Approach using « second generation » immune checkpoint inhibitors for the treatment of triple-negative breast cancer

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INTRODUCTION

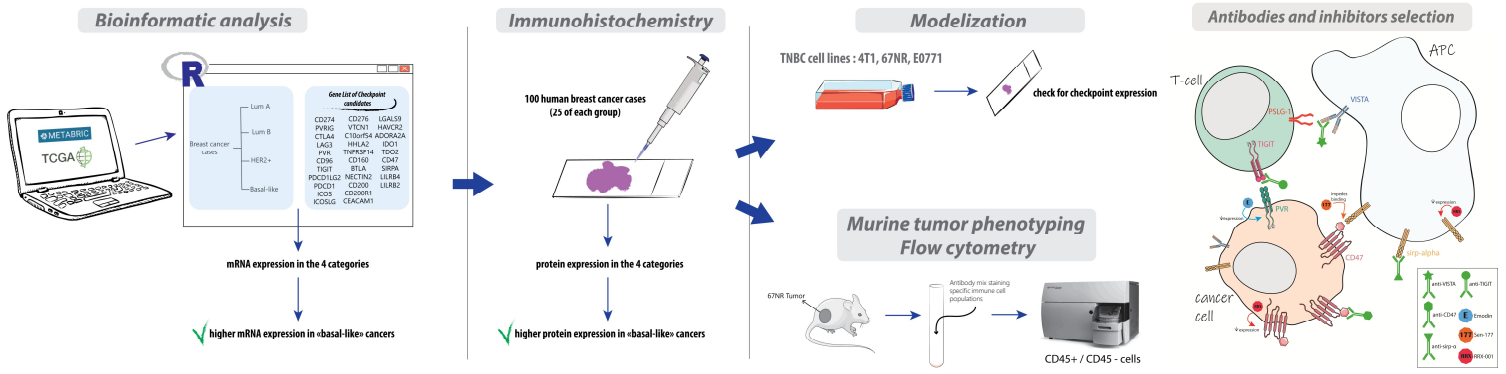
In the last decade, immune checkpoint blockade has known notable advances with anti-CTLA4 and PD-1/PD-L1 agents. However, only a subset of patients benefit from these therapies, while the majority shows limited or absence of response. Breast cancers, highly heterogeneous in both their prognostic and response to treatment, are typically divided into 4 molecular subtypes: *luminal A, luminal B, HER2* and *basal-like*. The latter, also called triple-negative breast cancer (TNBC) represents 10-20% of invasive breast cancers.

To this day, no TNBC-specific treatment exists, due to the absence of expression of ER, PR and HER2. Tumor cells being able to express several different immunosuppressive proteins – e.g. VISTA, CD47, PVR,...-, the blockade of a novel immune checkpoint could represent a promising strategy to treat so far unresponsive cancers.

AIM OF THE STUDY

The aim of this research project is to highlight new immune checkpoints and to study the impact of their inhibition on triple negative breast cancer models.

EXPERIMENTAL DESIGN



RESULTS

Protein expression of VISTA, sirp-α, CD47 and PVR is higher in human TNBC

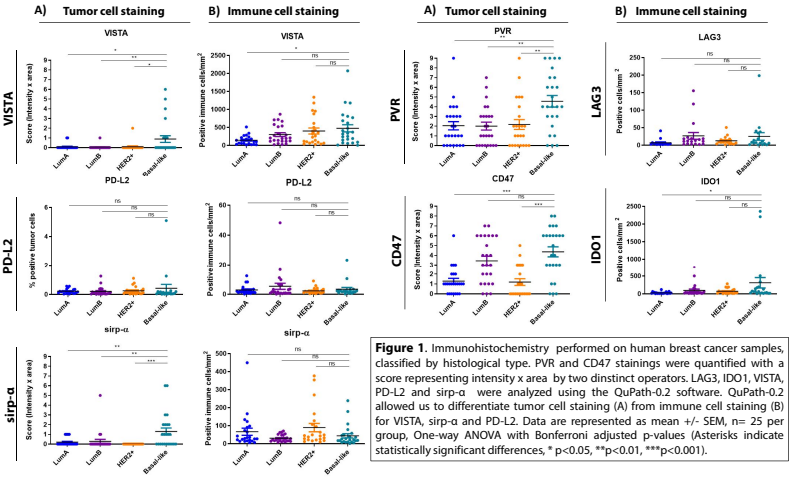


Figure 1. Immunohistochemistry performed on human breast cancer samples, classified by histological type. PVR and CD47 stainings were quantified with a score representing intensity x area by two dinstinct operators. LAG3, IDO1, VISTA, PD-L2 and sirp-α were analyzed using the QuPath-0.2 software. QuPath-0.2 allowed us to differentiate tumor cell staining (A) from immune cell staining (B) for VISTA, sirp-α and PD-L2. Data are represented as mean +/- SEM, n= 25 per group, One-way ANOVA with Bonferroni adjusted p-values (Asterisks indicate statistically significant differences, * p<0.05, **p<0.01, ***p<0.001).

