Tumor Treating Fields (TTFields) Enhance Antitumor Activity of Immunochemotherapy in Non-Small Cell Lung Cancer Models

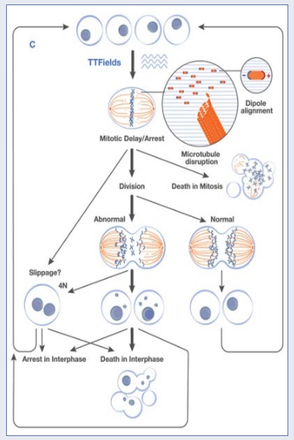
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Introduction

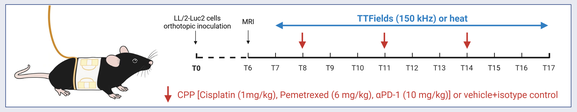
- Tumor Treating Fields (TTFields) are low-intensity electric fields that disrupt cellular processes critical for cancer cell division and tumor progression.[1-2]
- TTFields have been shown to induce immunogenic cell death (ICD) in cancer cells and elicit a systemic anti-cancer immune response.[3-4]
- TTFields therapy is approved together with standard chemotherapy for treatment of glioblastoma, pleural mesothelioma, and locally advanced pancreatic cancer; and together with PD-1/PD-L1 inhibitors or docetaxel for metastatic non-small cell lung cancer (NSCLC) after progression on platinum-based therapy.[1-2,5-6]



- The ongoing LUNAR-2 clinical trial (NCT06216301) is evaluating first-line TTFields administered concomitantly with pembrolizumab and platinum-based chemotherapy in metastatic NSCLC.
- The current study aimed to investigate TTFields concomitant with cisplatin, pemetrexed, and anti-PD-1(CPP) in NSCLC preclinical models.

Methods

- Human A549 NSCLC cells were treated with TTFields (150 kHz, 1.7 V/cm RMS, 72 h), with or without cisplatin (1.5 µM) and pemetrexed (6.3 µM) (CP). Subsequently, the cells were analyzed by flow cytometry for cell count, and for the ICD markers surface calreticulin (CRT) exposure and ATP depletion (quinacrine (QA) staining).
- C57BL/6 mice were orthotopically implanted with LL/2 lung carcinoma cells. Seven days later, after confirming tumor localization by MRI, the mice were randomized into 4 groups: control, TTFields, cisplatin/pemetrexed/anti-PD-1 (CPP), and TTFields together with CPP. Treatment consisted of TTFields (150 kHz) or sham (heat) continuously for 10 days, and vehicle or cisplatin (1 mg/kg), pemetrexed (6 mg/kg), and anti-PD-1 (10 mg/kg), administered via intraperitoneal injection every 72 hours (three doses total), 24 hours after treatment initiation.



- At study end, peripheral blood was collected. Tumors were excised, measured with calipers, and then processed into single cell suspensions.
- Serum was examined for HMGB1 levels with a dedicated ELISA kit and for pro-inflammatory cytokines using LEGENDplex panel testing IL-6, TNFα, CXCL10, CCL2, CCL4, CXCL9.
- Single-cell RNA sequencing data were analyzed to identify malignant and immune-cell populations, which were visualized using uniform manifold approximation and projection (UMAP). Treatment-associated transcriptional changes were further assessed by comparing cell-state distributions, differentially expressed genes (DEGs), and enriched biological pathways across groups.
- Tumor-derived single-cell suspensions and peripheral blood were stained with Zombie NIR and for the following immune markers: CD45, CD11b, CD3e, CD4, CD8a, CD11c, CD44, CD62L, F4/80. Tumor-derived single-cell suspensions were further stained intracellularly for granzyme B.
- To detect IFN-γ-producing cells, single-cell suspensions were restimulated with PMA/ionomycin for 2 h, followed by intracellular IFN-γ staining.
- Significance: *p < 0.05, **p < 0.01, ***p < 0.001

Results

FIGURE 1. TTFields enhance CP-induced cytotoxicity and ICD-associated responses in NSCLC cells *in vitro*

