Treatment of non-small cell lung carcinoma (NSCLC) cells with Tumor Treating Fields (TTFields) and DNA-dependent protein kinase (PK) inhibitors

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Introduction

- Double-stranded breaks (DSBs) are considered the most deleterious form of DNA damage and can be repaired in cells through three main repair pathways: homologous recombination (HR), non-homologous end-joining (NHEJ), and microhomology-mediated end-joining (MMEJ). [1]
- Tumor Treating Fields (TTFields) are non-invasive electric fields that disrupt cellular processes critical for cancer cell viability and tumor progression. Recent research showed that TTFields downregulate expression of proteins from the Fanconi Anemia-BRCA pathway, thus lowering the cell's repair capacity via the HR pathway, leading to replication stress and DNA damage. [2-4]
- Nedisertib and CC-115 are potent inhibitors of DNA-PK, a main protein mediating the repair of DSBs through the NHEJ pathway. CC-115 also inhibits mTOR, which regulates cell proliferation and survival. [5-6]
- The current study examined the potential of the concomitant treatment of TTFields and DNA-PK inhibitors to impair DSBs repair and whether this effect can promote accumulation of DNA damage, triggering cancer cell death.

Methods

- **TTFields application:** A549 and H1299 human non-small cell lung cancer (NSCLC) cells were treated with TTFields (1 V/cm RMS, 150 kHz) using the inovitro system.
- **Co-application experiments:** TTFields were applied to the cells for 72 h in the absence or presence of various concentrations of the DNA-PK inhibitors nedisertib or CC-115. To determine the type of TTFields-drug interaction, the expected additive effect was calculated from the effects of the individual treatments and compared to the actual measured value for treatments co-application.
- **Cell count:** Treated cells were counted using a flow cytometer, and cell count calculated relative to control cells.
- **Overall effect:** Treated cells were harvested, re-plated, and grown up to 14 days. Then, colonies were stained (0.5% crystal violet solution), counted, and clonogenic effect calculated relative to control. The overall effect was calculated by multiplying cell count and clonogenic effect.
- **Apoptosis:** Treated cells were double-stained with FITC-conjugated annexin V (AnnV) and 7-aminoactinomycin D (7AAD), and data acquisition was performed with a flow cytometer.
- **Cell Cycle:** Cells were fixed with ethanol, pelleted, washed, and stained with 7-AAD. Data acquisition was performed by flow cytometry at 665/30 nm.
- **DNA damage:** Treated cells were fixed (4% paraformaldehyde), permeabilized (0.1% Triton X-100) and stained with anti-γH2AX antibody. Slides were mounted in the presence of DAPI nuclear stain, and images collected on a fluorescent microscope.
- **Western blot:** Cell extracts were prepared using RIPA buffer and subjected to Western blot with appropriate antibodies.

References: 1. Yue, X., et al., (2020). Frontiers in genetics 11: 607428. 2. Karanam, N. K., et al. (2020). Transl Res 217: 33-46. 3. Mumblat, H., et al. (2021). Lung Cancer 160: 99-110. 4. Fishman, H., et al. (2023). J Neurooncol 163(1): 83-94. 5. Chen, F., et al. (2022). Cell Death Discov. 8; 293-301. 6. Wang, M., et al. (2021) Acta Pharm Sin B. 11(12): 3935-3949.

Results

FIGURE 1. In **A549** cells, TTFields synergistically enhanced the effect of nedisertib and CC-115 and led to a G1 arrest

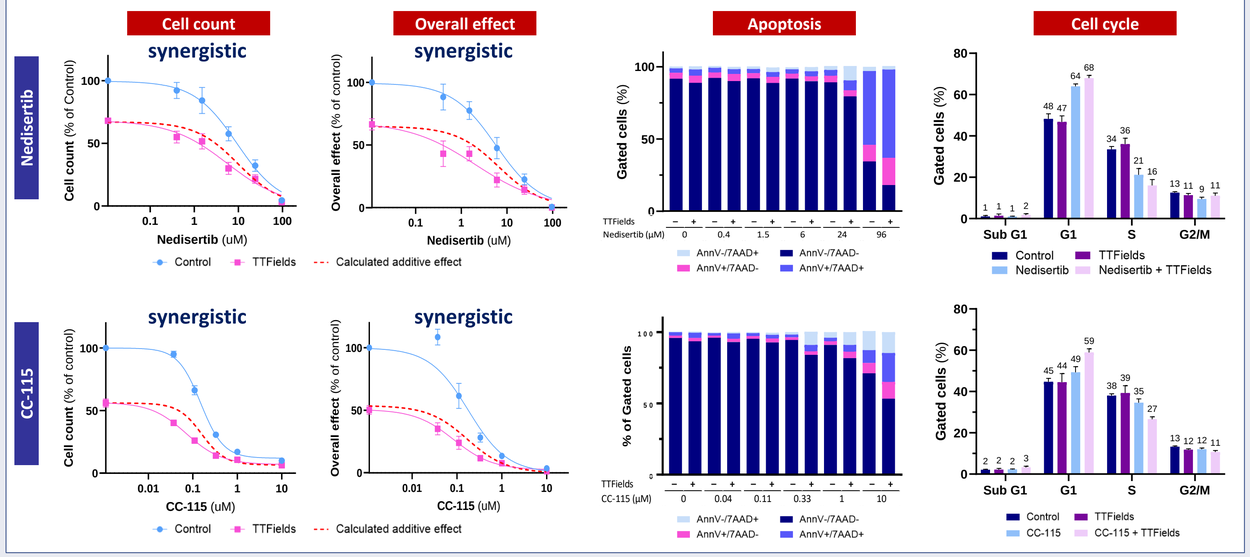


FIGURE 2. In **H1299** cells, TTFields enhanced the effect of nedisertib and CC115 mostly additively, and led to a G1 arrest

