Breast cancer is the leading cancer site in women in all countries of Europe. Early detection continues to improve survival, but prognosis worsens significantly after metastasis. Metastasis and chemoresistance are linked phenomena, but their molecular bases are unknown. The evidence suggests a role for Caveolin1 (Cav1): i) Cav1 mediates biomechanical remodelling of the extracellular matrix by cancer-associated fibroblasts (CAFs), promoting invasion ii) Cav1 is involved in drug/radiation resistance, iii) The Cav1 content of breast-TC exosomes correlates with metastatic potential.

Our project delves into all these aspects reaching important conclusions and promising data that we will continue to study. First, we study Cav1 expression during tumour progression and metastasis and we approach this aim from different perspectives, form patients to animal models and cells in culture.

On a cohort of 83 early stage breast cancer patients with positive estrogen receptors and negative human epithelial receptor 2 (HER2) treated with weekly nab-Paclitaxel as neoadjuvant treatment. In the univariate analysis we found changes in the expression of Cav1 in stroma to correlate with good pathological complete response. The follow-up of these patients over a period of three to five years will allow us to assess whether this Cav1 expression analysis may have a prognostic value.

Biopsies of 11 up to 16 triple negative breast carcinoma (TNBC) samples where successfully transplanted into inmunodeficient mice in serial recipients. In all cases, Cav1 was highly expressed in tumour cells. These results are consistent with previously published data, in which the expression of Cav1 is associated with the basal type, and tumour cells that undergo epithelial to mesenchymal transition (EMT). Tumour circulating cells (CTC) isolated from peripheral blood of tumour bearing mice are being analysed for Cav1 levels.

We found that Cav1 is present in exosomes. Production of exosomes in breast cancer cell lines with metastatic potential *in vivo* was analysed. Induction of Cav-1 and alpha-SMA after incubation of fibroblasts with tumour-derived exosomes was studied, as well as collagen contraction ability, matrix deposition and matrix remodelling. Furthermore, the proteome of Cav1-expressing exosomes was performed and quantified, showing changes in a panel of different proteins. Serum exosomes of mice bearing tumours were isolated and analysed for the expression of Cav1 and exosomes markers such as CD63 and glypican.

It was previously described that Cav1 mediates biomechanical remodelling of the ECM by CAFs favouring tumour invasion. To modulate Cav1 expression, we generated a lentiviral vector able to silence Cav-1 on stromal cells. After infection, primary CAFs obtained from human breast cancer biopsies reduced Cav1 expression by 80-90%. Further studies on tumour growth and metastasis dissemination are now under evaluation.

Patient derived xenografts (PDX) are tumours generated from the transplant of biopsies of cancer patients into inmunodeficient mice. PDX are fast becoming the new gold standard models for oncological drug development because they grow maintaining

human tumour characteristics. We developed a Cav1 mutant mouse in an immunodeficient strand and as a result, we generated animals to study human tumour metastasis in a Cav-1 deficient stroma. 22 biopsies of breast, lung, pancreatic and hepatocellular carcinoma were transplanted in Cav1 KO and wt animals.

Tumour Cav1 has been involved on mechanism of drug resistance in breast cancer. We have collected cell lines and primary tumour cell cultures from breast carcinomas and analysed them.

To explore the possibility of using Cav1Beta on patients that cannot benefit from neoadjuvant chemotherapy, our next goal was to generate an antibody specific for Cav1Beta. In silico Cav1-KLH conjugated peptides were designed to immunize rabbits. Fusion procedures of splenic beta cells with a rabbit plasmacytoma allowed us to obtain several antibody-producing hybridomas. Biochemical and immunofluorescence analysis determined that the obtained monoclonal antibodies recognize both cav1 isoforms. Different strategies should be attempted to obtain appropriate tools for the screening of resistant patients.

In summary, with this project we have obtained results that will open new therapeutic strategies not only about breast cancer but also on other types of epithelial cancers, such as lung or pancreas.