Combination of Tumor Treating Fields (TTFields) with neoantigen-targeted anticancer vaccines to effectively treat murine pancreatic tumors

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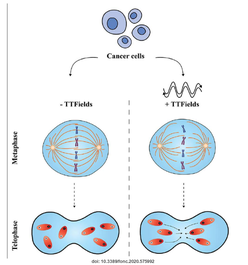
Purpose

- ✓ Tumor-treating-fields (TTFields) therapy uses alternating electric fields delivered via transducer arrays placed regionally close to the tumor site to non-invasively inhibit tumor growth.
- ✓ The mechanisms responsible for the anticancer effects of TTFields in vivo are not yet clear . One hypothesis is that TTFields therapy enhances tumor immunogenicity.
- ✓ The overall goal of this study is to determine whether TTFields alone or in combination with a neoantigen targeted vaccine, PancVax, can stimulate antitumor immunity in a murine model of pancreatic cancer.

Introduction

TTFields Therapy is based on the delivery of low-intensity (1 – 3 V/cm), intermediate-frequency (100 – 500 kHz) alternating electric fields to the tumor using transducer arrays placed on the skin around the region of the body containing the tumor.



Francesca Anna Carrieri et al., Front. In Onc.

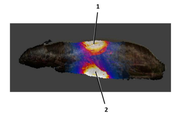


Figure 28: Electric Fields Simulation on Animal Torso in Areas A and B

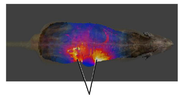


Figure 29: Electric Fields Simulation on Animal Right Flank SC

NOVOCURE inovivo™ System User Manual

This study is designed to test whether TTFields can improve the immune response to tumor-specific neoantigens.

Methods and Materials

- ➢ Early endeavors have been focused on the downstream analysis of adaptive immune cell populations following TTFields treatment. A splenocyte stimulation assay was used to comparatively assess pancreatic tumor-bearing mice treated with TTFields and a panel of controls.
- ➢ To assess T-cell responses to a defined peptide antigen, splenocytes were first isolated from mice, red blood cells were lysed, and the single-cell suspension was cultured in complete medium. Cells were then stimulated ex vivo with individual or pooled peptides that constitute minimal CD8 epitopes that are expressed from the Panc-02 pancreatic cancer cell line.
- ➢ Cytokine flow cytometry and enzyme-linked immunospot (ELISpot) assays were utilized to quantify the production of interferon-gamma (IFN-γ) expressing T cells.

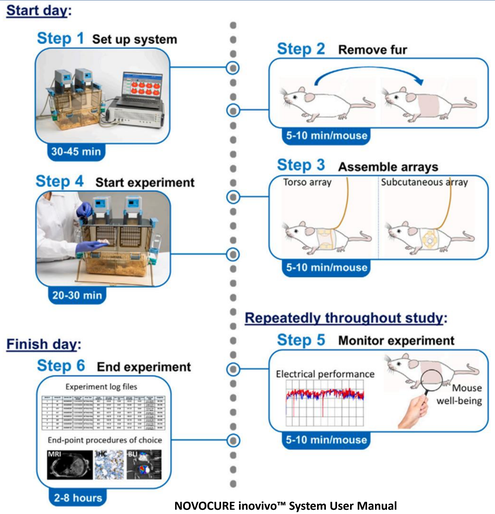


Figure 1. Here, we present a protocol for applying TTFields to torso orthotopic and subcutaneous mouse tumor models using the inovivo system. Tumor Treating Fields (TTFields) are electric fields clinically approved for cancer treatment, delivered via arrays attached to the mouse's skin.

Results and Discussion

- ❖ Preliminary data suggest that TTFields, employed as a monotherapy, resulted in detectable levels of activation of immune cells by tumor specific neoantigens.
- ❖ These experiments also showed that the combination of TTFields with PancVax, a neoantigen vaccine comprising of 10 different Panc-02-specific peptides, recruited 6.2-fold higher number of IFN-γ T cells compared to TTFields alone as assessed by the ELISpot assay. Interestingly, the concomitant treatment of TTFields + PancVax led to a 9.5-fold increase in T cell activity compared to PancVax alone.
- ❖ This early-stage study suggests that there may be a synergistic effect on antigen-specific T cell expansion when TTFields and a neoantigen-targeted vaccine are used concomitantly.

