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The Clinical Application of Tumor Treating Fields Therapy in Glioblastoma

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Coverletter

Beth Israel Deaconess Medical Center

A teaching hospital of Harvard Medical School

August 12, 2018

Ronald Myers, PhD
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RE: JoVE manuscript submission

Dear Dr. Myers:

We would like to submit our work, entitled "Tumor Treating Fields Therapy: Cell Biology, Electric Field Modeling, and Clinical Application" to JOVE Medicine Section for consideration for publication. This work has not been and is not being considered for publication elsewhere. We believe that our submission is well suited for the JoVE format. All authors have agreed submission of this manuscript.

As you may know already, Tumor Treating Fields are low frequency alternating electric fields that are approved by the FDA for the treatment of patients with newly diagnosed and recurrent glioblastomas. However, the anti-cancer mechanism behind this technology is not well understood. An earlier publication by Omar et al. in J. Vis. Exp. e51638 (2014) described the application of the transducer arrays performed using a plastic model of a human head and the first generation NovoTTF-100A System. In our manuscript, we provided a comprehensive discussion of several scientific domains that are relevant for our understanding of this technology, including electric field effects on cancer cell biology, physics of electromagnetism and MRI-guided computational modeling. We also included videos of blebbing of cells during mitosis. Most importantly, we will demonstrate in a patient head the application of the transducer arrays (including nuances that are pertinent to skull defects and surgical scars on the scalp), the operation of the second generation Optune System, and the identification of various pitfalls in the process that may result in complications.

We also would like to submit here a list of three potential reviewers. They are:
Dr. Rimas V. Lukas (Rimas.Lukas@nm.org) at Northwestern University Feinberg School of Medicine
Dr. Jay-Jiguang Zhu (Jay.Jiguang.Zhu@uth.tmc.edu) at the University of Texas Medical School at Houston
Dr. Suyash Mohan (Suyash.Mohan@uphs.upenn.edu) at the University of Pennsylvania School of Medicine

We look forward to hearing from you.

Sincerely yours,

Eric T Wong, MD
Associate Professor of Neurology
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October 21, 2018

Phillip Steindel, Ph.D.
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RE: JoVE manuscript revision JoVE58937R2

Dear Dr. Steindel:

We thank you the response from you and the reviewers on our manuscript, entitled "The Clinical Application of Tumor Treating Fields Therapy in Glioblastoma", to JOVE Medicine Section for re-consideration for publication. Please note that we agree with the reviewers' comments that the title is too broad in scope and therefore we revised the title more relevant to the subject matters discussed. In addition, we have uploaded our point-by-point response to the reviewers' comments in a separate WORD file. A clean copy of the revised manuscript and one with the track changes are enclosed. All authors have agreed submission of this manuscript.

We hope that our revised manuscript is satisfactory. We look forward to hearing from you.

Sincerely yours,

Eric T Wong, MD
Associate Professor of Neurology
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Director, Brain Tumor Center & Neuro-Oncology Unit
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November 1, 2018

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RE: JoVE manuscript revision JoVE58937R3

Dear Dr. Steindel:

We thank you for the 3 editorial comments for our manuscript, entitled "The Clinical Application of Tumor Treating Fields Therapy in Glioblastoma" and accommodated all of them. A clean copy of the revised manuscript and one with the track changes are enclosed. All authors have agreed submission of this manuscript.

We hope that our revised manuscript is satisfactory. We look forward to hearing from you.

Sincerely yours,

Eric T Wong, MD
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TITLE:

The Clinical Application of Tumor Treating Fields Therapy in Glioblastoma

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KEYWORDS:

brain tumor, glioblastoma, mitosis, alternating electric fields, tumor treating fields, transducer arrays

SUMMARY:

Glioblastoma is the most common and aggressive primary brain malignancy in adults, with most tumors recurring after initial treatment. Tumor Treating Fields (TTFields) therapy is the newest treatment modality for glioblastoma. Here, we describe the proper application of TTFields-transducer arrays on patients and discuss theory and aspects of treatment.

ABSTRACT:

Glioblastoma is the most common and lethal form of brain cancer, with a median survival of 15 months after diagnosis and a 5 year survival rate of only 5% with current standard of care. Tumors often recur within 9 months following initial surgery, radiation and chemotherapy, at which point treatment options become limited. This highlights the pressing need for the development of better therapeutics to prolong survival and increase the quality of life for these patients.

Tumor Treating Fields (TTFields) therapy was developed to take advantage of the effect of low frequency alternating electrical fields on cells for cancer therapy. TTFields have been demonstrated to disrupt cells during mitosis and slow tumor growth. There is also growing evidence that they act through stimulating immune responses within exposed tumors. The advantages of TTFields therapy include its noninvasive approach and increased quality of life compared to other treatment modalities such as cytotoxic chemotherapies. The Food and Drug Administration approved TTFields therapy for the treatment of recurrent glioblastoma in 2011

and for newly diagnosed glioblastoma in 2015. We report on the effects of TTFields during mitosis, the results of electric fields modeling, and proper transducer array placement. Our protocol outlines the clinical application of TTFields on a patient post-surgery, using the second-generation device.

INTRODUCTION:

Glioblastoma

Glioblastoma is the most common primary malignant brain tumor in adults. Due to its properties as a cytologically malignant, mitotically active, angiogenically proliferative and necrosis-prone neoplasm typically associated with rapid pre- and post-operative disease evolution and near-universal fatal outcome, the World Health Organization designated glioblastoma as a grade IV neoplasm[1]. Despite basic and translational research efforts, there is no curative treatment for glioblastoma. The 5 year survival rate of patients diagnosed with glioblastoma remains approximately 5%, highlighting the pressing need for more effective therapeutic interventions[2].

Mechanisms of tumor treating fields: electric field

TTFields are low-intensity, intermediate-frequency (100-300 kHz) alternating electric fields that permeate tumor-bearing tissues and are produced by insulated electrodes adhered externally to the patient's skin[3]. TTFields are thought to interfere with biological processes of tumor cells by exerting electromagnetic forces on intracellular molecules with high dipole moments during mitosis. TTFields exposure during mitosis resulted in aberrant mitotic exit leading to abnormal chromosome segregation, cellular multinucleation, and caspase dependent apoptosis of daughter cells[4]. These effects were frequency dependent and contingent on the incident direction of the field with relation to the mitotic plates of affected cells. The cells with mitotic plates perpendicular to the fields exhibited the greatest severity of damage. The intermediate frequency range is unique because it constitutes a transition region in which strength of the intracellular electric field, which is shielded at lower frequencies, increases significantly[5]. The threshold at which this increase occurs depends on the dielectric properties of the cell membrane[5]. For glioma cells, the optimal frequency of the TTFields with respect to both cell counts in culture and clonogenic assays is 200 kHz[6].

Using patient-specific MRI measurements, a personalized mapping of electric fields can be developed by incorporating the volume, electric conductivity, and relative permittivity of different tissue structures in the brain[7,8]. Furthermore, an end-to-end, semi-automatic segmentation-based workflow can also be utilized to generate a personalized finite element model for the delineation of intracranial TTFields[9]. Electric fields maps demonstrating the distribution of electric fields within the patient brain may have utility for guiding optimal placement of transducer arrays to maximize field strength within the tumor.

