RESEARCH ARTICLE | SEPTEMBER 15 2022

Potential prognostic factor in alternating electric fields therapy based on absorbed energy in tissue

Geon Oh ; Yunhui Jo ; Yongha Gi; Heehun Sung ; Jaehyun Seo; Hyunwoo Kim; Jaemin Lee; Myonggeun Yoon

AIP Advances 12, 095311 (2022)
https://doi.org/10.1063/5.0100820

Potential prognostic factor in alternating electric fields therapy based on absorbed energy in tissue

Cite as: AIP Advances 12, 095311 (2022); doi: 10.1063/5.0100820
Submitted: 28 May 2022 • Accepted: 22 August 2022 •
Published Online: 15 September 2022 •
Publisher Error Corrected: 26 September 2022

Geon Oh,[1] Yunhui Jo,[2] Yongha Gi,[1] Heehun Sung,[1] Jaehyun Seo,[1] Hyunwoo Kim,[1] Jaemin Lee,[3] and Myonggeun Yoon[1,4,a)]

AFFILIATIONS

[1] Department of Bioengineering, Korea University, Seoul 02841, Republic of Korea
[2] Institute of Global Health Technology (IGHT), Korea University, Seoul 02841, Republic of Korea
[3] Department of Internal Medicine, Anam Hospital, Korea University College of Medicine, Seoul 02841, Republic of Korea
[4] FieldCure Ltd., Seoul 02852, Republic of Korea

[a)] **Author to whom correspondence should be addressed:** radioyoon@korea.ac.kr

ABSTRACT

The present study aimed to determine a method for estimating a potential prognostic factor in alternating electric fields for the treatment of solid tumors based on cell survival curves that evaluate cell proliferation capability. In AGS, B16F10, U373, and HPAF-II cancer cell lines, the proportional relationships of the electric field magnitude and the duration of application with the proliferation of cancer cell lines was identified by *in vitro* alternating current electric field experiments performed under various conditions. A prognostic factor applicable to alternating electric field therapy was developed by identifying proportional relationships of the electric field magnitude and the duration of application with the proliferation of the four cancer cell lines. Through the experimental results, the absorbed energy in tissue has been suggested as a potential prognostic factor in alternating electric field therapy. The absorbed energy in tissue can be used as a reference to quantify the inhibition of cell proliferation related to control, enabling systematic assessment of alternating electric field therapy which, to date, has not been possible.

INTRODUCTION

Alternating current electric fields (AC-EFs) at low intensity (<3 V/cm) and intermediate frequency (100–300 kHz) were reported to inhibit the growth, *in vitro* and *in vivo*, of a variety of human and rodent tumor cell lines, including Patricia C, U-118, U-87, H-1299, MDA231, PC3, B16F1, F-98, C-6, RG2, and CT-26.[1] These AC-EFs, called tumor treating fields (TTFs), may inhibit cell proliferation via two mechanisms: interference with spindle formation due to an electric field and defects in cytokinesis caused by dielectrophoresis.[2] Thus, TTFs selectively kill cells that frequently divide, such as cancer cells, and have little effect on cells that rarely divide, such as normal cells.[3]

These TTFs were approved by the U.S. Food and Drug Administration (FDA) for the treatment of recurrent glioblastoma in 2011 and the newly diagnosed glioblastoma in 2015 and has received a CE mark in Europe.[4] Glioblastoma is a malignant tumor with a very high mortality rate, and since it is a tumor that is difficult to treat with conventional tumor treatment methods, such as surgery and chemotherapy, the therapeutic effect of TTFs was sufficient to attract public attention. At present, TTFs are used in about 1000 hospitals in the USA, Germany, and Switzerland.[4] In addition, the number of patients receiving this treatment is rapidly increasing every year, with 152 patients receiving TTF therapy in 2014 and ~14 000 in 2020, a greater than 90-fold increase over six years.[4]

The effect of TTF therapy varies with the magnitude of the electric field applied to cancer cells inside the body. Specifically, the greater the electric field magnitude, the greater the number of cancer cells killed.[5] Thus, current TTF therapy consists of the application of the maximum allowable electric field magnitude that does not cause any side effects to the skin. In addition, since

treatment duration is also proportional to the effect of TTFs, patients are treated for 18–24 h per day, up to the limit of patient tolerability.[5,6] However, since the relationship among these parameters is not absolutely linear, it is necessary to clarify this clearly.

