Pancreatic cancer cells are sensitized to FOLFIRINOX treatment by co-application with Tumor Treating Fields (TTFields)

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Poster 316P

Introduction

- Pancreatic cancer remains a leading cause of cancer-related death, with limited treatment options available for the advanced stage.[1]
- Tumor Treating Fields (TTFields) are electric fields that have shown *in vitro* and *in vivo* efficacy in pancreatic cancer models, with an optimal frequency of 150 kHz,[2] and have been shown to induce a state of BRCAness in various cancer types.[3,4]
- TTFields have improved survival when used together with Gem/NabP in a recent phase 3 clinical trial (PANOVA-3, NCT03377491).[5]
- FOLFIRINOX is a combination regimen of fluorouracil, oxaliplatin, irinotecan and leucovorin, serving as a first-line chemotherapy option in the advanced setting.[1]
- BRCA-mutated tumors seem to be more sensitive to FOLFIRINOX.[1]
- This *in vitro* study examined the potential concomitant use of TTFields with FOLFORINOX for pancreatic cancer treatment.

Methods

- **TTFields application:** AsPC1 and BxPC3 BRCA-wild type human pancreatic cancer cells were treated with 150 kHz TTFields (1 and 0.7 V/cm RMS, respectively), with or without various FOLFIRINOX concentrations, for 24-72 hrs using the inovitro system.
- **Co-application experiments:** TTFields were applied for 72 hrs in the absence or presence of increasing concentrations of FOLFIRINOX, and tested for cell count, colony formation, and apoptosis.
- **Cell count:** Treated cells were counted using a flow cytometer, and cell count was calculated relative to control.
- **Overall effect:** Treated cells were harvested, re-plated, and grown for an additional 7-14 days. Colonies were stained (0.5% crystal violet solution), counted, and colony formation was calculated relative to control. Overall effect was calculated by multiplying with the corresponding cell count.
- **RNA sequencing:** RNA was isolated from TTFields-treated and control samples, and a list of differentially expressed genes (DEGs) by time point and cell type was generated. Gene Set Enrichment Analysis (GSEA) was performed on the gene lists pre-ordered by their z score, using the GSEA software against the Reactome gene set, showing a subset of pathways divided by their Reactome head pathway.
- **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 confocal microscope.

References: 1. M.N. Rosen, et al. (2021) *World J Gastroenterol.* 27 (17): 1943-1958. 2. Giladi, M., et al. (2014). *Pancreatology* 14(1): 54-63 3. N.K. Karanam, et al. *Transl. Res.* 2020; 217: 33. 4. H. Mumblat et al. *Lung Cancer.* 2021; 160: 99. 5 H. M. Babiker et al. (2023) *J. Clin. Oncol.* 41(16_suppl): p. TPS4199-TPS4199

Results

FIGURE 1. TTFields augmented the cytotoxic effects of FOLFORINOX in pancreatic cancer cells