Mechanisms of tumor treating fields: cell biology

The precise mechanisms by which TTFields drive mitotic disruption are not completely understood, but two potential mechanisms by which electric fields may affect mitosis have been proposed. One involves the electric field's direct action on proteins with high dipole moments

resulting in their functional perturbation; the second is dielectrophoresis of ions, causing a mislocalization of ions within the dividing cell that may interfere with cytokinetic furrow ingression[3]. Two proteins with high dipole moments have been proposed as targets, the α/β-tubulin monomer and the Septin 2, 6, 7 heterotrimer, with dipole moments of 1740 D[10] and 2771 D[11], respectively. It has been suggested that TTFields decrease the ratio between polymerized and total tubulin, preventing proper mitotic spindle assembly and perturbing the cells at the transition from metaphase to anaphase[4]. Cells exposed to TTFields show normal progression until metaphase, but then exhibit reduced septin localization to the anaphase spindle midline and cytokinetic furrow[11]. The cells undergo uncontrolled membrane blebbing that leads to aberrant mitotic exit[12]. The resulting post-mitotic cells exhibit abnormal nuclear architecture such as micronuclei, signs of cellular stress, and an overall decrease in cellular proliferation including G0 arrest followed by apoptosis[11]. Research has shown an up-regulation in calreticulin and secretion of HMGB1 in TTFields treated cells, both hallmarks of immunogenic cell death[13,14]. Kirson et al. showed treatment of tumors reduced the metastatic potential, and the metastases within TTFields-treated animals showed an increase in CD8+ cells[15]. Together, these data support a mechanism of action that extends beyond direct effects on mitosis, and likely initiates antitumor inflammatory responses.

TTFields device and treatment options

Both first and second generation TTFields devices deliver alternating electric fields to the supratentorial brain for the treatment of glioblastoma. The device was first approved by the FDA in 2011 for the treatment of patients with recurrent glioblastoma and approved in 2015 for the treatment of patients with newly diagnosed glioblastoma[16,17]. Glioblastoma treatment should be undertaken in a multimodal fashion, with neurosurgical intervention, radiation oncology input, and chemotherapy administration. 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[18,19].

In the newly diagnosed setting, standard treatment approach consists of concurrent radiation and temozolomide followed by maintenance temozolomide. In 2004, a randomized phase III trial showed improved median and 2-year survival for patients with glioblastoma treated with radiotherapy and concomitant and adjuvant temozolomide[20]. Benefits of adjuvant temozolomide with radiotherapy lasted throughout at least 5 years of follow-up[21]. However, patient 06-methylguanine-DNA methyltransferase (MGMT) methylation status identified those most likely to benefit from the addition of temozolomide[22]. In another randomized clinical trial of patients with glioblastoma who had received standard radiation and concomitant temozolomide chemotherapy, the addition of TTFields to maintenance temozolomide chemotherapy resulted in improved outcomes compared to those that received maintenance temozolomide alone[23]. Additionally, research has shown that TTFields work irrespective of patient MGMT promoter methylation status; therefore TTFields may constitute a clinical intervention that also works in patients with unmethylated MGMT status[24]. Taken together, these studies suggest broad implications on the effectiveness of TTFields for the treatment of glioblastomas. Specifically, after radiation, incorporating TTFields in combination with temozolomide provides an effective treatment option for newly diagnosed patients with glioblastoma.

In the recurrent setting, there exists no standard treatment approach. However, bevacizumab and TTFields therapy are the two FDA-approved treatment modalities[25,26]. The EF-11 phase III trial of TTFields monotherapy (with 20-24 h/day usage) versus active chemotherapy in patients with recurrent glioblastoma showed comparable overall survival, while toxicity and quality of life favored TTFields[27]. Therefore, bevacizumab alone, TTFields monotherapy, or a combination of both constitutes treatment options for those with recurrent glioblastoma.

Clinical application

A previous JoVE publication demonstrated the application of the first generation device using a plastic model of a human head[25]. Here, we demonstrate the application of the second-generation device on a glioblastoma patient undergoing treatment. The protocol for using the device begins with configuring transducer array layout placement on the scalp using MRI measurements and a treatment planning system. The transducer array layout map delineates the orientation and location of each of the four arrays on the patient's head. The arrays are designed to adhere to the scalp to allow the transducers to deliver the 200 kHz frequency TTFields from an electric field generator. Patients receive treatment continuously and the arrays are typically exchanged every 3 to 4 days. In this paper we show the effects of TTFields on mitotic cells, the distribution of electric fields within the brain, and the step-by-step application method of the second-generation device on a human head to demonstrate treatment of a patient with glioblastoma.

PROTOCOL:

The presentation of this protocol follows ethical guidelines at Beth Israel Deaconess Medical Center and written authorization was obtained from the patient.

1. Application of the second generation TTFields device

NOTE: The system consists of the portable electric field generator, transducer arrays, a connection cable and box, a rechargeable battery, charger for portable batteries, and a plug in power supply.

1.1. Treatment planning procedure

1.1.1. Acquire MRI images of the patient's brain. The MRI scan includes the margins of the scalp for treatment planning. Incomplete delineation of the full thickness of the scalp interferes with the electric field calculations.

1.1.2. 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). Measure the superior to tentorium, right to left, and right to midline based on coronal view head size (mm).

1.1.3. Focusing on the primary lesion, measure the front to back without nose, right to left, right

to midline, right to close tumor margin, right to far tumor margin, front to close tumor margin, and front to far tumor margin based on axial view tumor size (mm). Measure the superior to tentorium, right to left, right to midline, right to close tumor margin, right to far tumor margin, superior to close tumor margin, and superior to far tumor margin based on coronal view tumor size (mm).

1.1.4. Open treatment planning software, enter username and password, and select new patient transducer array.

1.1.5. Enter in the measurements taken above, and click **Generate Transducer Array Placement**. Save the transducer array layout for future use on patient visit.

1.2. Applying transducer arrays to scalp

1.2.1. Prepare the scalp for transducer array placement by cutting hair and shaving hair stubbles with an electric razor down to the surface of the scalp until no hair remains. Avoid using a razor with blade(s) to prevent cuts on the scalp.

1.2.2. Wipe the scalp with 70% isopropyl alcohol.

1.2.3. Remove the transducer arrays from plastic packaging and begin planning placement upon the scalp according to the predetermined specific array layout scheme (see section 1.1). Locate the surgical scar and avoid placing transducer arrays upon scar.

1.2.4. If the scar is located under a predetermined transducer array, then shift the four array placements 2 cm clockwise or counterclockwise.

1.2.5. Determine the desired location of the connection wires as preferred by the patient (either right or left side of the body). Apply the transducer array that is nearest to the surgical scar first, while positioning the connection wire to the preferred side.

1.2.6. Apply the next transducer array to either the right lateral or the left lateral in a clockwise or counterclockwise fashion, keeping the location of the connection wire consistent. Apply the third and fourth transducer arrays in the same clockwise or counterclockwise fashion.

1.2.7. Place gauze strips underneath the metallic interface in between the array and the connection wire. Use silk tape to hold the gauze strip in place.

1.2.8. Braid the four connection wires together and fasten with silk tape. Place fishnet retainer over the head in order to hold the arrays in place.

1.3. Assembling the TTFields device system

1.3.1. Connect each of the four white and black color-coded connection wires to a corresponding

white or black port on the connection box, making sure each audibly snaps into place.

1.3.2. If using the portable battery, connect the battery charger to a wall outlet and switch the power button on in order to initially charge the battery.

1.3.3. Insert charged battery into the electric field generator by plugging it in through its connector to a socket labeled **DC IN** on the front panel of the device. Make sure the arrows on the battery connector face up towards the **DC IN** label.

1.3.4. If not using the portable battery, plug the electric field generator into a wall outlet.

1.3.5. Turn on the power button located at the bottom of electric field generator to start the device. Switch on the **TTFields** button located at the top of the electric field generator. The patient may experience a warm sensation.