TTF treatment has physical properties similar to radiation therapy, which involves electromagnetic waves. In radiation therapy, the absorbed dose is defined according to the degree of energy absorption of the material, and this is used to determine the prescribed dose. As such, the dose for radiation therapy is clearly defined, but it is not clearly defined for TTF therapy. Thus, unlike treatment plans in conventional treatment methods such as radiotherapy, the optimal electric field magnitude and duration of application cannot be accurately determined, which makes it difficult to provide treatment standards or guidelines. In general, the maximum allowable electric field with the maximum allowable treatment duration is currently applied to each patient without consideration of the degree of tumor progression, tumor development stage, or tumor type. Therefore, to systematize and optimize the implementation of TTFs in cancer patients, the dose should be clarified in TTF therapy.

A recent study of TTF therapy in 340 patients with brain tumors showed that patients with power loss densities (PLD) ≥ 0.77 mW/cm^3 had significantly better prognoses than patients with power loss densities <0.77 mW/cm^3.[7] Here, power loss density is defined as the energy absorption rate per unit volume and is proportional to the square of the applied electric field. In other words, power loss density is simply the absorbed energy density per unit time in each tissue. This means that power loss density does not include factors related to total treatment time, such as electric field application time, which is proportional to the cancer cell killing effect of TTF therapy.[8] Therefore, although power loss density can be considered a good prognostic factor, there are limitations in presenting power loss density as a dose in the treatment through TTF therapy.

In a study published in 2022, the TTF dose based on the product of TTF intensity and the average treatment time used to evaluate the effectiveness of TTF therapy for the treatment of glioblastoma and showed that tumor response is directly correlated with the TTF dose.[9] The concept of TTF dose is considered a more reasonable prognostic factor than the power loss density since it considers both the magnitude of the energy absorption rate in each tissue and the duration of the electric field. However, to verify the appropriateness of the proposed dose concept for TTF therapy, the concept of TTF dose should be also verified with quantitative analysis based on *in vitro* experiments.

In the present study, to determine the relationships of the magnitude and the duration of the electric field with the inhibitory effect of cell proliferation, electric fields varying in magnitude and duration were applied to several cancer cell lines. This led to a potential prognostic factor for TTF therapy suitable for prescribed dosages.

METHODS

AC-EFs of magnitudes of 0.6–1.2 V/cm and a frequency of 150 kHz were generated between a pair of insulated electrodes connected to a function generator (AFG-2112, Good Will Instrument Co. Ltd., New Taipei City, Taiwan) and a high-voltage amplifier (A303, A. A. Lab Systems Ltd., Ramat Gan, Israel). To maintain the cells under the influence of AC electric fields, a pair of insulated electrodes was attached to the bottom of each culture dish. In each experiment, a new pair of insulated electrodes was used, and the electric field magnitude was adjusted according to the treatment condition. To ensure that the conditions of the AC-EFs were adequately applied, voltage and frequency were measured using an oscilloscope (GDS-2102A, Good Will Instrument Co. Ltd.) before, during, and after each experiment, with visual confirmation on the stability of the insulated electrodes (Fig. 1). In general, the temperature in the treated media can be increased due to the heat generated by the applied electric fields. The measurement showed that the maximum increase in temperature of the treated media due to the application of AC-EFs was found to be ~0.3 °C, which seems negligible in our experiment.

