

## QUIESCENT CANCER CELLS RESIST T CELL ATTACK BY FORMING AN IMMUNOSUPPRESSIVE NICHE

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**Background** Triple Negative Breast Cancer (TNBC) is the most aggressive form of breast cancer and lacks expression of hormone receptors and HER2, preventing its treatment with targeted therapies. Until recently, chemotherapy was the only systemic therapy for these patients, limiting their options. Recently, two phase III clinical trials showed that immune checkpoint blockade (ICB) in combination with chemotherapy resulted in improved progression-free survival in metastatic PD-L1-positive TNBC, which led to approval of these regimens as a first line treatment.<sup>1,2</sup> Thus, immunotherapy is currently a promising approach for TNBC, however, only a fraction of patients benefits from it. An outstanding question is what mechanisms lead to resistance to these therapies.

**Methods** We investigated resistance to T cell killing in breast cancer cells that unequivocally expressed the targeted antigen by using the Green Fluorescent Protein (GFP) as a visible tumor neo-antigen. This was possible by exploiting our GFP-specific CD8+ T cells called Jedi.<sup>3</sup>

To understand how T cell infiltration and function was decreased in specific regions of the tumor, we used single cell RNA-sequencing (scRNA-seq) with precise spatial resolution. We adapted a technique involving photo-labelling, FACS-sorting and scRNA-seq<sup>4</sup> to large breast tumors, which we named Photo-conversion of Areas to Dissect Micro-Environments (PADME-seq).

**Results** By isolating antigen (GFP) positive breast cancer cells that survived during attack by CD8+ Jedi T cells, we uncovered quiescence as their principal feature. Quiescent cancer cells (QCCs) were found forming clusters with reduced immune infiltration and demonstrated a higher tumorigenic potential. By employing PADME-seq, we profiled cells infiltrating clusters of QCCs and, in parallel, cells in other regions. This enabled, for the first time, a comparison of intra-tumor infiltrates from functionally distinct niches within the same tumor mass by scRNA-seq. We uncovered that QCCs form a niche that contains immune-suppressive fibroblasts, dysfunctional dendritic cells, and highly exhausted T cells. Such ecosystems were orchestrated by a distinct quiescent population of cancer cells through activation of a hypoxia-induced program.

**Conclusions** QCCs constitute immunotherapy-resistant reservoirs by orchestrating a local hypoxic immune-suppressive milieu that blocks T-cell function. Eliminating QCCs holds the promise to counteract resistance to immunotherapy and prevent disease recurrence in TNBC.

### REFERENCES

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