Preclinical Evaluation of Tumor Treating Fields (TTFields) with Immunochemotherapy in small cell lung carcinoma

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Introduction

- Small cell lung cancer (SCLC) is a highly aggressive malignancy treated with platinum-etoposide chemotherapy, immunotherapy, and concurrent thoracic radiotherapy.[1,2]
- Tumor Treating Fields (TTFields) are electric fields that exert physical forces to disrupt cellular processes critical for cancer cell viability and tumor progression, approved for treatment of glioblastoma, pleural mesothelioma, metastatic non-small cell lung carcinoma, and locally advanced pancreatic cancer.[3,4]
- TTFields have been shown to downregulate DNA damage repair (DDR) pathways and induce immunogenic cell death (ICD) in various cancer cell types.[3,4]
- The current preclinical study aimed to investigate the efficacy of TTFields together with SCLC standard treatments.

Methods

- **TTFields in vitro:** Human SCLC cells were treated with 150 kHz TTFields, with or without cisplatin and etoposide (CE). Treatment conditions: **H196 cells**: 1.62 V/cm RMS TTFields, 0.15 µM cisplatin, 0.3 µM etoposide, 120 h; **DMS-53 cells**: 1 V/cm RMS TTFields, 0.25 µM cisplatin, 0.5 µM etoposide, 72 h.
- **Cell count and overall effect:** Cells were counted by flow cytometry. Cells were harvested, re-plated, and grown for 14-28 days. Colonies were stained with crystal violet. Overall effect was calculated by multiplying colonies with respective cell count.
- **FA-BRCA protein expression:** Cell Extracts were prepared using RIPA buffer and subjected to Western blot.
- **DNA damage:** Cells were fixed, permeabilized and stained with anti-γH2AX antibody. Slides were mounted with DAPI nuclear stain, and images collected on a fluorescent microscope.
- **Calreticulin detection:** Cells were stained with anti-CRT antibody and 7-AAD and acquired by flow cytometry.
- **HMGB1 detection:** Supernatants were collected and quantified by HTRF assay.
- **TTFields in vivo:** Male C57Bl/6 mice were orthotopically inoculated with 50,000 murine KP3 SCLC cells. Seven days later, TTFields or sham-heat were continuous applied for 10 days. Cisplatin (2 mg/kg), etoposide (8 mg/kg), and anti-PD-L1 (10 mg/kg) were administered i.p. according to the timeline in Figure 4.
- At study end, sera was collected. Tumors were examined with MRI, excised, weighed and measured with calipers. Tumors were then processed into single cell suspensions.
- Sera were examined for HMGB1 levels with a dedicated ELISA kit.
- Tumor sections were examined for γH2AX and TUNEL using immunohistochemistry.
- Tumor single cell suspensions were stained for γH2AX and for immunophenotyping with Zombie NIR and the following markers: CD45, CD11b, CD3e, CD4, CD8a, CD11c, CD44, CD62L, F480. For the detection of cells pre-expressing IFNγ, single cell suspensions were re-stimulated using PMA/Ionomycin for 2 hours, followed by intracellular staining of IFNγ.
- Significance: *p < 0.05, **p < 0.01, ***p < 0.001 , ****p < 0.0001

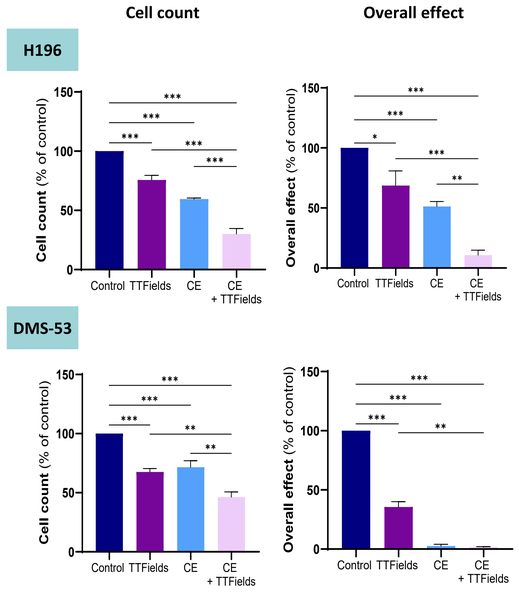
Conclusions

- Our results demonstrate efficacy of TTFields for the treatment of SCLC in preclinical models, and a potential advantage for TTFields concomitant with standard chemoimmunotherapy treatments.
- The enhanced efficacy of TTFields with chemoimmunotherapy may be attributed to induction of DNA damage, downregulation of key DDR pathways, and induction of ICD by TTFields.