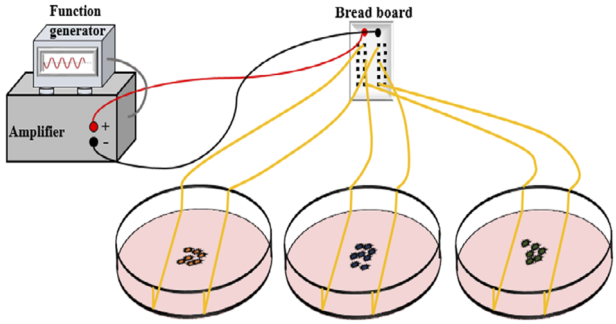

FIG. 1. Schematic diagram of the experimental setup.

To evaluate the effect of TTFs, different cell lines (AGS, HPAF-II, U373, and B16F10) were used (Table I). AGS and HPAF-II cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured according to the instructions supplied by the ATCC. U373 and B16F10 cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea) and cultured according to the instructions supplied by the Korean Cell Line Bank. For the colony formation assay using these cell lines, TTFs were applied to cells for 72 h under various treatment conditions. After 14–20 days, the colonies were stained with 0.4% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). The plating

TABLE I. Characteristics of the cell lines used.

Cell line	Organism	Tissue	Disease
U373	*Homo sapiens*, humans	Brain	Glioblastoma
B16-F10	*Mus musculus*, mice	Skin	Melanoma
AGS	*Homo sapiens*, humans	Stomach	Gastric carcinoma
HPAF-II	*Homo sapiens*, humans	Pancreas	Ductal adenocarcinoma

efficiency indicates the percentage of seeded cells of a particular cell line that formed colonies under specific culture conditions. The surviving cell fractions were calculated as colonies counted/(cells seeded × plating efficiency/100).

To find a prognostic factor that can be used for TTF therapy, absorbed energy density was considered by combining the magnitude and duration of electric fields. First, the absorbed energy density per unit time, which is equal to power loss density, was calculated based on the conductivity of the cell culture medium and the magnitude of the applied electric field.[7] The formula is shown as follows:

$$\text{Absorbed energy density per unit time} = \frac{1}{2}\sigma E^2, \tag{1}$$

where σ is the conductivity (Siemens/m) of the cell culture medium[10] and E is the magnitude of the applied electric field. By definition, multiplying the absorbed energy density per unit time by the total treatment time yields the absorbed energy density, as shown in the following equation:

$$\text{Absorbed energy density} = \frac{1}{2}\sigma E^2 \times \text{treatment time}. \tag{2}$$

RESULTS

To determine the relationships between the electric field magnitude and the inhibitory effect of cell proliferation, electric fields of magnitudes of 0.6, 0.9, and 1.2 V/cm were applied to four cell lines in their appropriate cell culture media, with a conductivity of 0.017 S/cm, for 72 h. These experiments confirmed the correlation between the squared electric field magnitude and the surviving fractions of the four tumor cell lines. The surviving fractions decreased in proportion to the increase in the square of the electric field magnitude, with the four tumor cell lines showing similar trends, despite differences in the slopes of their declines (Fig. 2).