1.3.6. To achieve an optimal response, Have patient use TTFields therapy on a continuous basis for a minimum compliance of 75%, or 18 hours a day. Treatment duration of less than 18 hour per day has been associated with suboptimal results.

1.4. Exchange of transducer arrays

NOTE: Array exchange procedures in this section are repeated every 3 to 4 days.

1.4.1. Use baby oil to remove adhesive from skin. Pull off arrays by applying slow and even tension with both hands.

1.4.2. Wash scalp with gentle shampoo. Check scalp for dermatitis, erosions, ulcers, or infection. Apply anti-septic ointment as needed.

1.4.2.1. If ulcers or infections are present, discontinue treatment until ulcer heals or infection clears.

1.4.3. Shave off regrown hair.

1.4.4. Clean scalp with 70% isopropyl alcohol. Reapply the transducer arrays (see section 1.2).

2. Removal of systemic agents that may interfere with anti-tumor immunity

2.1. Lowering or discontinuation of dexamethasone

NOTE: Dexamethasone is a synthetic fluorinated glucocorticoid that has anti-inflammatory effects in humans by impairing cell-mediated immunity.

2.1.1 Wean dexamethasone in a stepwise fashion due to its hysteresis effect.

2.1.2 Apply trimethopreme-sulfamethaxazole (400–80 mg single-strength tablet daily or 800–160 mg double-strength tablets three times per week) to prevent the development of pneumocystic pneumonia during the weaning process.

2.1.3 Cut the dose half quickly every 7–10 days to achieve a daily dose of 4 mg/day. If the patient is already at 4 mg/day or lower the dose, cut the dosage slower, at a rate of every 10 to 14 days until discontinuation.

2.1.4 Look for signs of adrenal suppression (i.e., lethargy, cold intolerance, weakness and hypersomnia). If signs of unacceptable neurologic deficits and/or adrenal suppression appear, the previous dose of dexamethasone is re-applied.

NOTE: Other means for reduction of dexamethasone are being sought (see concurrent bevacizumab administration).

2.2. Concurrent bevacizumab administration

NOTE: Bevacizumab is a humanized anti-vascular endothelial growth factor (VEGF) monoclonal IgG_1 antibody. The antibody has a potent antiangiogenic effect by sequestering VEGF, rendering it unable to bind to the cognate receptors VEGFR1 and VEGFR2 and to exert its proangiogenic effect. Immature blood vessels also have high permeability and elimination of these newly generated vasculature within the glioblastoma microenvironment also helps to reduce cerebral edema. Bevacizumab has a long half-life of about 20 days[28] and therefore it can be administered to patients every 2 to 3 weeks as an intravenous infusion. The indication for bevacizumab is to obviate the prolonged use of dexamethasone.

2.2.1. Exclude bevacizumab from patient who had recent hemorrhage (either intracranial or extracranial), myocardial infarction or stroke, major surgery (including craniotomy) within 4 weeks, uncontrolled hypertension, pregnancy or lactation. Exercise caution in patients with chronic kidney disease, proteinuria, bleeding disorder, uncontrolled angina, cardiac arrhythmia, congestive heart failure, prior chest wall irradiation, prior anthracycline exposure or other concurrent illness deemed unfit by the treating physician.

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, administer bevacizumab at a dose of 2.5, 5.0, or 10 mg/kg. There is class 2 evidence that bevacizumab at doses of <10 mg/kg work as well as 10 mg/kg[29,30]. Start TTFields treatment either before or after initiation of bevacizumab.

2.2.4. Infuse the initial dose of bevacizumab over 60 minutes in 100 cc of normal saline. If there is no adverse event, administer subsequent doses over 30 minutes.

2.3. Other systemic immunosuppressive agents to avoid

NOTE: There are a number of anti-cancer drugs that also have significant immunosuppressive properties. They are listed below.

2.4. Avoid Everolimus, which is an mTOR inhibitor.

NOTE: Everolimus is approved to treat subependymal giant cell astrocytoma, advanced hormone-receptor-positive, Her2-negative breast cancer, pancreatic neuroendocrine tumors and renal cell carcinoma. However, the addition of Everolimus has been shown definitively in a randomized study to hasten the death of glioblastoma patients, most likely by impairing their anti-tumor cell-mediated immunity[31]. It is also used to prevent rejection of transplant organ recipients.

2.5. Avoid Sirolimus, which is also known as rapamycin.

NOTE: Temsirolimus is a pro-drug that can be metabolized to sirolimus. It is an mTOR inhibitor with immunologic interference properties similar to everolimus. It is also used to prevent rejection of transplant organ recipients.

REPRESENTATIVE RESULTS:

TTFields cause disruption during mitosis leading to an asymmetric distribution of chromosomes and misalignment of metaphase plates during mitosis, (compare **Figure 1A** and **Figure 1B**). TTFields are thought to exert their effect by perturbing the function of high dipole moment possessing proteins such as α/β-tubulin or septin. One proposed model for TTFields action on mitotic cells is that they perturb septin function. Normally, septin acts to organize the cytokinetic furrow and to reinforce the structurally important interaction between the subcortical actin cytoskeleton and the overlying plasma membrane that is needed to resist intracellular hydrostatic forces produced during furrow ingression. This results in a loss of structural integrity within the dividing cells that is necessary for normal mitosis, resulting in the disruption of chromosomal segregation and cytokinetic furrow function leading to aberrant mitotic exit (**Figure 1C**).

Electric field intensity is not homogenous within the brain of patients undergoing TTFields treatment[32]. Electrical conductivity and relative permittivity of individual tissue types and their volume results in a variation of electric field intensity and distribution within the brain, shown in **Figure 2A,B**. Therefore, transducer array placement may have an effect on electric field strength in the region of the tumor. An example of this variability is shown in **Figure 2C**, which predicts the electric field strength within the patient's brain at adjacent axial, coronal, and sagittal slices.

Figure 3A shows the personalized output of the treatment planning software for the proper placement of the arrays on a patient, shown in **Figure 3B**. Scalp sensitivity to the arrays can be

alleviated by topical application of corticosteroids and by shifting the arrays as described in **Figure 4**.

The above protocol was used to treat a 56-year-old woman who developed a hemorrhage in the left frontal brain. She underwent a gross total resection of the hemorrhagic mass and the pathology showed IDH-1 mutated glioblastoma with hypercellularity, cellular atypia, mitotic figures and necrosis. She subsequently received external beam radiotherapy and daily temozolomide. Dexamethasone was stopped early at the second week of radiation. She experienced pancytopenia due to temozolomide administered during the adjuvant phase of treatment, requiring growth factor support as well as platelet and blood transfusions. Increased gadolinium enhancement was noted on head MRI 5 months after diagnosis and bevacizumab was started. Eight months after diagnosis TTFields therapy was also added. She has been maintained on the regimen of bevacizumab and TTFields for 48+ months after the diagnosis of her glioblastoma. The MRI images of this patient revealed stable disease for 48 months after initial diagnosis of glioblalstoma, shown in **Figure 5**. She has survived thus far with a high Karnofsky score of 80.

