbing of cells during mitosis going to be included in the manuscript? If yes, please list them in the figure legends and reference them in the manuscript.

Yes, the videos will be included in the manuscript to help the reader understand the mechanisms of action of TTFields.

15. References: Please do not abbreviate journal titles.

Done.

16. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

Done.

**Reviewers' comments:**

**Reviewer #1:**

Manuscript Summary:

The manuscript "Tumor Treating Fields Therapy: Cell Biology, Electric Field Modeling, and Clinical application" submitted by Riley MM et al. describes a method how to clinically apply TTFields to treat patients suffering from glioblastoma. TTFields have been proven to be effective in the EF-14 clinical trial and are approved for glioblastoma therapy. Their usage is spreading in the clinic and therefore, a comprehensive instruction how to correctly place the electrodes for their application is reasonable. The authors describe in detail how to plan the treatment, how to apply and exchange the arrays and how to combine the treatment with Bevacizumab. In addition, they provide cell culture data showing that TTFields interfere with mitotic processes. Although the manuscript is well and comprehensively written, I have some major concerns which should be addressed by the authors in a revised version of the manuscript.

Major Concerns:

1. The presented "cell biology" results showing that TTFields interfere with mitotic processes are not related at all to the method described. They are rather background information and therefore could be removed completely from this manuscript. In addition, these data are not new. That TTFields disrupt mitosis has been published by others several times, originally by Giladi M et al., Sci Rep 2015, 5: 18046-18055. In addition, it is neither described how these data were generated, nor which cells were used. However, since the provided life imaging of cell divisions with and without TTFields application are new and since I assume they were generated using the "innovitro life" system, I would suggest to describe this method and generated life imaging data in a separate manuscript.

We noted this comment Reviewer 1. We respectfully disagree with Reviewer 1 that this background information on TTFields should be removed from this manuscript. In our view, this background information is rather important for clinicians to appreciate, first, the importance of applying the treatment consistently rather than intermittently so that TTFields are present at the time when the tumor cells are undergoing mitosis. Second, it is also important for the clinician to understand that the blebbing process stresses the tumor cells in a way that makes them vulnerable for immune clearance. As a result, the patient should avoid immunosuppressive drugs like dexamethasone, everolimus and sirolimus if possible, as well as to utilize bevacizumab as a steroid sparing agent. In the literature, there is no article that comprehensively address these issues in the context of treating patients with glioblastoma.

2. JoVE published an article by Oman, A. in 2014 regarding the use of tumor treating field therapy in combination with bevacizumab for the treatment of recurrent glioblastoma. This article has not been cited although the protocols outlined in Lines 197- 223 (Applying Transducer Arrays to Scalp), Lines 226-248 (Assembling the TTFields Device System) as well as Lines 283- 310 (Concurrent Bevacizumib Administration) are very similar to the 2014 article. Following on that, in general, there appears to be not much difference at all between the previous article and the article being reviewed apart from the "Cell Biology" methods which are not actually a part of the outlined protocol.

In the publication by Omar AI in 2014, he described placement of the arrays on a plastic head model. In patients there are nuances with respect to the placement of the arrays and we have pointed them out in the protocol. Specifically, we stated that the first array should be placed on the side with the surgical scar because placement of this array is most complicated. The disks should not exert pressure on the surgical scar or the underlying metallic hardware. The plastic head model does not have a scar but our patient does. The subsequent arrays should be placed in a clockwise or counterclockwise fashion. We will show the specific procedures in our video. Furthermore, there was no mention of the treatment planning software in Omar’s article. This is important from our standpoint because quite often the MRI images of the head do not include the full thickness of the scalp. When that occurs, the array configuration may not be optimized.

Minor Concerns:

1. The title leans more towards a review article title than a protocol / methods paper. "Cell Biology" methods are not actually included, thus, can be misleading. Although lines 94-115 discusses the mechanisms of tumor treating fields and representative results are provided , the figure given in the results section, lines 371-378, does not explain how the data were achieved. Thus, it might be more appropriate to focus the title on the method described.

We appreciate Reviewer 1’s comment. Therefore, we changed the title to “The Clinical Application of Tumor Treating Fields Therapy in Glioblastoma”.

1. Section 4 "Exchange of Transducer Arrays", lines 249-258. In our institution after washing the scalp and checking the skin for alterations, it is treated with a cream (e.g. Bepanthen). In addition, re-grown hair is shaved off. These important steps are missing.

We thank Reviewer 1 for these comments.

In section 1.4.2, we included “Apply anti-septic ointment as needed”. We do not want to state the brand name Bepanthen.

We also added an extra line, now 1.4.4, on “Regrown hair is shaved off.”

1. Lines 340-365 discuss electric field intensity in relation to transducer array placement. It should be emphasized that the planning software suggests the optimal placement of the arrays to achieve maximum results, since this is not clear.

We thank Reviewer 1 for this comment. We respectfully disagree. NovoTAL is a proprietary software and the algorithm is not available for examination by investigators. Therefore, there is no way to determine whether or not the transducer array configuration generated represents the optimal arrangement. Nevertheless, clinical data from phase III clinical trial in newly diagnosed glioblastoma patients showed that placement of the arrays for TTFields treatment makes a survival difference compared to those without.