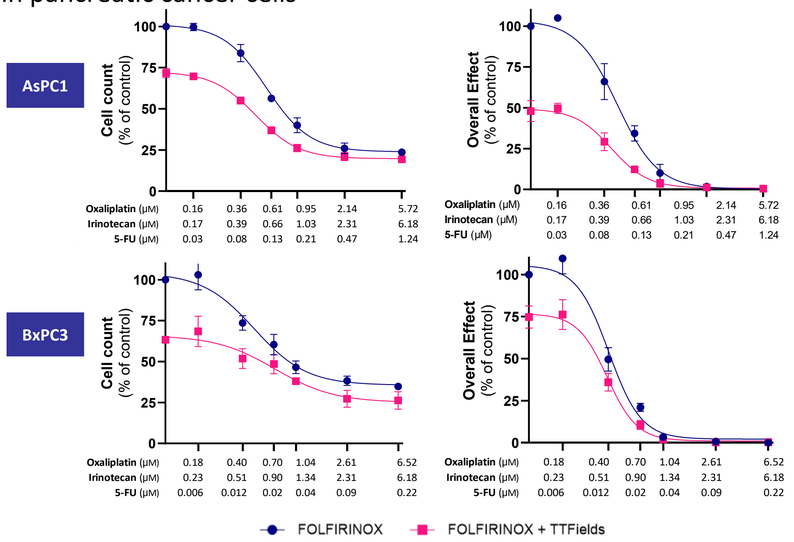
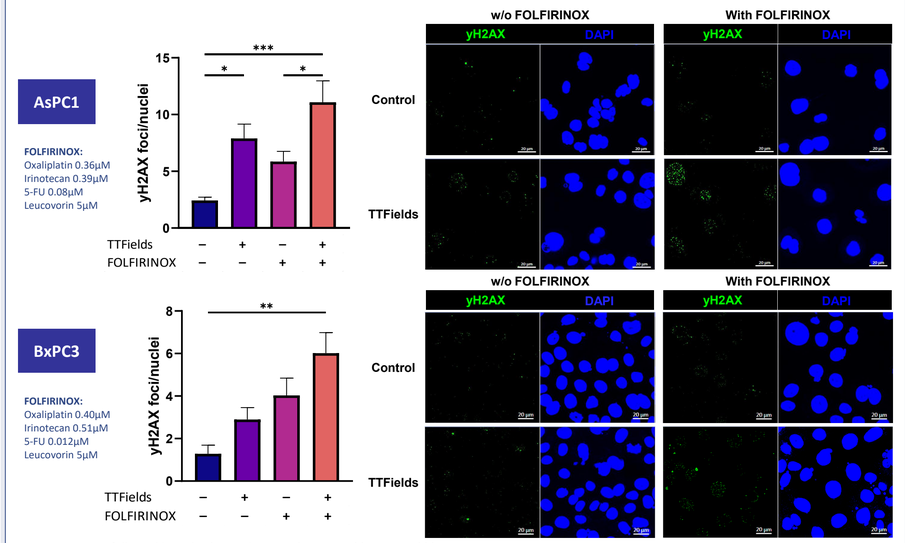
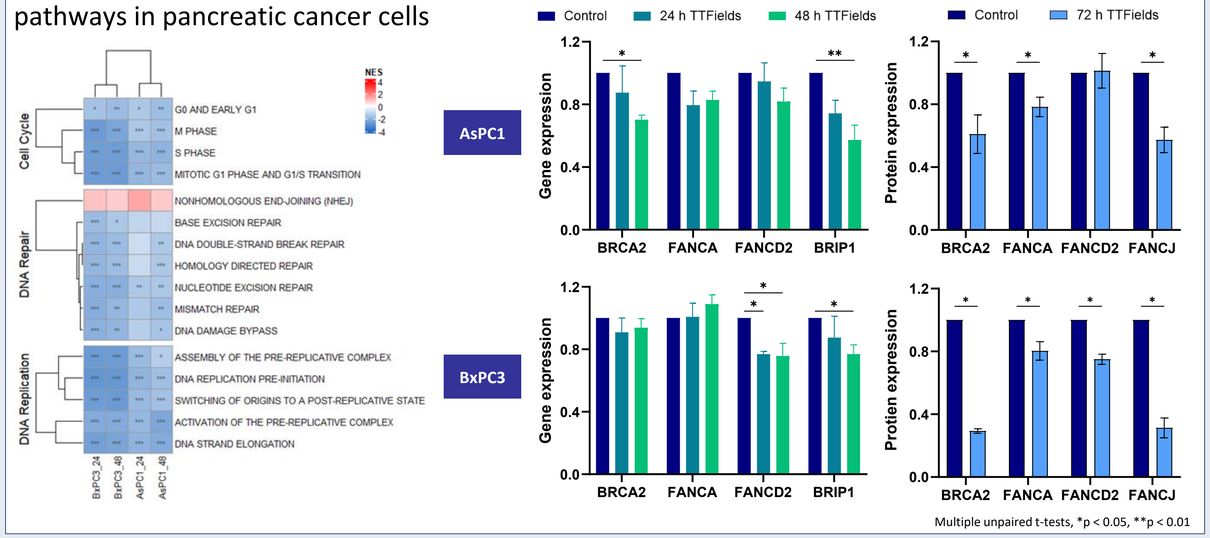


FIGURE 3. TTFields induced DNA damage in pancreatic cancer cells



one-way ANOVA followed by Tukey's post hoc test *p < 0.05, **p < 0.01, ***p < 0.001

FIGURE 2. TTFields downregulated cell cycle-related pathways and DNA replication and repair pathways in pancreatic cancer cells



Multiple unpaired t-tests, *p < 0.05, **p < 0.01

Conclusions

- TTFields application together with FOLFIRINOX in pancreatic cancer cells lacking background BRCA mutations exhibits potential improvement relative to FOLFIRINOX alone.
- The improved efficacy of FOLFIRINOX with TTFields may be rationalized based on the induction of DNA damage and downregulation of key DNA repair pathways.
- Future studies should explore the possibility of reducing FOLFIRINOX treatment dose, potentially alleviating toxicity related to FOLFIRINOX treatment.

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