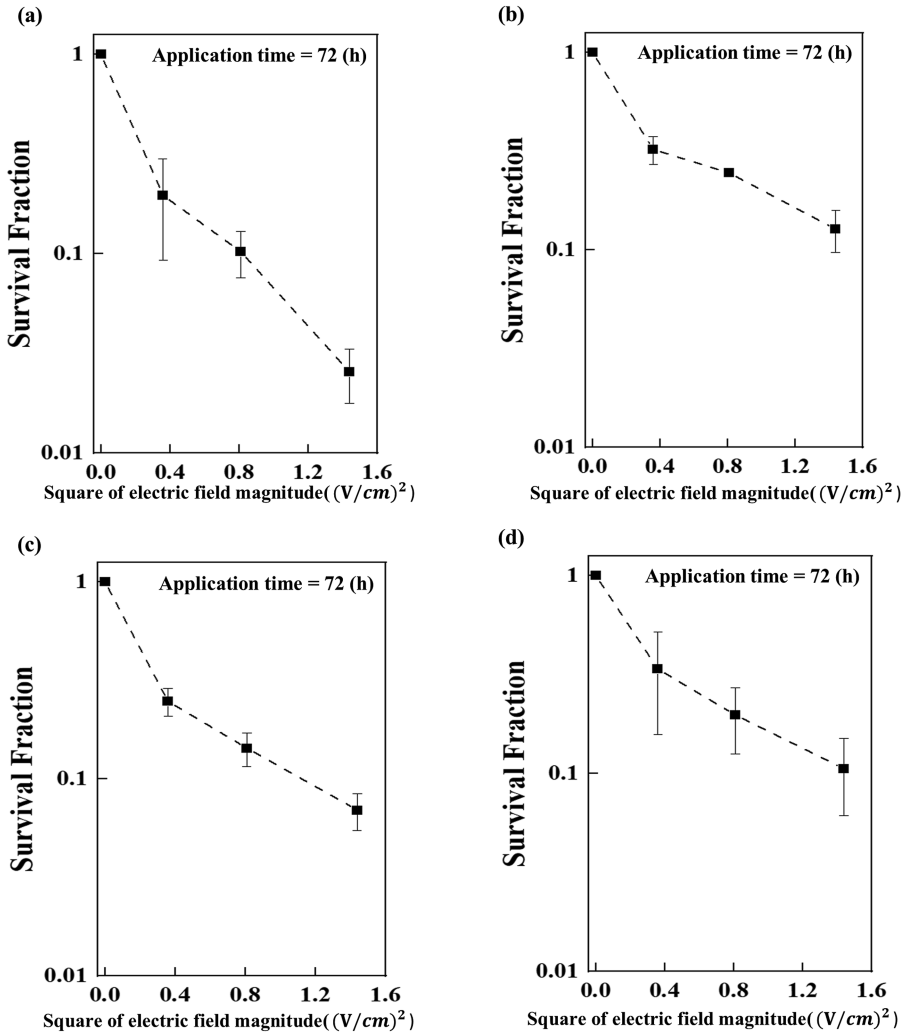

FIG. 2. Relationships between the square of the applied electric field magnitude and the surviving fractions of four tumor cell lines, (a) AGS, (b) B16F10, (c) U373, and (d) HPAF-II, after 72 h of TTFs.

To investigate the relationship between the duration of electric field application and the inhibitory effect, electric fields were applied to cell lines for 3, 6, 12, and 24 h, followed by a period of 24 h when the electric field was not applied, with the procedure repeated for three days. Therefore, various total treatment durations such as 9, 18, 36, and 72 h were applied to cell lines for three days. As seen in Fig. 3, the surviving fractions decreased in proportion to the increase in the duration of electric fields, with the four tumor cell lines showing similar trends, despite differences in the slopes of their declines.

After exposure to electric fields, the correlation between the absorbed energy density (J/cm^3) calculated using Eqs. (1) and (2) and the surviving fraction of the four types of cancer cells was assessed. Here, the magnitude of the total treatment time was varied from 9 to 72 h, whereas the magnitude of the absorbed energy density per unit time was maintained at 3.06 mW/cm^3 in the cell culture media. As the absorbed energy density increased from 0.98 to 1.97, 3.93, and 7.88 × 10^5 (J/cm^3), the surviving fraction decreased proportionately, with similar trends observed for all four types of cells (Fig. 4).

DISCUSSION

The findings of these results suggest that both the magnitude and duration of electric fields are proportional to the inhibitory effect of cell proliferation. For each tumor cell type, the absorbed energy density is correlated with the surviving fraction of tumor cells and is proportional to the suppression of tumor cell growth. The experimental evidence suggests that the absorbed energy density, which is a single variable dependent on both the magnitude and duration of electric fields, is a good candidate for a dosage unit for TTF therapy.