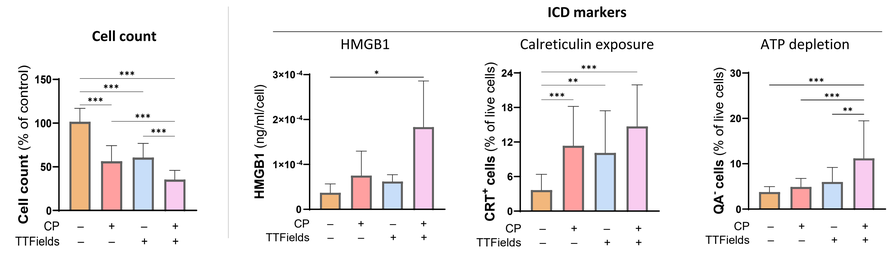


FIGURE 2. TTFields together with CPP improve tumor control in an orthotopic NSCLC model, accompanied by elevation of serum HMGB1 and CXCL9 levels

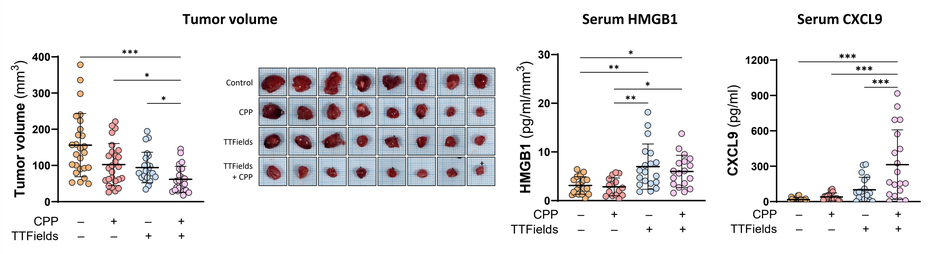


FIGURE 3. TTFields together with CPP promote immune- and stress-associated transcriptional programs in malignant NSCLC cells

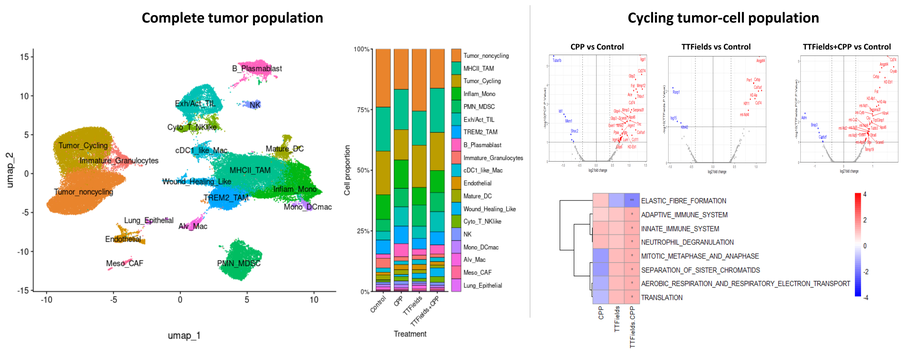


FIGURE 4. TTFields alter the innate immune landscape and induce wound-healing-like macrophages with inflammatory features augmented by CPP

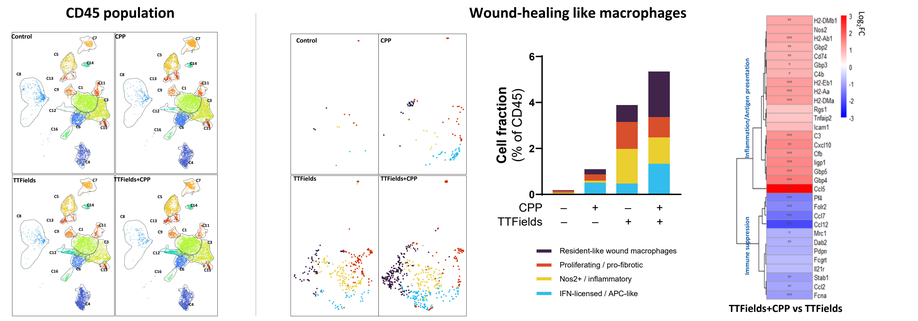


FIGURE 5. TTFields enhance adaptive antitumor immunity and, when applied with CPP, increase IFN-γ and granzyme B levels, reduce Tregs, and elevate the CD8/Treg ratio