FIGURE AND TABLE LEGENDS:

Figure 1. TTFields disrupt mitosis during cell division. (**A**) Phase contrast microscopy was used to observe HeLa cells during mitosis. DRAQ5 is a DNA stain and was used to monitor chromosomal behavior. Image taken from a video of cells undergoing normal mitosis, included as an additional supplement. The procedures for obtaining video images were described in previous work[11]. (**B**) Phase contrast and DRAQ5 under TTFields show cell blebbing and aberrant mitosis. Scale bar 20 μm. Image taken from a video of cells undergoing mitosis during TTFields treatment, included as an additional supplement. The procedures for obtaining video images were described in previous work[11]. (**C**) Proposed model for TTFields-induced mitotic disruption. TTFields perturb septin association with the cytokinetic furrow and the subcortical actin cytoskeleton. This creates insufficient furrow contractility and makes cells vulnerable to plasma membrane rupture from the underlying cytoskeleton, resulting in membrane blebbing. This leads to aberrant mitotic exit including mitotic slippage (failure to divide) and asymmetric cell division.

Figure 2. In situ electric field intensities vary within tissues based on electric conductivity and relative permittivity of the tissues they pass through. (**A**) Electric Field-Volume Histogram (EVH) shows the magnitude of electric field strength. (**B**) Specific Absorption Rate-Volume histogram (SARVH) shows the rate of energy absorbed in different tissues. (**C**) Representative field mapping of a patient with a left frontal glioblastoma, showing field strength within distributions on axial, coronal and sagittal slices. Green arrows indicate location of tumor. Relative electric field intensity is arbitrary.

Figure 3. Clinical application on a glioblastoma patient after surgery, radiation and temozolomide. (**A**) Treatment planning software output showing placement of the 4 arrays. (**B**) Array placement on the patient.

Figure 4. Array placement variation during treatment. (**A**) The individual lateral arrays should be rotated in aggregate by 2 cm from their primary position in a clockwise fashion, and the frontal and posterior arrays moved forward by 2 cm from the (**B**) primary positions for array placement position, which are based on the output from the treatment planning software for the individual patient. (**C**) The individual electrodes in each array should be rotated in aggregate by 2 cm from the primary position in a counterclockwise fashion, and the anterior and posterior arrays moved in aggregate by 2 cm backwards.

Figure 5. Patient MRI scans before and after TTFields treatment. MRI scans at diagnosis (left column), MRI scans after surgery, radiation, and temozolomide (middle column), and MRI scans after 43 months of TTFields treatment (right column).

DISCUSSION:

This article demonstrates the proper application of the second generation TTFields device to treat glioblastoma patients. The significance of TTFields therapy with respect to alternative treatments includes reduced toxicity, increased quality of life, and higher median overall survival especially when combined with temozolomide chemotherapy. Furthermore, we show in a step-by-step fashion the proper application of the transducer array onto the scalp, while avoiding pitfalls that may cause complications. In addition, we provide a detailed account of the cell biology effects of TTFields as well as electric field mapping as TTFields penetrate into the brain.

A few steps in the protocol are particularly critical for the successful implementation of the device. 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. It is important to locate any surgical scars and avoid placing transducer arrays on the scar to obviate complications from scalp breakdown. During each exchange, check the scalp for dermatitis, erosions, ulcers or infection and, if needed, stop application of arrays until ulcers are healed and infections are resolved[33,34].

The improvement of life expectancy depends most on high patient compliance of 18 hours per day or more. A post hoc analysis of the EF-11 phase III trial data showed significantly longer median overall survival in TTFields therapy patients with a compliance rate ≥75% (≥18 hours daily) versus those with a <75% compliance rate (7.7 versus 4.5 months, $p = 0.042$)[35]. Patients who are less than 75% compliant appear to receive little benefit, while those who pass the 75% compliance cut-off exhibited significant benefit. Physician guidance and family support plays an important role in achieving higher patient compliance, and advice on application can be given so that the patient is more comfortable wearing the arrays for longer periods of time. Ambient temperature should remain in a comfortable range while wearing the arrays. Regular intervals of array changes, hair shaving of the scalp, and placement of a breathable net on the head to hold the arrays in place may also improve comfort leading to higher compliance.

There is accumulating evidence that TTFields treatment works better when combined with other therapies. TTFields were used as a monotherapy in the EF-11 pivotal phase III trial, and the median overall survival was 6.6 months for the TTFields arm compared to 6.0 months for the

chemotherapy arm. Although these initial results showed no statistically significant improvement in overall survival over standard-of-care treatment, fewer severe adverse events and improved quality-of-life measures were noted in the TTFields arm which formed the basis for its approval for recurrent glioblastoma by the FDA[27]. The later EF-14 phase III trial on newly diagnosed glioblastoma showed a median overall survival of 20.9 months in the TTFields-temozolomide arm versus 16.0 months in the temozolomide-alone arm[36,37]. Another study on TTFields in clinical practice using the PRiDe registry showed a median overall survival of 9.6 months, which was significantly longer than the median overall survival in the control arm of EF-11[35]. Furthermore, preclinical data have shown that adding in alkylating agents like temozolomide improves tumor cell kill in tissue culture[24]. The PRiDe registry and EF-14 data support this concept because these patients had better outcomes when they received concurrent temozolomide and/or other treatments added to TTFields. Wong et al. showed similar results by comparing TTFields therapy and bevacizumab alone or in combination with a regimen consisting of 6-thioguanine, lomustine, capecitabine, and celecoxib (TCCC). The TCCC group exhibited prolonged overall survival, median 10.3 months versus 4.1 months for TTFields and bevacizumab alone[38]. Collectively, these data support the addition of adjuvant therapies to increase the effectiveness of the device in treating glioblastoma.

In the EF-14 trial, the patients that received TTFields in the experimental arm had a longer overall survival compared to the controls, but there was no difference between the experimental and control arms in the EF-11 trial. The EF-14 trial added a known therapeutic agent, temozolomide, which appears to combine synergistically with TTFields treatment. Another potential explanation for this difference may be due to the chemotherapy näive status of newly diagnosed patients, which may enable them to mount a more effective anti-tumor immune response. Although the mechanism of an immune response from TTFields remains unclear, dexamethasone is given as an immunosuppressive and has been shown to lower median survival when combined with TTFields[39–41]. In conclusion, lowering patients' dose of dexamethasone while on TTFields would increase the number of immune cells in the blood of glioblastoma patients and could lead to a stronger response and improved treatment result. TTFields may also sensitize tumor cells to the effects of ionizing radiation[42,43]. However, the selection of combination therapy should be individualized with respect to the neurologic and medical conditions of the patient.

The TTFields device was approved by the FDA for the treatment of adult patients with recurrent and newly diagnosed glioblastoma at age 22 years and older; the efficacy of this device for patients under 22 years is unknown. Furthermore, the side effects are unknown when the patient is using TTFields concurrently 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. Known allergic reaction to electrode gels, open wounds, skull defects, and pregnancy are also contraindicated. Patients with major skull defects, such as absence of a large segment of the calvarium from craniectomy, may have a higher penetration of TTFields[44]; however, craniectomy is not routinely performed on glioblastoma patients.

Poor patient compliance is a major limitation to this treatment modality. Factors that may

decrease compliance include concurrent medical or psychiatric illness (i.e., depression)[45–47], lack of support from caretakers, scalp breakdown due to erosions or infection, skin swelling, and dermatitis.

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. Nevertheless, TTFields therapy shows promise in multiple solid tumor types, including some of the most aggressive forms of cancer. TTFields serve as an effective antimitotic treatment in preclinical pancreatic cancer models and have a long-term negative effect on the survival of these cancer cells. These results make TTFields an attractive candidate for testing in patients with pancreatic cancer[48]. TTFields have also shown encouraging preclinical results for the treatment of ovarian cancer[49] and non-small cell lung cancer[15,50]. Therefore, TTFields are being applied in ongoing phase III clinical trials for primary (NCT02973789) and metastatic (NCT02831959) lung cancer, pancreatic cancer (NCT03377491), and mesothelioma (NCT02397928). Hopefully, TTFields will provide additional treatment options for these difficult-to-treat malignancies.