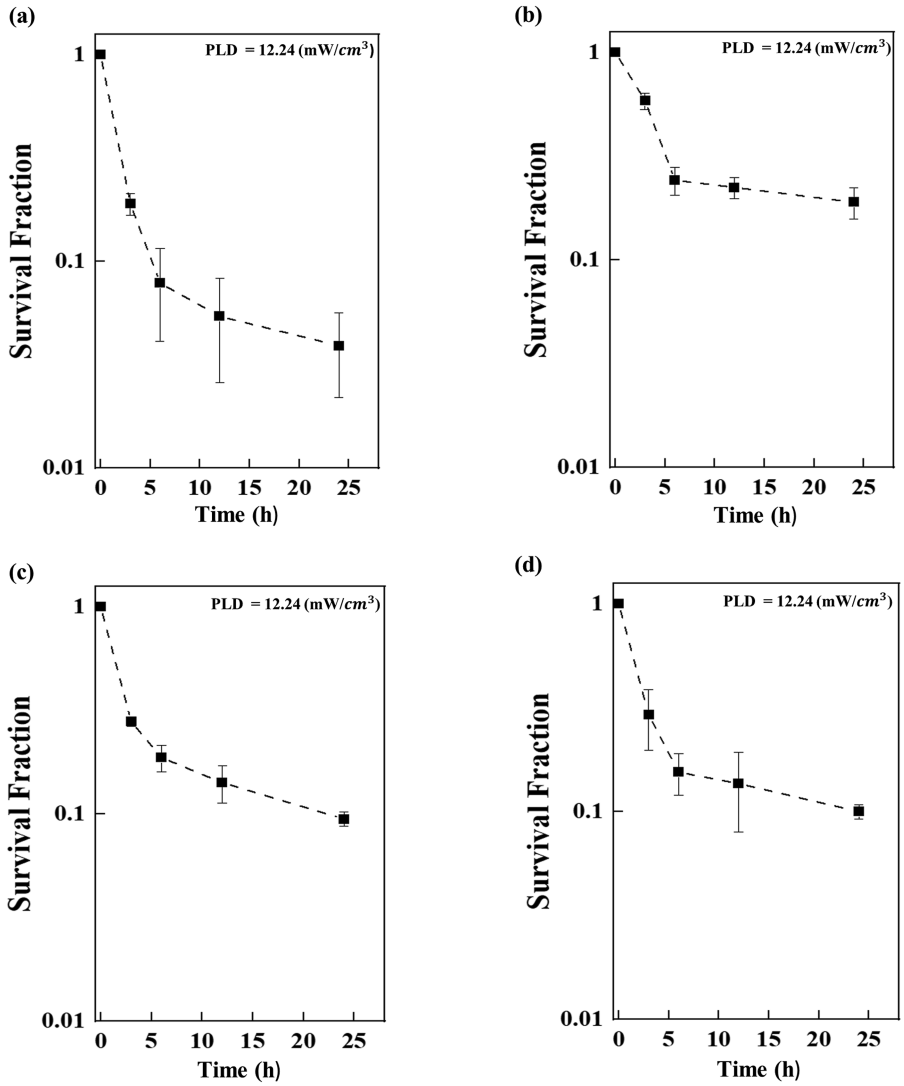

FIG. 3. Relationships between application times and survival rates of the four types of cancer cells, (a) AGS, (b) B16F10, (c) U373, and (d) HPAF-II, subsequent to TTFs after maintaining a constant power loss density (PLD) for 72 h.

