#C079

NUVISAN
The Science CRO

Comprehensive immunophenotypic profiling sheds light on the dynamic interplay between immune cells in the 4T1 breast cancer model upon anti-PD1 and anti-CTLA4 immunotherapy

Christos Nikolaou, Krzysztof Brzezinka, Simon Heller, Wiebke Winkler, Stefan Kaulfuss, Carlo Stresemann, Alexandra Eichten, Oliver von Ahsen, Martin Lange

NUVISAN Innovation Campus Berlin GmbH | Muellerstr. 178 | 13353 Berlin | Germany

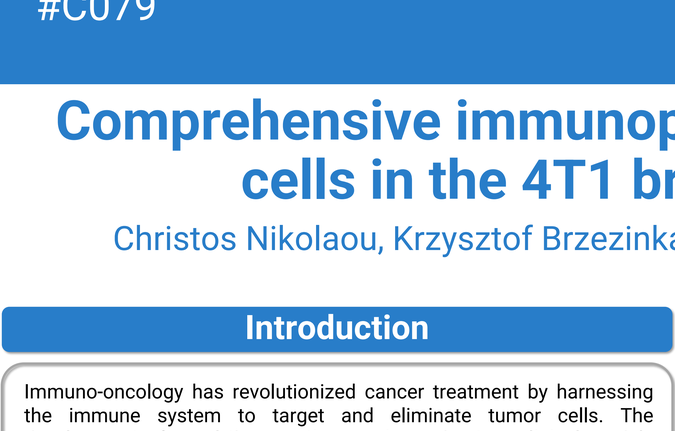
Introduction

Immuno-oncology has revolutionized cancer treatment by harnessing the immune system to target and eliminate tumor cells. The development of novel therapeutics requires robust preclinical models and immune cell assays.

In this study, we employed a combination of full spectrum flow cytometry (Cytek®) and spatial profiling by Immunohistochemistry (IHC) to investigate the impact of Immune checkpoint inhibitors (ICIs) anti-PD1 (programmed cell death protein 1) and anti-CTLA4 (cytotoxic T-lymphocyte-associated protein 4) treatments on the 4T1 breast cancer model. We comprehensively analyzed 25 surface markers on immune cells to gain insights into the dynamic changes induced by immunotherapy.

Methods

IHC assay development for immune cell markers was performed using commercially available antibodies and healthy mouse control and syngeneic mouse tumor tissues. Antigen retrieval and antibody dilutions were optimized for signal specificity.



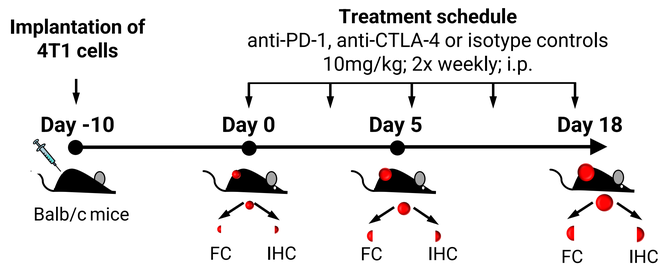
Images of IHC stainings using optimized conditions on mouse spleen

Flow cytometry panel design: Epitope recovery was optimized by comparison of mechanical dissociation, enzymatic (collagenase 1, 4 or Dispase II) digest, fixation and freezing. Mouse immuno-panel consists of CD3, CD4, CD8, CD11b, CD19, CD25, CD38, CD44, CD45R, CD69, CD62L, CD137(4-1BB), CD206, TCRgd, SLAMF7, PD1, Ly6c, Ly6g, F4/80, CTLA4, NKp46, MHCII, CD45, Live/Dead and was measured on the Cytek® Aurora full spectrum cytometer.