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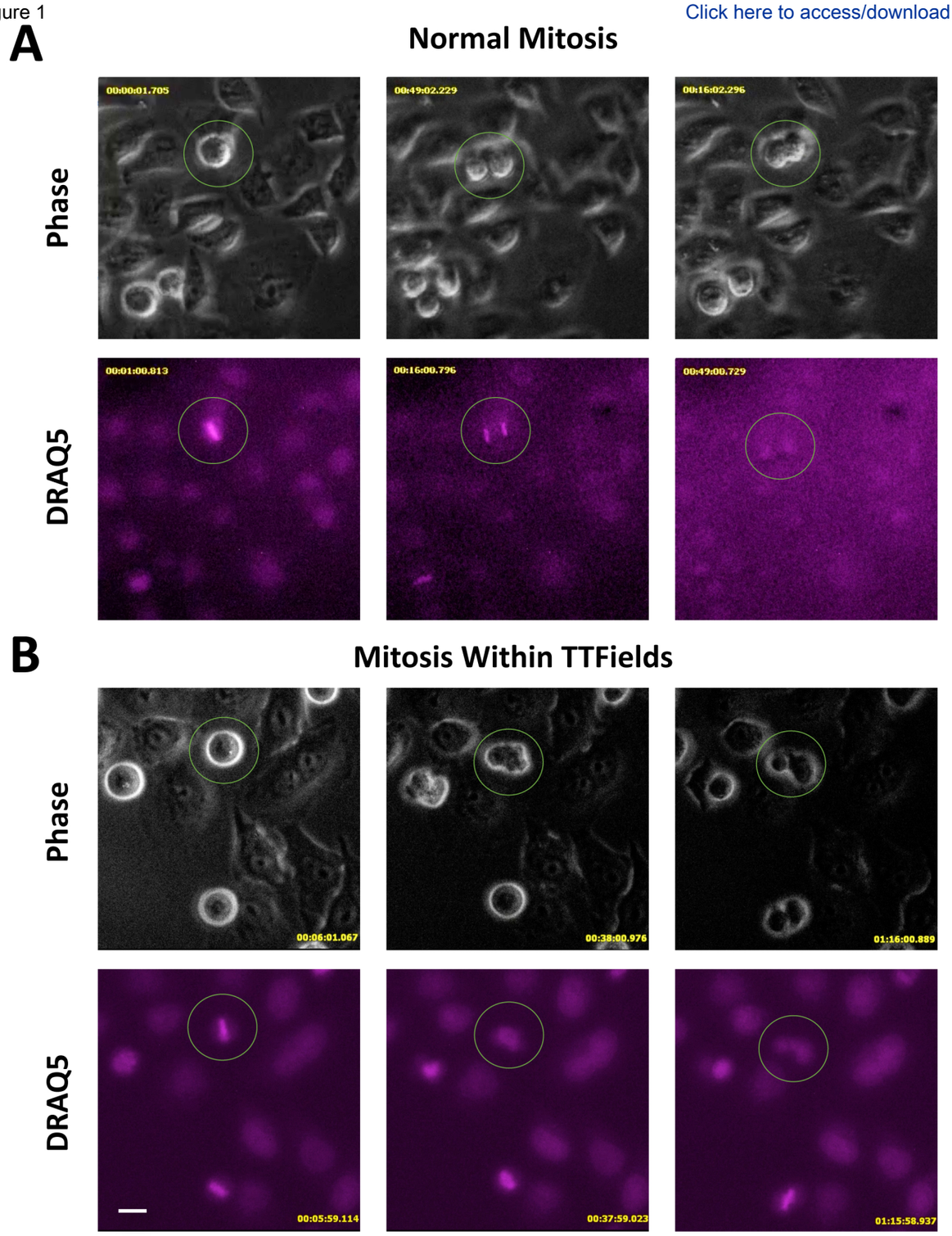


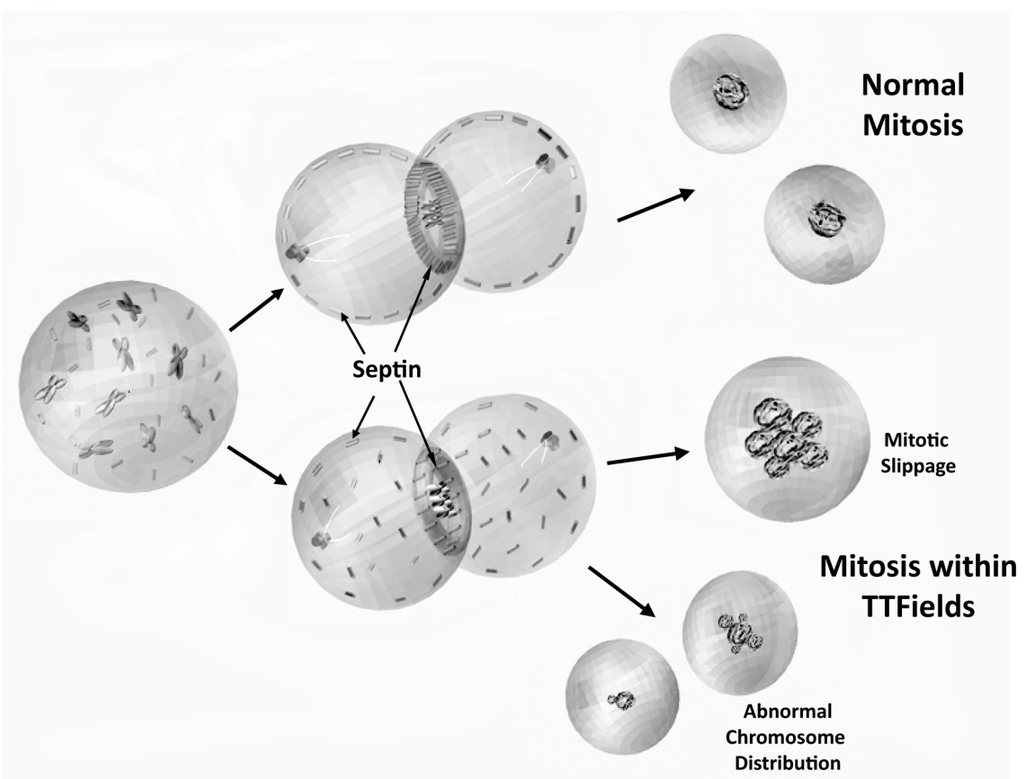
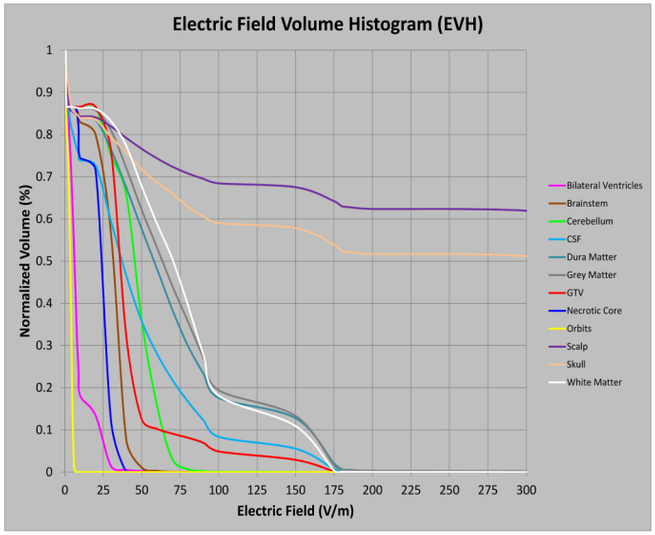


