## 7P Immunomodulatory effects of Tumor Treating Fields (TTFields) on lung cancer models

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**Background:** Tumor Treating Fields (TTFields) are a clinically approved anti-mitotic treatment modality delivered via noninvasive application of low intensity, intermediate frequency, alternating electric fields. In this study, we evaluated whether TTFields-induced cell death can be perceived as immunogenic.

**Methods:** Human and murine lung carcinoma cell lines were treated with TTFields using the inovitro system. Immunogenic cell death was evaluated by changes in the levels of calreticulin (CRT) on the surface of treated cells, phosphorylation of the translation initiation factor eIF2α, and secretion of ATP and High mobility group box 1 (HMGB1). Activation of immune cells was evaluated using co-culture of bone marrow

derived dendritic cells (DCs) with TTFields treated cells. For in-vivo studies, mice orthotopically implanted with lung tumors were treated with TTFields, the immune checkpoint inhibitor anti-PD-1 or a combination of the two modalities. Tumor volume was monitored and flow cytometry analysis was performed for phenotypic characterization of infiltrating immune cells.

**Results:** We demonstrate that cancer cells that died under TTFields application exhibited release of HMGB1, ATP secretion from cells, and ER stress leading to CRT translocation to the cell surface, all of which are cardinal signs of immunogenic cell death. TTFields treated cells promoted in vitro phagocytosis by DCs and DC maturation as well as initiation of inflammation in vivo. The combined treatment of lung tumor-bearing mice with TTFields plus the immune checkpoint inhibitor anti-PD-1 led to a significant decrease in tumor volume compared to anti-PD-1 alone or to the control group. Significant increases in CD45+ tumor infiltrating cells were observed in the TTFields plus anti-PD-1 group. These infiltrating cells demonstrated upregulation of surface PD-L1 expression. Correspondingly, cytotoxic T-cells isolated from these tumors showed higher levels of IFN-γ production relative to untreated mice.

**Conclusions:** Our results demonstrate the potential of TTFields therapy to induce immunogenic cell death and increase the efficacy of anti PD-1 therapy by further enhancing antitumor immunity.

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