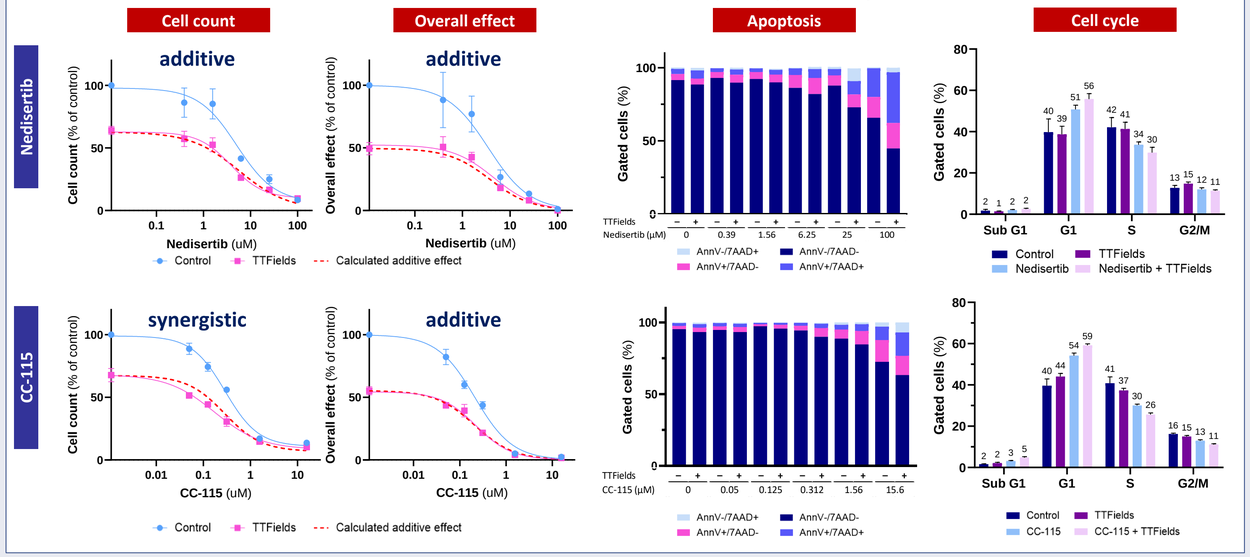
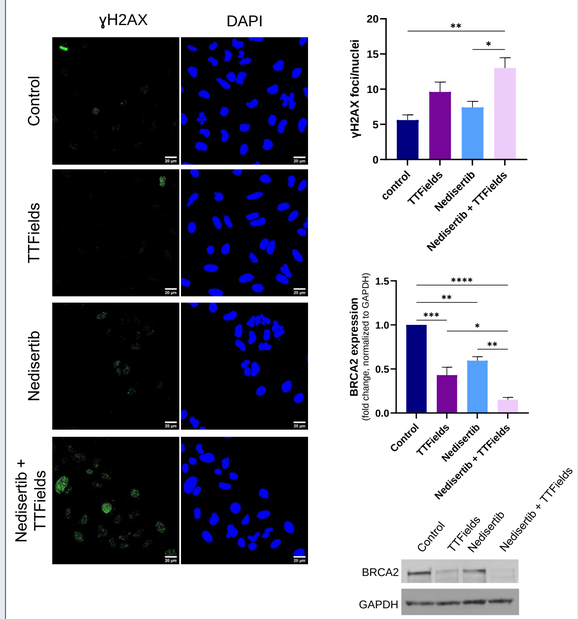


FIGURE 3. In **A549** cells, concomitant application of TTFields with nedisertib elevated DNA damage and decreased BRCA2 expression in A549 cells



*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; One-way ANOVA, Tukey's test

Conclusions

- Our results suggest a potential advantage for TTFields concomitant with DNA-PK inhibitors. The stronger interaction between TTFields and CC-115 compared to that with nedisertib seen in both cell lines, may possibly be attributed to the dual inhibitory effect of CC-115.
- The lower effect of both drugs in H1299 cells compared to A549 cells may be explained, at least in part, by the fact that H1299 cells have an amplification mutation in PRKDC, the catalytic subunit of DNA-PK.
- The benefit of the concomitant application may be attributed to effects on DNA damage and repair.
- Future studies will test whether treatment with TTFields plus DNA-PK inhibitors may sensitize cancer cells to irradiation, a treatment modality that induces DSBs.

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