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Evaluation of the immune signature in response to chemotherapy in MMTV-PyMT mouse model.

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Introduction and Objective

The knowledge of the leukocyte infiltrate allows us to understand and evaluate the efficiency of different antitumor therapies. Currently, anthracyclines followed by taxane are used for the chemotherapeutic treatment of breast cancer, but there is no biological basis to support this order. The NeoSamba Clinical Trial (INCA) demonstrated that there was better overall and disease-free survival in patients treated with the reverse order of chemotherapy arms: taxane followed by anthracyclines (FAC).

Based on the rationale of the NeoSamba protocol, our goal is to evaluate the impact of neoadjuvant treatment with combinations of FAC followed by taxane and the reverse order (taxane followed by FAC) on the leukocyte infiltrates in the model of breast cancer development in PyMT animals.

Methodolody

PyMT mice were treated for three weeks with each chemotherapy arm (FAC - 5-fluorouracil, doxorubicin, cyclophosphamide and/or taxane - docetaxel) with an interval of one week between arms. Tumors (untreated; treated only with FAC or with taxane; FAC-T and T-FAC) were collected when they reached a volume between 800mm³ and 1400mm³ or after the end of treatment and were referred for flow cytometry, immunohistochemistry and RNA sequencing (RNA-seq).

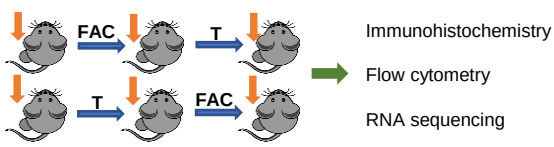

Figure 1. Scheme of treatment of PyMT mice with early and advanced tumors based on the sequence of anthracyclines (FAC) and taxane (T) proposed in the study of NeoSAMBA (BINES et al. 2020).
The tumors will be divided into three parts: One for immunohistochemistry analysis, another one will be stored in RNAlater and frozen for RNA-seq analysis and finally, a part will be dissociated, and the cells will be sent for immunostaining.

Results

We performed initial studies to characterize the leukocyte tumor infiltrate in untreated and FAC-only treated mice. We could observe some differences in the two groups initially analyzed. In the FAC group, there was an overall decrease in the cancer stem cell population compared to the untreated control. We also observed decreased percentage of TIM-3+ cells amongst CD3+ and also on CD4+ cells in the FAC group when compared with untreated animals. Furthermore, we observed that TIM-3 is mostly expressed in CD4+ cells while PD-1 is expressed in CD8+ cells, although we observed no differences between treated and untreated groups.

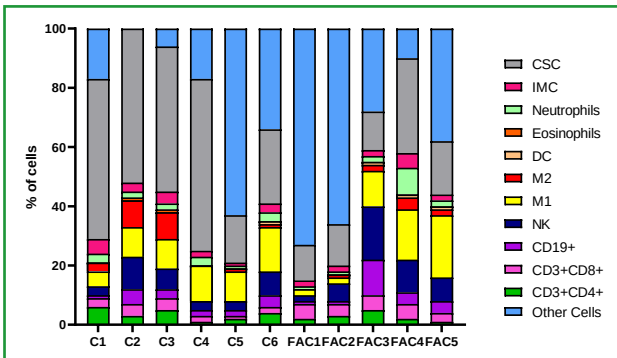

Figure 2 Initial characterization of immune infiltrate and cancer stem cell (CSC) from tumors PyMT mice untreated (control) and treated with anthracycline combination (FAC) by flow cytometry. IMC: Imature myeloid cells; DC: Dendritic cells; M1 and M2: Macrophages type 1 and 2; NK: Natural Killer cells.

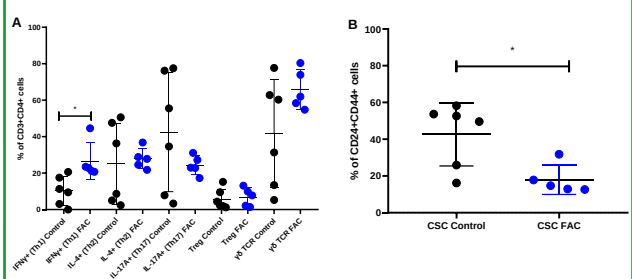

Figure 3 Flow cytometry analysis of subpopulations of CD3+CD4+ T cells (A) and CD24+CD44+ cancer stem cells (B) from tumors PyMT mice untreated (control) and treated with anthracycline combination (FAC).

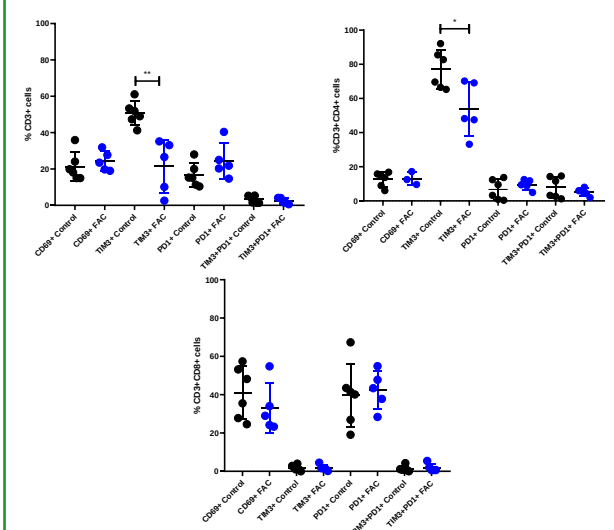

Figure 4. Flow cytometry analysis of activated (CD69+) and exhausted (TIM3+, PD1+ or TIM3+PD1+) T cell populations (CD3+, CD4+ or CD3+CD8+) from tumors of PyMT mice untreated (control) and treated with anthracycline combination (FAC).

Next steps

As next steps, we will continue the treatments of the animals and qualitatively and quantitatively evaluate the leukocyte infiltrates after each of the chemotherapy blocks. Gene expression analysis will be performed in the samples already collected. By doing so, we hope to characterize the impact of different chemotherapeutic regimens on the tumor immune response.

Contact

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Footnote

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