

## **Spatial Determinants of Immunotherapy Resistance in Cancer.**

Significant work over the last two decades has increased our understanding of the intricate, and sometimes opposing roles, that the immune system plays in cancer. While robust immune responses are required for sustained protective anti-tumor responses, tumor infiltrating CD8<sup>+</sup> T cells which have the potential to control tumor growth, often acquire a dysfunctional state due to chronic antigen exposure in a nutrient depleted, and suppressive tumor microenvironment (TME). T cell dysfunction is characterized by a gradual loss of effector function coupled with increased expression of immune checkpoint receptors, including CTLA4, PD-1, Lag3 and Tim-3. As a consequence, immune-checkpoint therapy (ICI), using blocking antibodies against these receptors, has emerged as an important treatment modality for cancer. ICI has demonstrated unprecedented responses in patients with several types of metastatic tumors that were otherwise refractory to available treatment options. However recent studies show that across all indications only ~13% of patients respond to immunotherapy.

To date, the majority of efforts to understand how cancer immunotherapy restores anti-tumor T cell responses have concentrated on assessing the reactivation of dysfunctional T cells. It was largely thought that the efficacy of checkpoint blockade is mediated by the direct ability of blocking antibodies to bind to co-inhibitory molecules and reverse the state of T cell dysfunction. However, this concept has been challenged with seminal work demonstrating that existing intra-tumoral CD8<sup>+</sup> T cells have limited capacity for rejuvenation with a relatively inflexible chromatin landscape, suggesting that the protective effect of ICI is unlikely to be due to reprogramming of dysfunctional T cells. Without a basic understanding of how ICI precisely works we cannot begin to understand why then, in the majority of patients, ICI fails. As such there is great interest in understanding ways to target other immune cells.

To address these questions, we have outlined a series of experiments to analyze the cellular and transcriptional changes associated with tumor infiltrating immune cells. This proposal is highly interdisciplinary implementing single-cell 'omics' strategies and systems immunology approaches to further unravel the players and rules governing immune responses during malignancy. In addition, we plan to develop a suite of computational tools that will allow us to better understand the organization of tumor microenvironment in terms of the cellular composition, spatial arrangement of cells and their communication.