References: **1.** Siegel RL, et al. (2022*). CA Cancer J Clin.* 72: 7-33. **2.** Petty WJ, Paz-Ares L. (2023). *JAMA Oncol.* 9(3): 419–429. **3.** Karanam NK, Story MD (2021). *Int J Radiat Biol.* 97(8): 1044-1054. **4.** Moser JC, et al. (2022). *Cancer Res.* 82(20): 3650-3658.

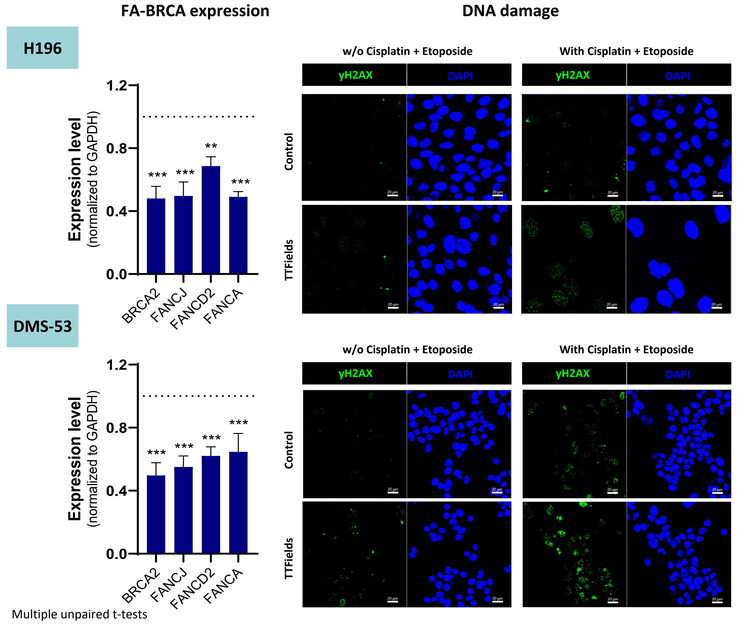
Results

FIGURE 1. TTFields plus cisplatin and etoposide (CE) were more efficacious than either TTFields or CE alone



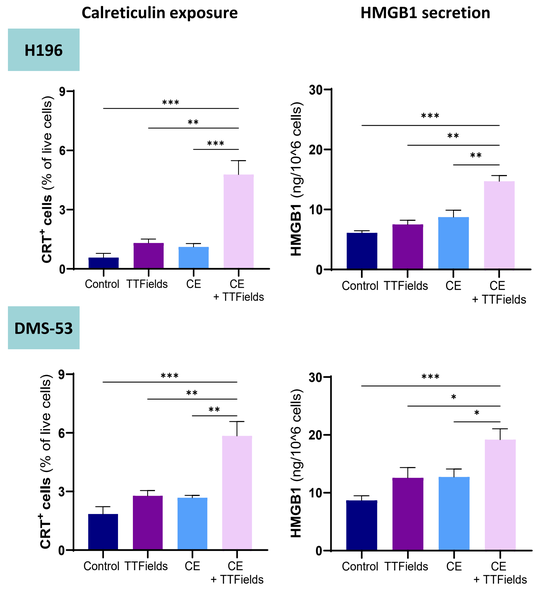
One-way ANOVA with Tukey's multiple comparison

FIGURE 2. TTFields downregulated proteins in the FA-BRCA DNA repair pathway and, when applied with CE, induced more DNA damage than either TTFields or CE alone



