Title: The impact of Tumor Treating Fields (TTFields) on cancer stem-like cells isolated from the sub-ventricular zone of glioblastoma patients.

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Abstract:

Treatment of glioblastoma (GBM) is challenging due to its heterogeneous nature, invasive potential, and poor response to standard-of-care treatments. Areas of residual disease represent the source of the recurrent tumor that is fatal for GBM patients. However, targeting the residual disease is not offered as part of the standard of care because these areas are difficult to identify. Our laboratory was the first to identify and characterize residual disease in the sub-ventricular zone (SVZ) of the lateral ventricles of GBM patients.

In this study, we are examining the impact of Tumor Treating Fields (TTFields) on treatment-resistant cancer stem-like cells (CSCs) isolated from the SVZ of 12 GBM patients using single-cell transcriptomics and functional phenotyping analysis. These cells are maintained in conditions that preserve the molecular profile of the original patient tumor, thus representing *bona fide* models to study the impact of TTFields on GBM residual disease.

Our results show that: CSCs isolated from the SVZ from different individuals show different sensitivity to TTFields, the anti-proliferative effects of TTFields are maintained at different plating cell densities, and when the frequency of TTFields increase from 50 kHz to 200 kHz, CSCs undergo morphological changes (*e.g.*, fewer spheres and fewer connections between cells). We used 100 µM Temozolomide and ionizing radiation (10 Gy) mimicking the standard of care of GBM patients, and the growth of the treatment-resistant CSCs was inhibited by TTFields (200 kHz).

To elucidate how TTFields impact the cellular and molecular characteristics of CSCs in their microenvironment, ongoing work focuses on single-cell transcriptomics and functional phenotyping analysis. These results extend our understanding of the mechanisms of action of TTFields, specifically on how TTFields have the potential to alter the cytokine-mediated cross-talk between CSCs of the SVZ and their microenvironment.