We will change the sentence in lines 352-353 to “transducer array placement has an effect on electric field strength in the region of the tumor” to give a stronger relevance to array placement in the patient.

4. Lines 371-378. (A) Which cells are shown in the figure has not been mentioned. Is it a cell line? If so, which one?

They are HeLa cells. This information was added to the legend of Figure 1.

5. There is no reference to the life imaging videos provided within the text or figure legends.

These videos have not been previously published. But they are similar to the ones published before in reference 11. Therefore, we will cite this reference in the legend for Figure 1.

6. Lines 272 and 280. Typographical errors.

In section 2.1.2, “Pneumocystic” is changed to “Pneumocystis”.

In section 2.1.5, “dexaxmethasone” is changed to “dexamethasone”.

7. Figure 4A. The frame of the arrow is partially masked by the frame showing the head.

The arrow has been moved to the front of the image.

**Reviewer #2:**

Manuscript Summary:

The authors have prepared a competent review article that nicely summarizes the current knowledge on TTFields technology and its applications. It presents the necessary details on the clinical procedures for the next generation of patient-directed TTFields devices as well as some details on modeling of electric fields onto human brains. We find it acceptable for publication but with some revisions and we would like the authors to address some general as well as specific comments and questions.

Major Concerns:

1. In the section on future applications, the authors state that the anti-mitotic effects of TTFields devices can be extended to cancers aside from glioblastoma (e.g. pancreatic cancer, mesothelioma or non-small cell lung cancer). In a number of these models, the target tissues or organs are capable of regeneration from the activation, proliferation and expansion of progenitor cells. Presumably, the brain possesses no such capacity (glial cells and glioblastoma notwithstanding). In your opinion, would TTFields therapy against the cancers of said organs or tissues also affect dividing progenitor cells and thereby compromise tissue regeneration and function post-therapy? In other words would TTFields affect the surrounding normal but regenerating tissues? If you have any views on this please add a statement or two in the article.

We thank Reviewer 2 for this comment. The authors think that TTFields have an anti-mitotic effect on any dividing cells undergoing mitosis. Therefore, the effect should apply to progenitor cells in addition to tumor cells. We added a sentence in the Future Applications section, “TTFields have an unequivocal anti-mitotic effect on dividing tumor cells. Quite possibly, this effect also extends to progenitor cells but preclinical or clinical data on normal tissue is lacking”, to address this point.

2. Do you think that differences in cellular size (aside from proliferative status) between tumor and normal tissue will be a variable in the effectiveness in the therapy?

The relationship between size and activity is probably not a basis for targeting cells for damage by TTFields.

3. We believe that the main point of the JoVE article is to be a presentation of the current knowledge on TTFields as well as a description of next-generation clinical application with a brief description on modeling of TTFields on human brains. Consequently, we were expecting perhaps an instructional video on the clinical set-up of the device on patients and steps for optimization. However, all videos that we have seen describe the effects of TTFields on cellular division. Is there something that we are missing? Or will this clinical video become apparent in the final online version of the publication?

The clinical video on a patient with glioblastoma undergoing TTFields treatment has not been shot yet. It will be done when the article is accepted for publication.

Minor Concerns:

Figure 1C

You may want to place the captions "Normal Mitosis" and "Mitosis within TTFields" by the side of their respective pathways (i.e. "Normal Mitosis" by the top branch or pathway while "Mitosis within TTFields" beside the bottom pathway) just to emphasize the differences in experimental conditions. Also make those two labels in larger fonts than anything else to make them even more distinct.

We will justify “Normal Mitosis” and “Mitosis within TTFields” to the left and include an arrow pointing toward the right side to indicate the progression of mitosis.

Figure 2C

We are a bit confused by how the electric field intensity of the TTFields on a human brain with left frontal glioblastoma was obtained. We read the phrase "arbitrary" but does this mean that the modeling is not based upon ab initio data from the patient that you are reporting? Alternatively, we thought that the data may be based upon the work from ref. 43 (Korshoej et al.) however reference 43 is not specifically cited in the relevant figure legends nor in passages within the text related to modeling of the alternating electric fields on the brain. It would be nice if we could obtain a clarification. Also, how validated is your modeling of the electric field? Is there a reason why known modelers of TTFields such as Jack Tuszynski or Kris Carlson are not involved nor cited?

We thank Reviewer 2 for this comment.

The electric field map was generated from our patient. We will take away the word arbitrary, which is confusing for the reader.

Jack Tuszynski’s is working on the modeling of electric field effect on tubulin while Kris Carlson is modeling the entire cell during division.

Reviewer 2 is correct that Korshoej et al performed anisotropic electric field modeling in patients. Therefore, we added a reference from him in the first sentence of the second paragraph under Representative Results (Korshoej et al, Impact of tumor position, conductivity distribution and tissue homogeneity on the distribution of tumor treating fields in a human brain: A computer modeling study. PLoS One 2017;12(6):e0179214).

Figure 5:

Is there a reason why the authors did not include a panel of Coronal T2 FLAIR scans?

Coronal T2 FLAIR was not acquired during MRI scanning of our patient.

In compliance with data protection regulations, please contact the publication office if you would like to have your personal information removed from the database.