Figure 1

Figure 2

A



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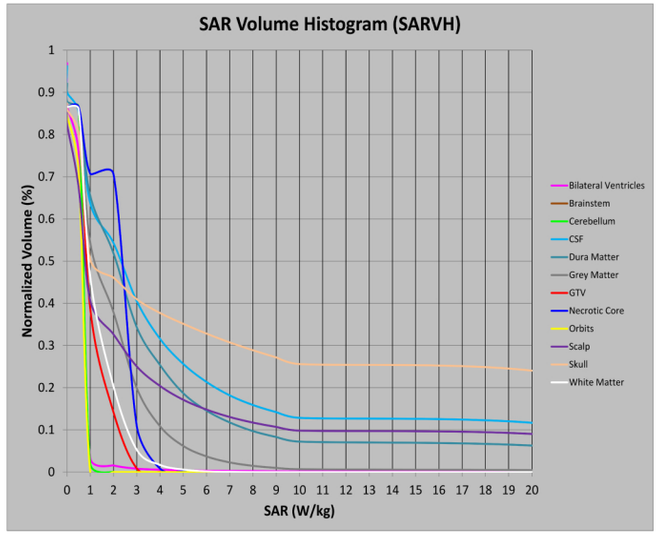

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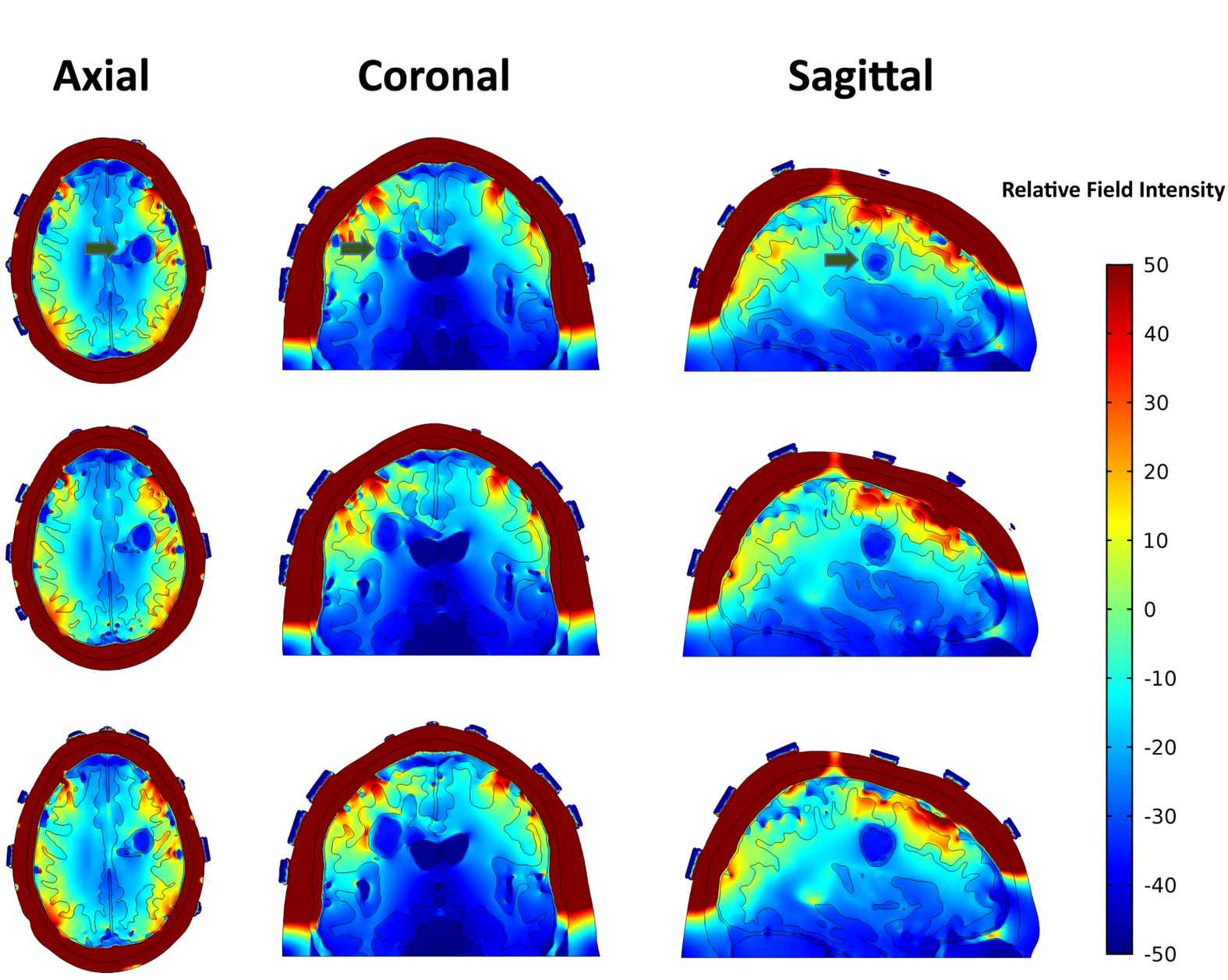