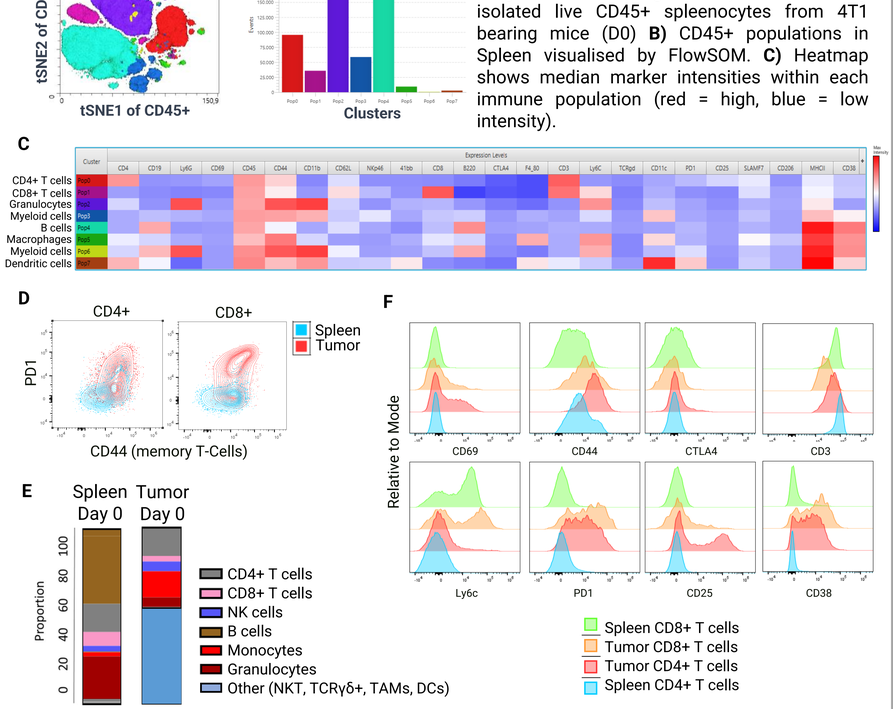
In vivo studies: 4T1 cells were implanted orthotopically into the mammary fat pad (fourth mamille) of > 8-week-old female Balb/c mice. When tumor volume reached a predefined average size, animals were allocated to treatment groups by stratified randomization procedure and treatment started according to study plan with Immune checkpoint Inhibitors (a-PD-1 and a-CTLA-4, 10mg/kg vs. isotype controls, 2x weekly, i.p.). Animal experiments were conducted in accordance with animal welfare laws, approved by local authorities (State Office for Health and Social Affairs, Berlin, Germany.

Results

In vivo study design of the murine 4T1 breast cancer model



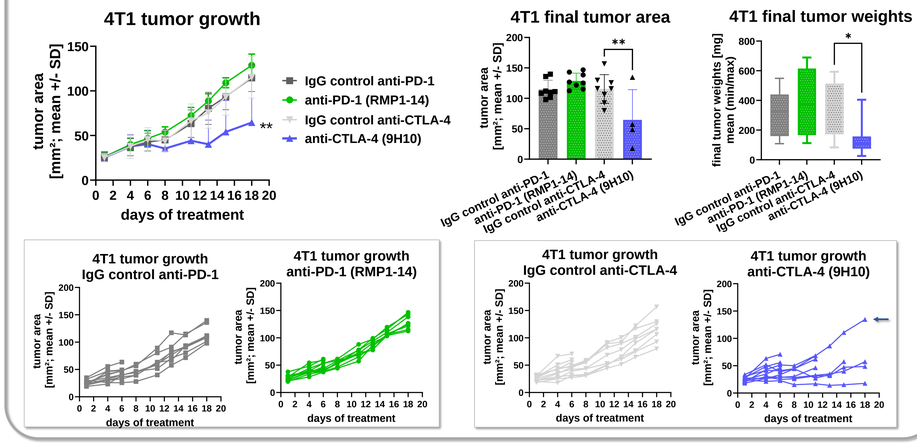
Immunophenotyping of spleen and 4T1 tumors