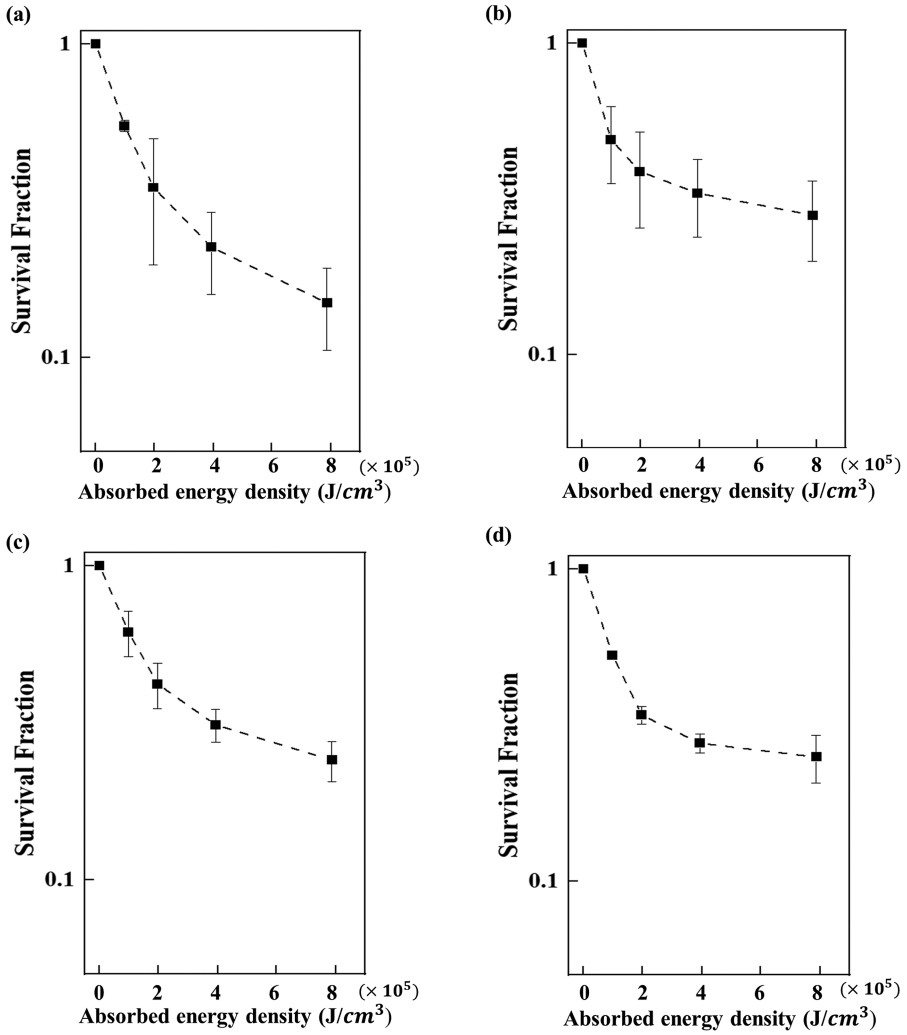

FIG. 4. Relationships between the absorbed energy density calculated by Eqs. (1) and (2) and the surviving fractions of four tumor cell lines, (a) AGS, (b) B16F10, (c) U373, and (d) HPAF-II, after 72 h of TTFs.

The experimental results suggest that power loss density may not be appropriate as a dosage unit for TTF therapy. At a constant power loss density (i.e., absorbed energy density per unit time), as seen in Fig. 4, the inhibition of cell proliferation is proportional to the duration of electric field application, indicating that dosage units necessary for TTF therapy should include both the magnitude and duration of exposure to an electric field. In radiation therapy, the inhibition of cell proliferation is also proportional to both the magnitude and duration of exposure to radiation. Due to this reason, the absorbed dose in radiotherapy was defined by considering both the duration of treatment and the energy absorption rate in each tissue.[11] Therefore, the absorbed energy density for TTF therapy is very much similar to the dose concept in radiotherapy in a sense that both consider the magnitude and the duration of treatment together.

Although the absorbed energy density for TTF therapy is similar to the absorbed dose in radiotherapy, they are slightly different. While the absorbed energy density in TTF therapy is the absorbed energy per unit volume of the tissue, the absorbed dose in radiotherapy is the absorbed energy per unit mass of the tissue. In the case of hyperthermia therapy, which uses radio frequency electromagnetic fields (RF EMFs) to treat tumors, the specific absorption rate (SAR) is generally used to measure the dosimetric quantity. The unit of SAR is defined as the amount of the absorbed energy rate per unit mass of biological tissue. Biologically, organs and tumors in the human body are composed of tissues with diverse characteristics, and they have different mass densities. In other words, although the same magnitude of energy is absorbed in a unit volume of tissue, the energy transferred to each cell in the unit volume of tissue will vary dependent on the mass density of the tissue.