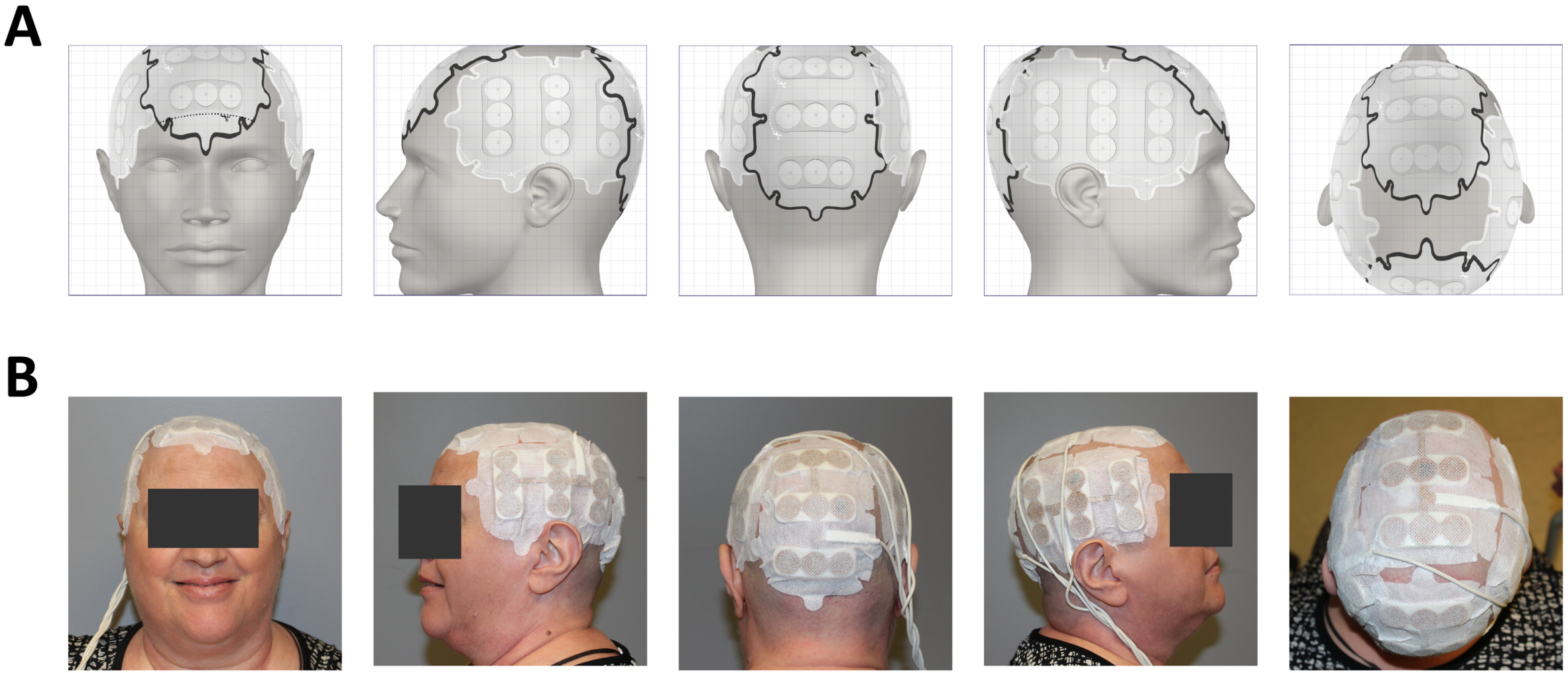


Figure 3

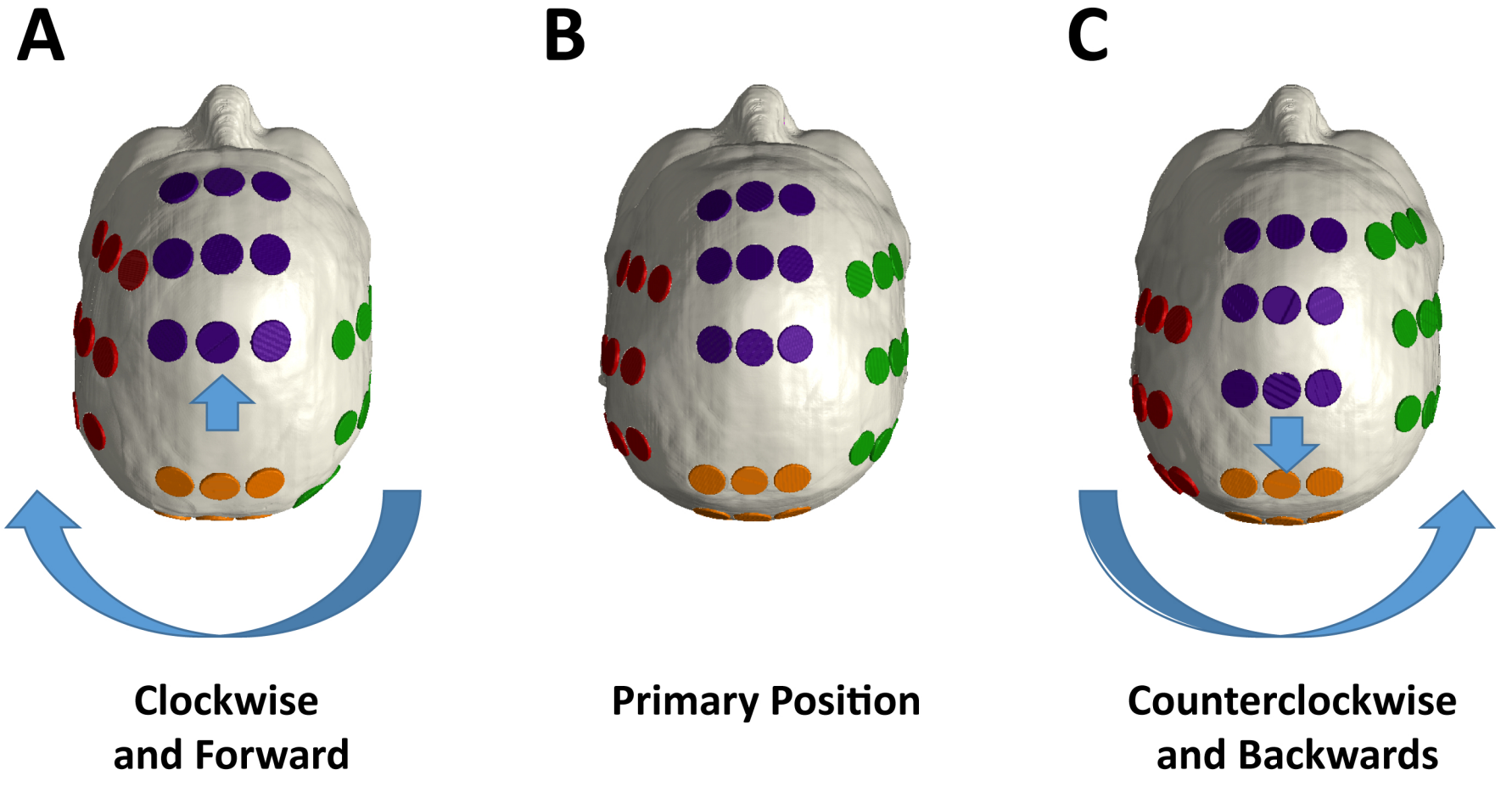


Figure 4

Figure 5

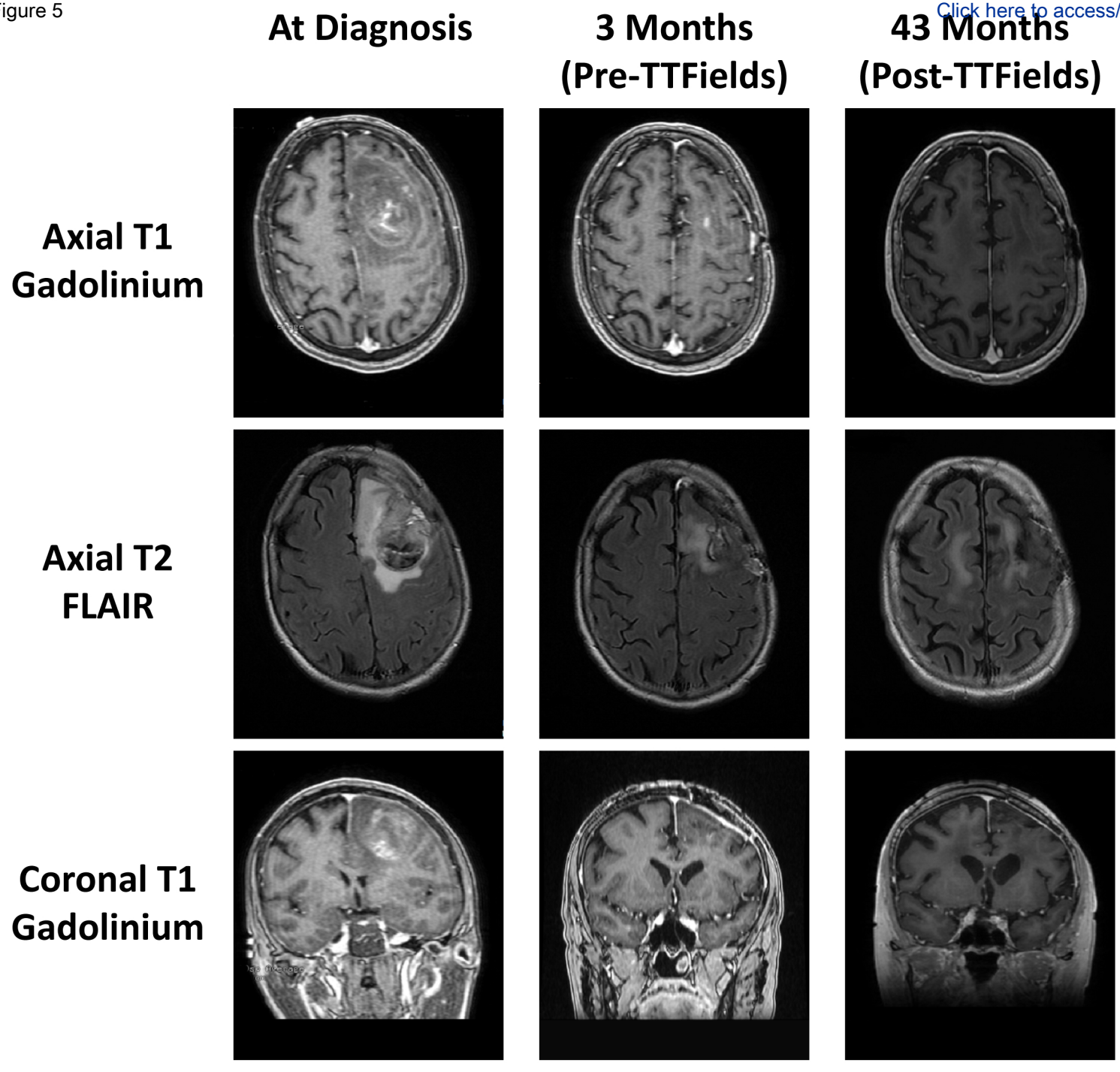
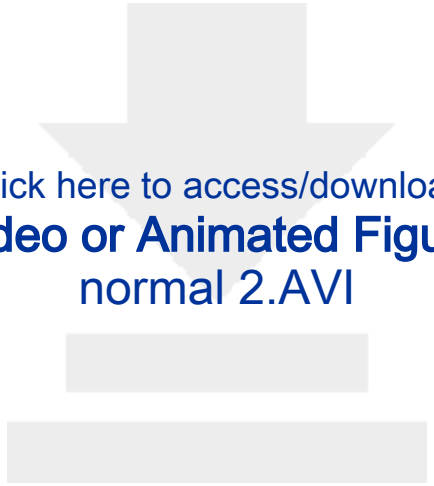