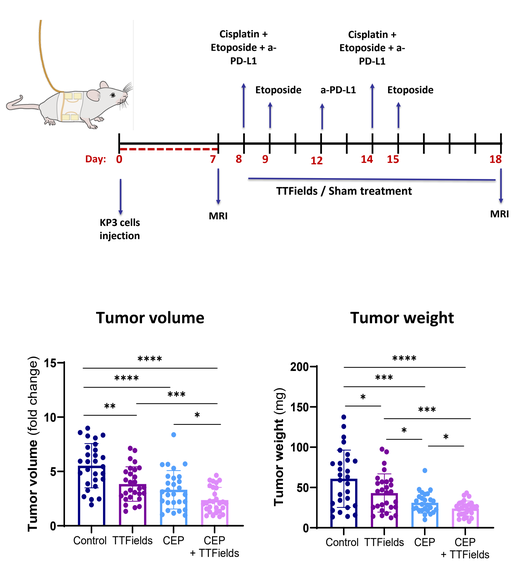
Multiple unpaired t-tests

FIGURE 3. TTFields plus CE enhanced ICD beyond either TTFields or CE alone



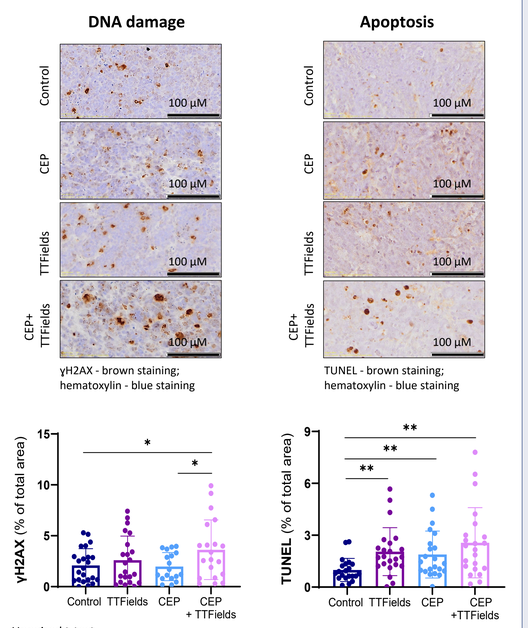
One-way ANOVA with Tukey's multiple comparison

FIGURE 4. TTFields reduced tumor growth versus control, with greater benefit when applied concomitantly with cisplatin, etoposide, and anti-PD-L1 (CEP)



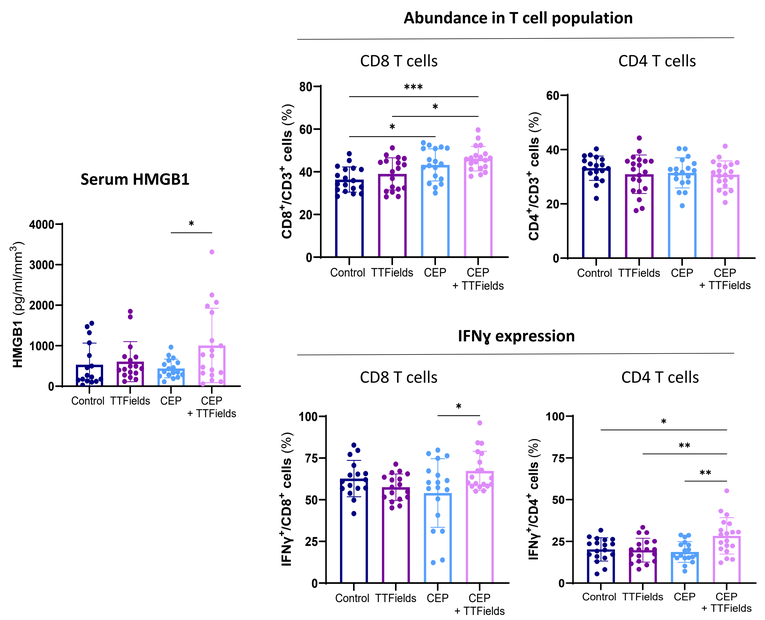
Unpaired t-test

FIGURE 5. TTFields plus CEP increased DNA damage and apoptosis relative to TTFields or CEP alone



Unpaired t-test

FIGURE 6. TTFields plus CEP elevated serum HMGB1, enhanced tumor infiltration of CD8 T cells, and increased interferon-γ production by tumor infiltrating CD4 T cells



One-way ANOVA with Tukey's multiple comparison