**Lay Description of Important Outcomes**

 Despite the improvement in the prognosis of HER2+ breast cancer, the efficacy of systemic therapy is limited in the brain compartment. Patients with brain metastasis (BM) are likely to die of BM progression or local therapy complications. Tucatinib is a new agent currently approved for HER2+ metastatic breast cancer with known efficacy in patients who have developed BM. However, after initial control, these patients often ultimately progress in the brain and there are few cases in which local treatment (surgery or radiation therapy) can be avoided. With this proposal we aim to study the microenvironment of the BM, intra- and peri-lesional, to characterize the unique conditions in which HER2+ tumors cell acquire the capacity to proliferate and invade the brain despite the systemic exposure to active drugs.

We designed this proposal as a sub-study of a “window of opportunity” pre-surgical trial called WinHER2, that allows us to collect brain metastasis tissue after medically necessary resection for BM. All patients receive 4 days of preoperative tucatinib to achieve the active concentration in the blood. We will correlate the concentration of the drug (hypothesized to be different in patients with progressing in the brain while on tucatinib versus patients with cancer not previously progressing on tucatinib) with microenvironment features (single cell RNA seq and electron microscopy).

 In the first two patients analyzed by electron microscopy, both progressing after stereotactic radiation and never exposed to the drug, tumoral cells appeared scattered in an abundance of fibrotic tissue, without apparent interaction with the normal brain cell populations. We aim to characterize activated molecular signature by single cell RNA sequencing of the normal brain tissue surrounding the metastasis (juxta-lesional) and intralesional. The hope is to identify potential biomarkers or new therapeutic targets to avoid the brain invasion by breast cancer cells in the central nervous system, with the hypothesis that the microenvironment brain cells are perturbated/modified by the cancer cells invasion.