Figure 5

Normal Mitosis 1.1



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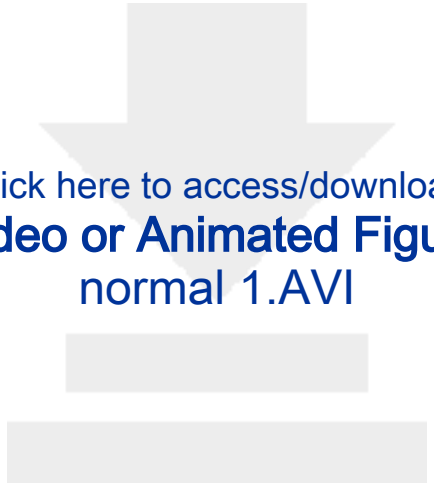
Normal Mitosis 1.2



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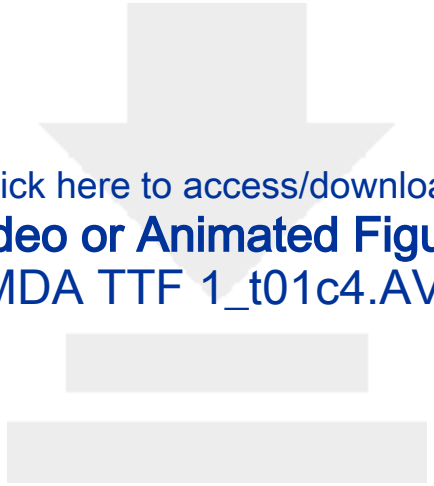

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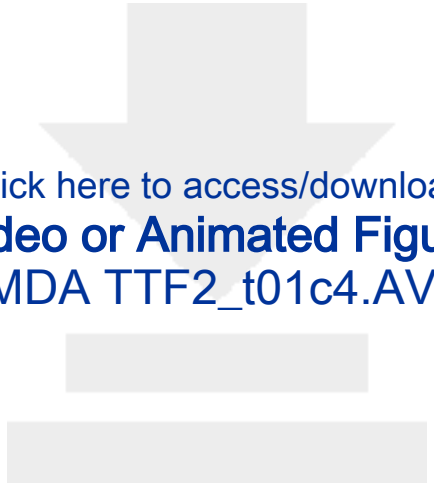

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Table of Materials

Name of Material/ Equipment	Company	Catalog Number
Baby Oil	Johnson & Johnson	Product Code 473542
Bevacizumab	Genetech, Inc.	Not applicable
Elastic Net	Medline Industries	NET012
Gentle Shampoo	Johnson & Johnson	Product Code 108249
Isopropyl Alcohol 70%	The Betty Mills Company	MON 23222701
Medical Tape	The Betty Mills Company	MON 38202201
Sterile Gauze	The Betty Mills Company	MON 71392000
Trimethoprim-sulfamethoxazole	Pfizer, Inc.	Not applicable
TTFields Device (Optune)	Novocure, Ltd.	Not applicable

Comments/Description

The system consists of the portable electric field generator, transducer arrays, a connection cable and box, a rechargeable battery, charger for portable batteries, and a plug in power supply.

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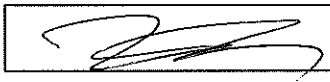
Signature:	
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Beth Israel Deaconess Medical Center

A teaching hospital of Harvard Medical School

August 21, 2018

Phillip Steindel, PhD
Review Editor
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One Alewife Center, Suite 200
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Tel: 617-674-1888

RE: JoVE58937 manuscript submission

Dear Dr. Steindel:

Thank you very much for your email communication on August 15, 2018 regarding our JoVE58937 manuscript, entitled "Tumor Treating Fields Therapy: Cell Biology, Electric Field Modeling, and Clinical Application". We have made changes in the manuscript by removing commercial identifiers and replaced it with generic terms. Specifically, NovoTTF-100A™ and Optune™ are now described as first and second generation TTFields devices, respectively. Furthermore, NovoTAL™ is now named as the treatment planning software for TTFields.

We hope that these changes are satisfactory and thank you very much for your consideration. We look forward to hearing from you.

Sincerely yours,

Eric T Wong, MD
Associate Professor of Neurology
Harvard Medical School

Director, Brain Tumor Center & Neuro-Oncology Unit
Beth Israel Deaconess Medical Center

Director, Neuro-Oncology Fellowship Program
Beth Israel Deaconess Medical Center

Member, Neuro-Oncology Protocol Committee
Dana Farber/Harvard Cancer Center

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".

2. 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."

3. 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.

Response to Editorial Comments:

1. Protocol: As this protocol involves human medical treatment, we must have a statement indicating that this protocol follows ethical guidelines (if not necessarily research guidelines).

The following statement has been added immediately after PROTOCOL heading in line 171:

"The presentation of this protocol follows ethical guidelines at Beth Israel Deaconess Medical Center and written authorization was obtained from the patient."

2. Protocol step 1.4: Around how often are these steps done?

Protocol step 1.4 is repeated every 3 to 4 days. We added section 1.4.6 as:

"1.4.6 Array exchange procedures from 1.4.1 to 1.4.5 are repeated every 3 to 4 days."

3. It's acceptable to present the cell biology results seen in Figure 1 without a corresponding protocol section, but there should be a little more information on how the figure and movies were obtained. Can all this information be found in reference 11 (as indicated in your response to Reviewer 1)? Please cite if so; otherwise, please outline any differences.

Figure 1 legend now includes a statement, "The procedures for obtaining video images were described in reference 11", at the end of sub-section (A) and (B).

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