Lay abstract

The prognosis of patients with HER2-positive breast cancer is dramatically affected by the seeding of cancer cells in the brain. The impact of brain involvement on quality of life and survival of breast cancer patients is challenging and therapeutic options are currently limited. Although new local treatment approaches in the field of surgery and radiotherapy have improved the local control of brain metastases (BM), the responses are often not durable and re-treatment not always feasible. In addition, there are short- and long-term neurological complications of treatment that can affect this subset of patients. Unlike other sites of disease spreading, most chemotherapeutic agents do not help prevent the progression in the brain. Indeed, the brain is a privileged comportment for the presence of a blood-brain and blood-tumor barrier that limit the drugs' access from the bloodstream. Therefore, BM can be considered the *"Achille's heel"* for breast cancer patients and new treatment approaches are urgently needed.

A novel agent called tucatinib has been recently approved and made available for patients with metastatic HER2-positive breast cancer and, uniquely, it is the first agent to demonstrate a significant survival benefit in patients with BM (stable and progressing). However, the biology behind the exceptional response to this agent in the brain along with mechanisms of resistance are still unknown. Therefore, we designed a clinical trial for patients with HER2-positive breast cancer who need surgery for BMs to study brain pharmacokinetics, the ability of tucatinib to penetrate the brain (IRB 22-168). The present proposal will study the brain tumor microenvironment. More specifically, we aim to explore whether the different immune or neuronal cells surrounding the metastases and inside the metastases, if present, have a role in the tucatinib brain penetration and correlates with imaging by a special type of MRI (perfusional brain MRI). To do so, we will use unique technology such as single cell RNA sequencing (scRNAseq) and electron microscopy. Additionally, normal brain tissue surrounding the metastases, will be respected and: (1) separated in single cells. The extracted RNA from the single cells will be sequenced to describe what genes are expressed; (2) ultra-structurally analyzed by electron microscopy to evaluate interactions with breast cancer cells and with peri and intra-tumoral vessels.

This proposal represents an ambitious and matrixed academic effort across different departments to study with new technologies the brain microenvironment in HER2-positive breast cancer. It may suggest biological rationale for new combinations/treatment approaches to maximize the potential of this agent in a group of patients with poor prognosis.