In case of the same type of tissue with different mass densities, this means that if the absorbed energy per unit volume is the same, the absorbed energy per each cell is higher in a tissue with lower mass density. In this case, the absorbed energy per unit mass of tissue seems a more reasonable dosimetric quantity unit. However,

if various types of tissues are considered, it is not clear whether the absorbed energy per unit mass of tissue is a more reasonable dosimetric unit than the absorbed energy per unit volume or not. Therefore, time and research are needed to select a more appropriate unit for TTF therapy. Until then, the absorbed energy per unit mass of tissue can be used together with the absorbed energy per unit volume of tissue as a dosage unit for TTF therapy. Fortunately, there might be no big difference between the absorbed energy per unit mass and the absorbed energy per unit volume of tissue since most of the tissues except bone tissue generally show similar mass density.

The finding of this study also indicates that there was a rapid decrease in the cell survival fraction corresponding to the applied electric field initially and then the gradient was decreased beyond a certain point (Fig. 2). One possible reason for this gradient change may be due to the "overkill" effect of the high intensity electric field since cell killing does not seem to be effective at the region of high intensity electric field. In addition, when a fixed power loss density is applied to various cell lines, there appears to be a critical point at ~5 h, which does not appear to appreciably change with the change in the cell line (Fig. 3). In electric field therapy, the magnitude of the electric field inside a cell is different based on its orientation to the applied electric field. While the electric field is maximum inside a cell when the axis of the cleavage furrow is perpendicular to the applied electric field, the electric field inside a cell becomes negligible when the axis of the cleavage furrow is parallel to the applied electric field.[1,5] Therefore, if some of the cells are not well aligned to applied electric fields, those cells should not be affected by the applied electric field irrespective of the treatment duration. This might be the reason why the graph becomes saturated beyond a certain point in Fig. 3. Although possible reasons were suggested for the shape in Figs. 2 and 3, further research is needed to investigate a simple model with some characteristic constants, which can explain the relationship among the treatment duration, intensity of the electric field, and inhibitory effect of the cell division.

There is another concern in this study. In TTF therapy, the inhibitory effects on cell proliferation of electric fields of different frequencies can vary, and a particular frequency band provides the best treatment effect for each type of cancer cells.[12] In other words, the same magnitude of the physical dose can induce different biological outcomes. Therefore, correlations between the frequency and the inhibitory effect of cell proliferation should also be evaluated to define the relevant biological factors associated with the cancer inhibitory effect of cell proliferation, thus determining the frequency based relative biological effectiveness (RBE). In addition, although the present study assessed the effects of TTF therapy on four types of cancer cells, other types of cells should be tested to determine the consistency of these findings. For example, a previous study suggested that TTF therapy induced cell death selectively in cancer cells but not in normal cells, resulting in tumor specificity.[13] While TTF therapy seems to mainly target proliferating cells only by inhibiting mitosis and cytokinesis, several *in vitro* studies have reported the non-mitotic effects of TTF therapy.[14,15] Therefore, it is important to consider the difference in cell survival rates between cancerous cell lines and non-cancerous cell lines.

In summary, the cell proliferation inhibitory effect in the four cell lines is correlated with the square of the electric field magnitude and the duration of electric field application. The results of this study suggest that the TTF dose can be measured by determining the absorbed energy density, a parameter that includes both the magnitude and duration of electric field application. These findings may provide a safe and effective method for the prescription of TTF therapy.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (Grant Nos. 2021R1A2C2008695 and 2022R1A2C1010337) and a Korea University grant.

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Ethics Approval

Our study did not require an ethical board approval because it did not contain human or animal trials.

Author Contributions

Geon Oh and Yunhui Jo contributed equally to this paper.

