Breast cancers are the leading cause of cancer in women worldwide (1). There are several molecular subtypes characterized by the expression level of genes encoding estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor: luminal A et B (ER+, PR+, HER+/-), HER2 (ER-, PR-, HER+) and triple-negative (ER-, PR-, HER-). Numerous therapeutic options are available for luminal and HER2 tumours (hormonotherapy and targeted therapy). But conventional chemotherapy using antracyclins (doxorubicine), taxanes (paclitaxel) or CMF (cyclophosphamide, methotrexate, 5-fluorouracile) constitutes the indicated treatment for triple-negative tumours (2).

Resistance to treatments is observed in all types of cancer with various therapeutic approaches (3). In the case of triple-negative tumours, the mechanisms of resistance explain the failure of chemotherapy and the high rate of recurrence that characterizes such type of agressive cancer. In the tumour, some cells acquire the resistant phenotype during the treatment while other cells, like cancer stem cells (CSC), display the capacity to escape the mechanisms of action of chemotherapeutic agents. It is even described that their number increases after a treatment with chemotherapy or radiotherapy (4). The mechanisms proposed to explain the resistance to treatments belong to two groups: non cellular mechanisms related tumour environment (poor vascularisation for instance) and cellular mechanisms including the molecular targets of the therapeutic agents, the enzymes, and the systems of intracellular transport (5).

Resistance to treatment is a pitfall for the efficiency of anticancer therapies and it is very important to develop strategies able to overcome it. In this context, we started a study dealing with the metabolic reprogrammation of breast cancer cells and its impact on the response to treatments. Indeed, it is described in several preclinical studies that a fasting period or a caloric restriction could increase the response to chemotherapy. In vitro, the culture of cells in starvation condition (IVS = in vitro starvation) is performed in a medium containing low glucose (0,5 g/L) and low serum (2,5%) and can mimic the fasting period in vivo. We obtained a grant from the ligue contre le cancer at the end of 2018 for this study. We observed that triple-negative breast cancer cells were sensitive to IVS. Moreover, in several triple-negative breast cancer cell lines, IVS did not potentiated the effect of chemotherapeutic agents and could even decrease their efficiency. For instance, Hs578T cells simply growing in IVS condition entered into apoptosis whereas the same cells treated with FEC (5-Fluorouracile/Epirubicine/Cyclophosphamide) in IVS condition, displayed a G2/M blockade but no apoptosis. In IVS, the cells escape to the apoptosis usually induced by chemotherapy.

Then our study suggests that IVS is able to reduce tumour growth as already reported in mice submitted to a therapeutic fast (6) but that in some cases, IVS could also inhibit the effect of chemotherapy.

The objectif of the PhD thesis will be to characterize the metabolic reprogrammation induced by IVS and the resistance to treatment with FEC. We will focus on triple-negative breast cancer cells (Hs578T and MDA-MB-231).

First, we will study the effects of IVS and FEC when the cells grow in three dimensions (mammospheres), a method that we developed recently. Mammospheres allow an enrichment in CSC, that often display resistance to treatment and are involved in recurrences (7).

Developing mammospheres, after 4 days of culture, will be grown in standard medium or in IVS condition, in presence or absence of FEC during 3 days. Mammospheres usually develop in a DMEM/F12medium without serum, IVS will be obtained by lowering glucose (0,5 g/L) and avoiding EGF. The number of mammospheres after 3 days of treatment and their diameter will be measured. We will study the expression of apoptosis, autophagy and senescence markers. We will also study the proportion of CSC in the different culture conditions by flow cytometry (staining CD44+/CD24-/ESA+) as well as the expression of totipotence markers (Oct4, Nanog, et Sox2) (8). These data will be essential in order to determine if the cell respond identically or differently in 2D or 3D cultures.

Secondly, we will perform a transcriptomic study in order to identify the molecular signatures of the cells submitted to IVS. The cells will be treated during 16 heures with 1 µM FEC or untreated (NT). The cells that were grown in 2D and the mammospheres will be collected in order to extract the RNAs. Microarrays will be hybridized and et scanned (Technologie Agilent) using the facility « Biologie Moléculaire et Toxicologie » of the Faculty of Pharmacy in Nancy. Our laboratory has the knowledge to realize these steps. The molecular profiles obtained will be compared and classified under a non supervised method with online available tools (GeneCluster et JavaTreeview). Data analysis will be done using other tools also available online (Gene Ontology, DAVID, PANTHER, GSEA, FuncAssociate…). The variations in the expression of target genes will be confirmed by RT-qPCR. The importance of these genes will be demonstrated by surexpression/invalidation approaches.

To conclude, this work should allow to identify new molecular targets that could decrease resistance or predict it.

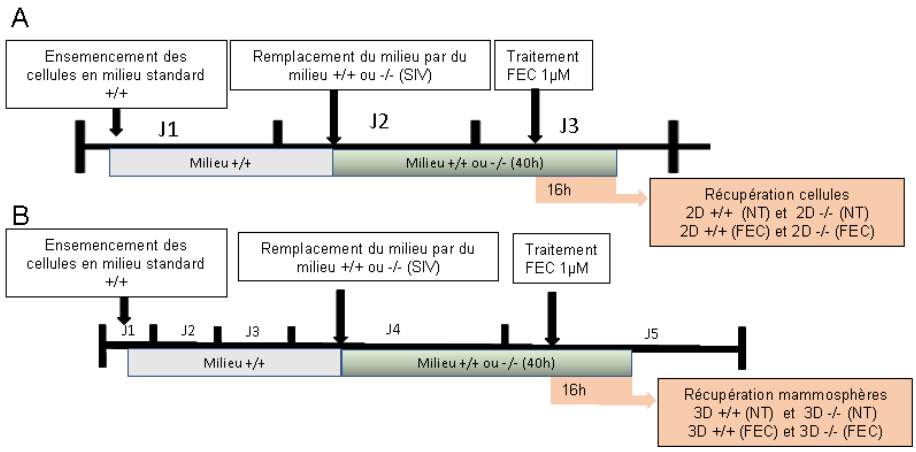

Figure 1: Treatment protocoles for RNAs collection in cells that were grown in 2D (A) or as mammospheres (B) in standard conditions +/+ or IVS, in presence or absence of FEC.

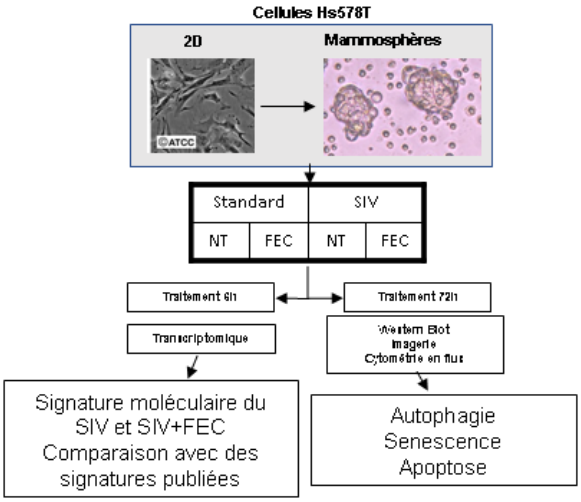

Figure 2: Summary of the experimental strategy

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