ALL-IN-ONE FLOW CYTOMETRY STAINING PANEL FOR IMMUNE-CELL PROFILING IN SYNGENEIC TUMOR MODELS

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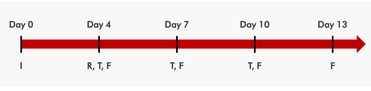
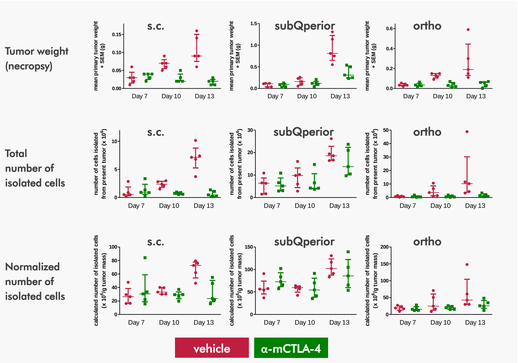
Introduction

Checkpoint inhibitor treatment has become a common therapy of various cancer types; however, clinical data indicate that only few patients respond to this regiment due to attenuated anti-tumor immune response. Thus, it has been recognized as important to consider the immune response already during preclinical drug development to anticipate such clinical drawbacks and investigate the changes of immune cell populations after treatment. Flow cytometry is widely used for this kind of analysis. However, a comprehensive analysis of numerous immune cell populations in one tumor is a major challenge when only limited material is available due to small tumor size of e.g. a tumor responding to the treatment or analysis at an early tumor developmental stage. Having a staining panel that allows for the analysis of all major immune cell populations in a single staining would be a major advantage.

Tumor Models

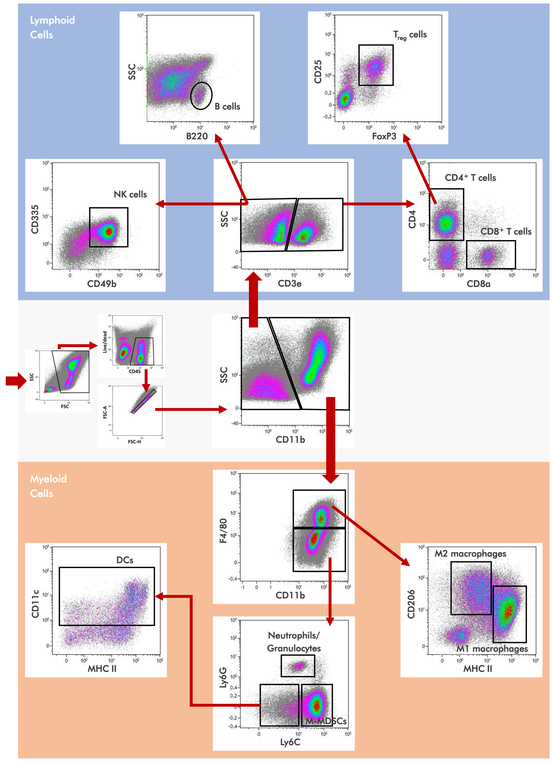
On day 0, syngeneic CT26wt colon tumor cells were implanted subcutaneously (s.c.), into the mammary fat pad (subQperior) and orthotopically (ortho), respectively. On day 4 after implantation, the tumor bearing mice were randomized in two groups and treatment started. Group 1 was treated with vehicle only, whereas group 2 received anti-mCTLA-4 antibodies every 3rd day. On the day of randomization (day 4, n=6, not shown), day 7, day 10 and day 13 after implantation five animals were euthanized and the tumor harvested for flow cytometry analysis.



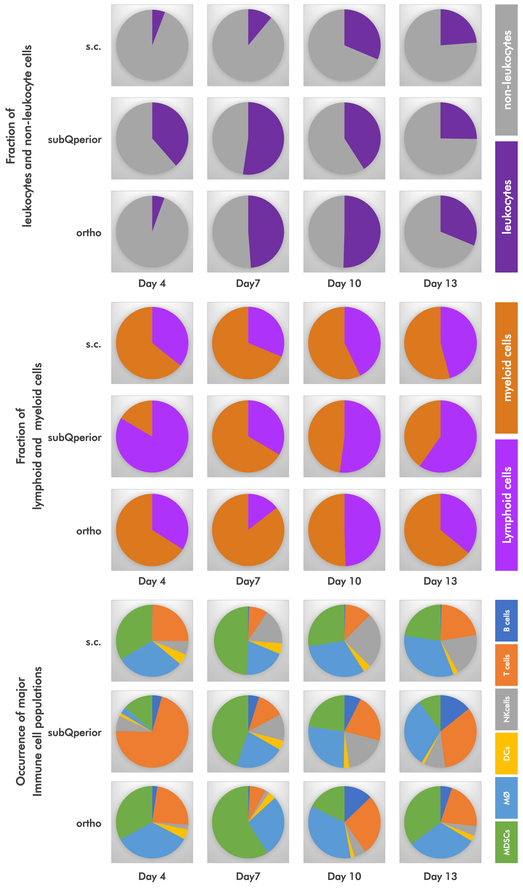


Flow Cytometry Gating Strategy

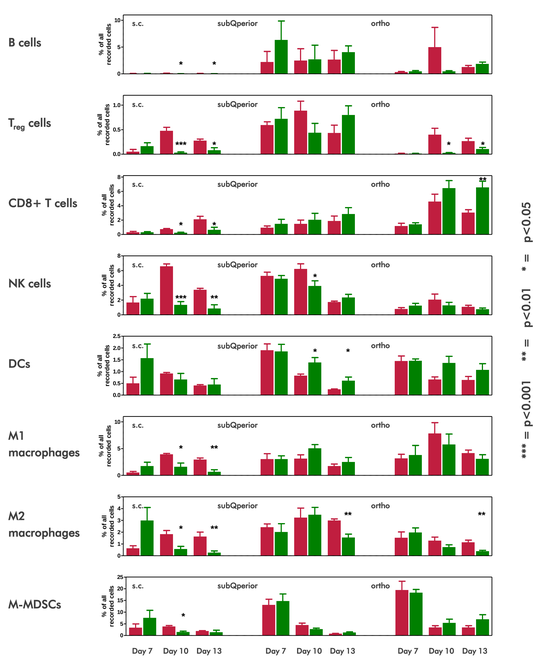
Tumors were collected and processed for flow cytometry analysis on day 4, 7, 10 and 13. Primary tumor material was disrupted, erythrocytes removed and up to 3 x 10^6 obtained single cells/well dispensed. The single cells were stained for live/dead and the antigens CD3, CD4, CD8a, CD45, CD25, CD11b, Ly6C, Ly6G, F4/80, CD11c, MHC class II, CD206, CD335, CD49b, B220 and FoxP3. The samples were analyzed by flow cytometry using a LSR Fortessa (Becton Dickinson). The gating strategy is depicted below starting at the large arrow in the middle.



Immune cell distribution in vehicle treated tumors



Effect of α-mCTLA-4 treatment on immune populations



Summary

- All CT26wt models responded well to immune-checkpoint inhibitor therapy
- Analysis of little tumors is possible
- With a superior gating strategy the all-in-one staining panel yields more accurate results
- Dynamic changes in the immune populations are observed during tumor growth in all models
- The subQperior model appears closer to the orthotopic than to the subcutaneous model, e.g. the total number of immune cells