

**Public/Lay Abstract:**

Triple-negative breast cancer (TNBC) is characterized by lack of expression of the estrogen receptor, progesterone receptor and HER2 receptor. Metastatic TNBC, where the cancer has spread to another part of the body, has a highly aggressive clinical course with shorter average survival compared to patients with other subtypes of metastatic breast cancer, representing a clinically unmet need.

Current research suggests that the immune system plays a critical role in long-term outcomes of TNBC. A subset of triple-negative tumors is infiltrated by high levels of T-cells, a type of immune cell that plays a key role in the recognition and destruction of tumor cells. Patients with TNBC and whose tumors have high levels of associated T-cells experience improved survival. In addition, TNBC is characterized by elevated expression of a protein called PD-L1, which interacts with PD-1 receptors on T-cells, preventing the immune system from recognizing and attacking the tumor. Drugs that block PD-1 therefore lift the "breaks" off of the immune system and help to restore the immune response. These PD-1 inhibitors have shown promising activity in TNBC, particularly in combination with chemotherapy.

Preliminary results indicate that the presence of high levels of immune cells in the tumor may predict response to immunotherapy. However, not all tumors with high immune infiltration respond to treatment and not all responders have high levels of immune cells. Thus, much remains unknown about the immunological effects induced by PD-1 blockade, the interaction between the tumor and immune system in TNBC, and how this information can be leveraged to help select patients who are more likely to benefit from PD-1 inhibition.

On the surface of T-cells, there is a structure called the T-cell receptor (TCR) that is responsible for recognizing proteins on tumor cells. By sequencing TCR in tissue or blood samples, it is possible to distinguish whether there is a more specific or so-called "clonal" immune response to the tumor. In other cancer types, high clonality of TCR in tissue or blood has been associated with clinical response to PD-1 inhibition. If these results are also confirmed in TNBC, TCR sequencing in blood samples from patients treated with PD-1 inhibition may serve as a marker of response. If so, we would potentially be able to use a minimally invasive technique, such as a blood draw, to predict which patients are most likely to respond to immunotherapy-based treatment, while avoiding the risks of invasive procedures required for tissue biopsies.

Currently, we are conducting a Phase II clinical trial in which 132 patients with metastatic TNBC are randomized to receive chemotherapy with or without the immunotherapy drug, nivolumab (PD-1 inhibitor). A research tumor biopsy and a blood draw are obtained prior to starting therapy, after the second dose of treatment and at the time of progression. To investigate whether T-cell clonality can help predict which patients are more likely to respond to treatment, we will perform TCR sequencing on these paired tumor and blood samples of patients enrolled on the trial. We will then compare the distribution of T-cells in patients who responded to carboplatin plus nivolumab and those who did not respond to the combination. We anticipate finding a similar increase in T-cell clonality in both tumor and blood samples of patients who respond to carboplatin plus nivolumab. If our hypothesis is confirmed, this could help stratify patients during the first weeks of treatment into: a) those who are more likely to respond to immune checkpoint inhibition and who would benefit from continuing therapy, and b) those in whom other strategies may be needed to enhance the effectiveness of immunotherapy.

The integration of genomic and immune profiling with clinical data may improve our knowledge of the mechanisms of responsiveness to immunotherapy in TNBC, using feasible and reproducible assays that can be incorporated into daily clinical practice. In addition, data generated from our study may trigger the development of novel treatment strategies and improve patient selection for targeted therapies, with the ultimate goal of reducing mortality in this patient population.