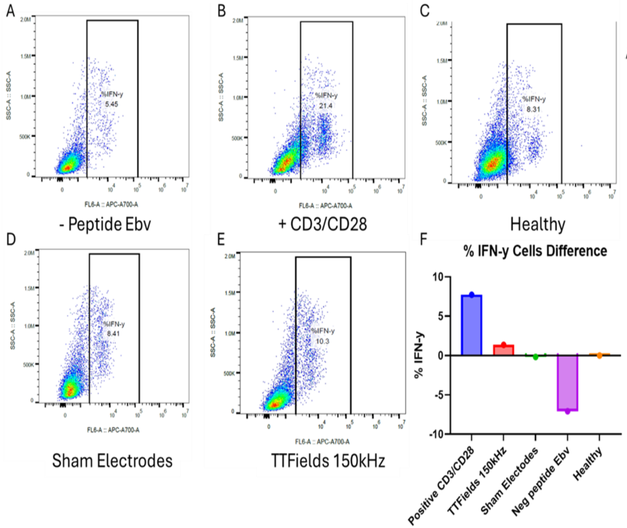


Figure 2. Splenocyte stimulation by peptide antigens. Flow cytometry was used to assess T cell activity via interferon-γ expression. Mouse splenocytes were stimulated with A, irrelevant peptide (Ebv peptide), B, CD3/CD28 beads, as negative and positive controls for T cell activation. C, Healthy mouse (no tumor, no treatment), splenocytes stimulated with Panc02 peptide #44; D, Sham-treated tumor, splenocytes stimulated with peptide #44; E, TTFields treated tumor, splenocytes stimulated with peptide #44 (P44). F, Plot of the percentage IFN-γ difference of each sample compared with the corresponding sample from the healthy mouse.

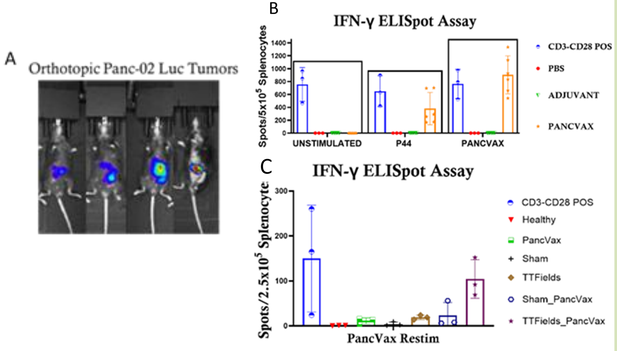


Figure 3. G) Bioluminescent image of five C57BL6 mice bearing orthotopic Panc-02 Luc tumors within the pancreas tissue. H) Ex-vivo splenocyte stimulation assay was conducted using healthy mice treated with either PBS, Adjuvant, or PancVax. Results showed high production of IFN- γ T cells for the P44 (peptide included in the PancVax vaccine) and PancVax restimulated conditions respectively in the PancVax group. I) Mice bearing orthotopic pancreatic tumors were sacked and their spleens extracted to conduct the ex-vivo splenocyte stimulation assay. IFN-γ Elispot Assay showing high production of IFN-γ population in the positive control of CD3-CD28 cohort consistently observed in the PancVax ex-vivo restimulation of the splenocytes.

Conclusions

- ○ There appeared to be synergistic antitumor effects when TTFields and the neoantigen vaccine PancVax were used in combination in a widely-used murine pancreatic cancer model.
- ○ These preliminary findings support the hypothesis that TTFields can influence neoantigen recognition and/or T cell activation, and could thereby serve as a catalyst for future studies that can explore the addition of immunotherapy in combination with radiotherapy for optimal anti-tumor outcomes.

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