Geon Oh: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (equal); Software (lead); Writing – original draft (lead). **Yunhui Jo**: Conceptualization (equal); Data curation (lead); Formal analysis (equal); Methodology (lead); Writing – original draft (equal); Writing – review & editing (equal). **Yongha Gi**: Formal analysis (equal); Software (equal). **Heehun Sung**: Data curation (equal); Software (equal). **Jaehyun Seo**: Data curation (equal). **Hyunwoo Kim**: Methodology (equal). **Jaemin Lee**: Supervision (equal). **Myonggeun Yoon**: Conceptualization (lead); Investigation (lead); Resources (lead); Supervision (lead); Writing – review & editing (lead).

DATA AVAILABILITY

The data that support the findings of this study are available within the article.

REFERENCES

[1] E. D. Kirson, Z. Gurvich, R. Schneiderman, E. Dekel, A. Itzhaki, Y. Wasserman, R. Schatzberger, and Y. Palti, Cancer Res. **64**, 3288 (2004).
[2] M. Pless and U. Weinberg, Expert Opin. Invest. Drugs **20**, 1099 (2011).
[3] F. A. Carrieri, C. Smack, I. Siddiqui, L. R. Kleinberg, and P. T. Tran, Front. Oncol. **10**, 575992 (2020).
[4] Novocure, Novocure Corporate Presentation, 2019.
[5] E. D. Kirson, V. Dbalý, F. Tovaryš, J. Vymazal, J. F. Soustiel, A. Itzhaki, D. Mordechovich, S. Steinberg-Shapira, Z. Gurvich, R. Schneiderman, Y. Wasserman, M. Salzberg, B. Ryffel, D. Goldsher, E. Dekel, and Y. Palti, Proc. Natl. Acad. Sci. U. S. A. **104**, 10152 (2007).
[6] D. Fabian, M. D. P. Guillermo Prieto Eibl, I. Alnahhas, N. Sebastian, P. Giglio, V. Puduvalli, J. Gonzalez, and J. Palmer, Cancers **11**, 174 (2019).

[7]M. T. Ballo, N. Urman, G. Lavy-Shahaf, J. Grewal, Z. Bomzon, and S. Toms, Int. J. Radiat. Oncol., Biol., Phys. **104**, 1106 (2019).
[8]Y. Jo, J. Sung, H. Jeong, S. Hong, Y. K. Jeong, E. H. Kim, and M. Yoon, Technol. Cancer Res. Treat. **18,** 1533033819845008 (2019).
[9]M. Glas, M. T. Ballo, Z. Bomzon, N. Urman, S. Levi, G. Lavy-Shahaf, S. Jeyapalan, T. T. Sio, P. M. DeRose, M. Misch, S. Taillibert, Z. Ram, A. F. Hottinger, J. Easaw, C.-Y. Kim, S. Mohan, and R. Stupp, Int. J. Radiat. Oncol., Biol., Phys. **112**, 1269 (2022).
[10]Q. Lang, Y. Wu, Y. Ren, Y. Tao, L. Lei, and H. Jiang, ACS Appl. Mater. Interfaces **7**, 26792 (2015).
[11]F. M. Khan and J. P. Gibbons, *Khan's The Physics of Radiation Therapy* (Lippincott Williams & Wilkins, 2014).
[12]Y. Porat, M. Giladi, R. S. Schneiderman, R. Blat, A. Shteingauz, E. Zeevi, M. Munster, T. Voloshin, N. Kaynan, O. Tal, E. D. Kirson, U. Weinberg, and Y. Palti, J. Visualized Exp. **123**, e55820 (2017).
[13]Y. Jo, S.-G. Hwang, Y. B. Jin, J. Sung, Y. K. Jeong, J. H. Baek, J.-M. Cho, E. H. Kim, and M. Yoon, Cell Death Discovery **4**, 46 (2018).
[14]M. Giladi, R. S. Schneiderman, T. Voloshin, Y. Porat, M. Munster, R. Blat, S. Sherbo, Z. Bomzon, N. Urman, and A. Itzhaki, Sci. Rep. **5**, 18046 (2016).
[15]E. Chang, C. B. Patel, C. Pohling, C. Young, J. Song, T. A. Flores, Y. Zeng, L.-M. Joubert, H. Arami, and A. Natarajan, Cell Death Discovery **4**, 113 (2018).