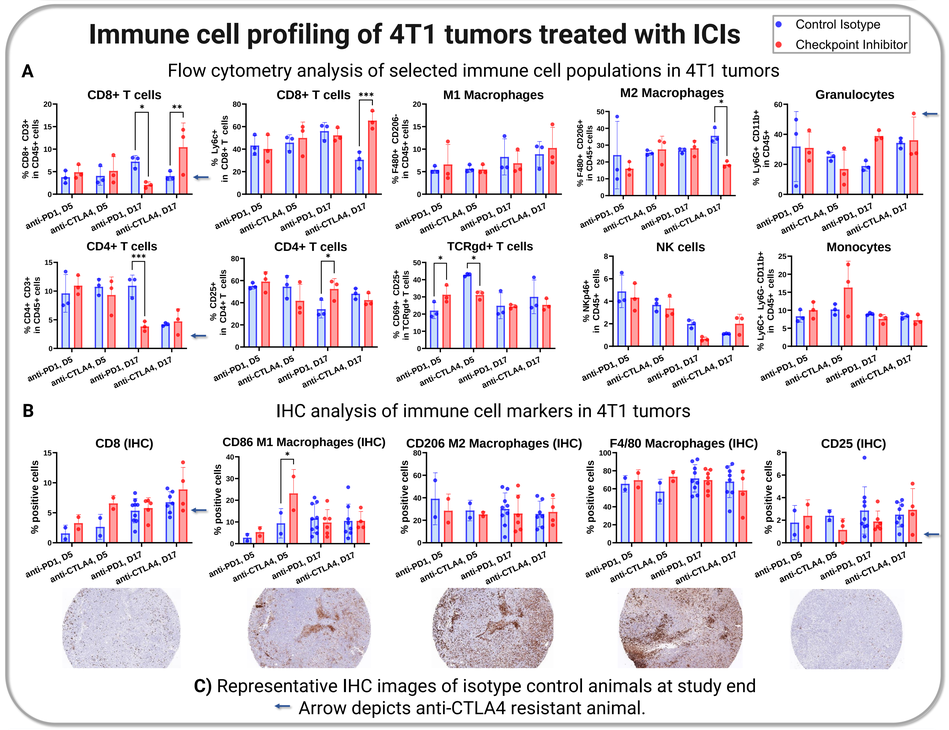
A) Identification of differentially distributed cellular phenotypes by t-Distributed Stochastic Neighbor Embedding (tSNE) from freshly isolated live CD45+ spleenocytes from 4T1 bearing mice (D0) **B)** CD45+ populations in Spleen visualised by FlowSOM. **C)** Heatmap shows median marker intensities within each immune population (red = high, blue = low intensity).

D) Representative plots for CD8+ and CD4+ T cells in spleen and tumor. Tumor infiltrated T cells have an effector/activated phenotype. **E)** Leukocyte composition in spleen and tumors of 4T1 tumor-bearing mice. Relative distributions of immune cell subsets identified within CD45+ leukocytes for spleen and tumor at D0 respectively. Median subset proportions are shown for each group as stacked bars (100% CD45+). **F)** Representative histograms of CD4+ and CD8+ T cells depict the expression of the indicated surface molecules on T cells, freshly isolated leukocytes from spleen and tumor at D0.

Anti-tumor efficacy of ICIs in the murine 4T1 breast cancer model



Immune cell profiling of 4T1 tumors treated with ICIs



C) Representative IHC images of isotype control animals at study end
← Arrow depicts anti-CTLA4 resistant animal.

Conclusion

Our comprehensive immunophenotypic analysis sheds light on the dynamic interplay between immune cells and the 4T1 breast cancer model during anti-PD1 and anti-CTLA4 immunotherapy. These findings provide valuable insights into the mechanisms underlying the therapeutic efficacy of immune checkpoint blockade in this aggressive cancer model.