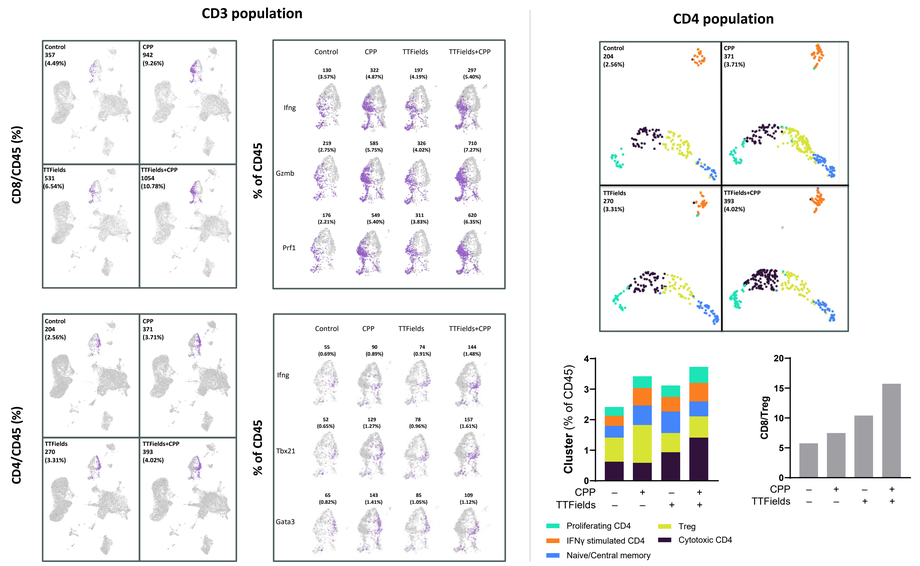
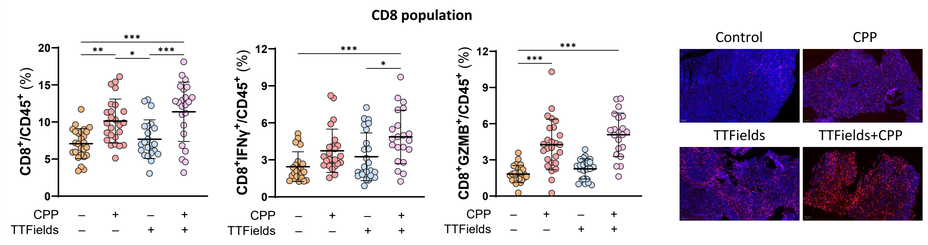


FIGURE 6. TTFields applied alongside CPP enhance intratumoral CD8⁺ T-cell infiltration and activation



Conclusions

- The addition of TTFields to cisplatin, pemetrexed, and PD-1 inhibitor provided the greatest tumor control in NSCLC.
- TTFields reinforced immunogenic cell-death–associated responses and enhanced intratumoral T-cell infiltration and activation.
- Single-cell RNA sequencing revealed treatment-associated remodeling of malignant-cell states and the tumor immune microenvironment, with the most pronounced changes following TTFields with immunochemotherapy.

References: **1.** Moser JC, et al. (2022). *Cancer Res*. 82(20): 3650-3658. **2.** Karanam N. and Story MD (2021). *Int J Radiat Biol*. 97(8): 1044-1054. **3.** Voloshin T, et al. (2020). *Cancer Immunol Immunother*. 69(7): 1191-1204. **4.** Barsheshet Y, et al. (2022). *Int J Mol Sci*. 23(22): 14073. **5.** Leal T, et al. (2023). *The Lancet Oncology*. 24(9): 1002-1017. **6.** Babiker HM, et al. (2025). *J Clin Oncol*. 43(21): 2350-2360.