VISTA, sirp-α, CD47 and PVR are expressed on both tumor and TME- cells of mouse syngeneic tumors

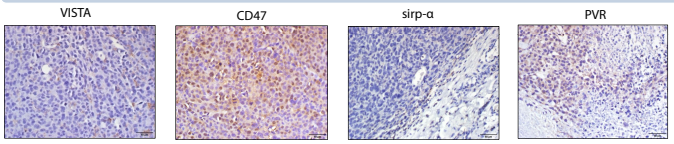


Figure 2. Immunohistochemistry staining of syngenic mouse tumors. Scale bar is 50 µm. TME= tumor microenvironment

Blocking one of the 4 checkpoints effectively slows down tumor growth in mice

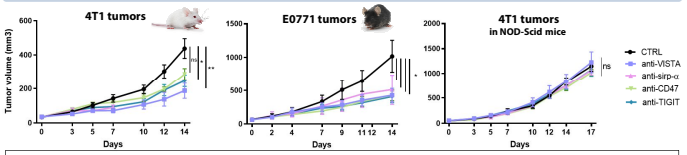


Figure 5. Tumor growth curves of 4T1/E0771 tumors in syngeneic mouse models. Tumor cells were injected orthotopically 10/14 days prior to treatment. Mice were treated with antibodies against VISTA, sirp-α, CD47 or TIGIT (4T1/E0771). n= 8 (4T1) and n= 10 (E0771) per group, One-way ANOVA with Dunnett adjusted p-values on measures at day 14 (Asterisks indicate statistically significant differences, * p<0.05, **p<0.01, ***p<0.001).

The TME of 4T1 tumors treated with anti-VISTA contains a decreased percentage of CD4+ and CD8+ Treg cells and an increased M1/M2 ratio

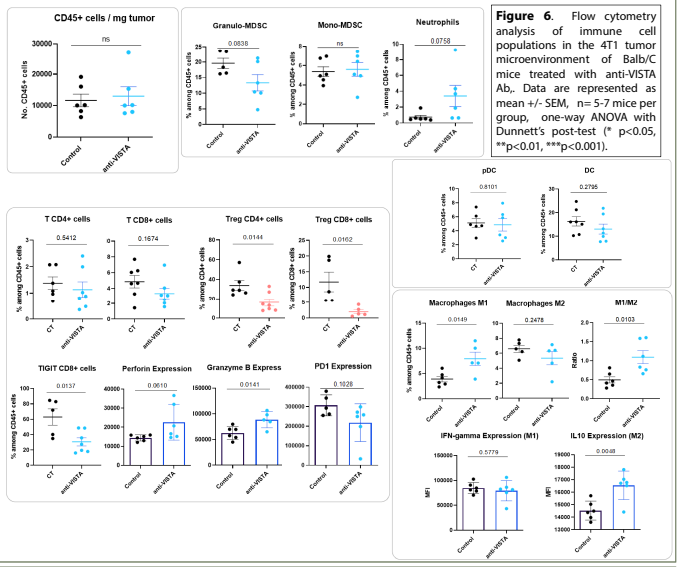


Figure 6. Flow cytometry analysis of immune cell populations in the 4T1 tumor microenvironment of Balb/C mice treated with anti-VISTA Ab,. Data are represented as mean +/- SEM, n= 5-7 mice per group, one-way ANOVA with Dunnett's post-test (* p<0.05, **p<0.01, ***p<0.001).

Apoptosis tests and proliferation assays of mouse TNBC cell lines using antibodies and inhibitors targeting our checkpoints of interest allowed us to select concentrations with no direct impact on the cancer cells

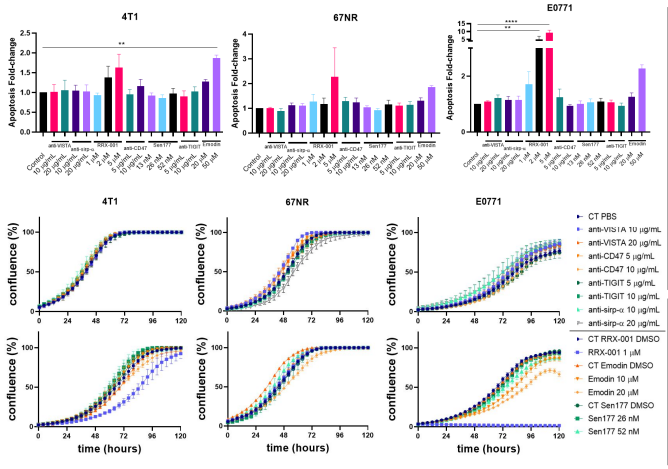


Figure 3. Apoptosis tests carried out on the three mouse TNBC cell lines 4T1, 67NR and E0771 using an Annexin V/PI staining and flow cytometry analysis. The cells were cultured for 24 hours in presence of monoclonal antibodies targeting VISTA, sirp-α, CD47 and TIGIT and inhibitors targeting the sirp-α/CD47 axis (RRX-001 and Sen177) and PVR (Emodin). Data are represented as mean +/- SEM, n=3, ANOVA with Dunnett's post-test (* p<0.05, **p<0.01, ***p<0.001).

Figure 4. Proliferation assays carried out on the three mouse TNBC cell lines 4T1, 67NR and E0771 using the Incucyte technology. Cells were cultured for 5 days in presence of antibodies against VISTA, sirp-α, CD47 and PVR and the inhibitors RRX-001, Sen177 and Emodin.

CONCLUSIONS AND PERSPECTIVES

Our observations have allowed us to narrow down the list of potential targets to VISTA, PVR, CD47 and sirp-α. Monoclonal antibodies and chemical agents targeting our checkpoints have been selected and their effects on proliferation and apoptosis of the tumor cells has been assessed. The antibodies and inhibitors have been tested in the appropriate syngeneic mouse models and further investigation needs to be conducted in order to unravel the precise mechanisms involved in the deceleration of tumor growth in treated mice.

Acknowledgements

The authors thank the FNRS, the Télévie, the Léon Fredericq foundation and their colleagues at ULiège who, together, made this thesis project possible.

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