

➤ Subcutaneous mouse tumor models

Subcutaneously implanted tumor cells represent a convenient means to test novel potential anti-cancer drugs *in vivo*. A large variety of human and murine cell lines derived from both, solid tumors or leukemias, covering a wide range of tumor geno- and phenotypes, have been adapted to grow in mice, and thus allow testing of a compound in the appropriate tumor model.

➤ CT26wt cells (CPQ-238)

Origin: colon / mouse BALB/c
Description: colon carcinoma

➤ Study outline

- subcutaneous implantation of CT26wt cells into the left flank
- randomization into treatment groups according to tumor sizes
- tumor sizes are measured via calipering twice weekly
- animal behavior is monitored daily
- animal weights are measured three times weekly
- Accessory services: tumor wet weight and volume measurement at necropsy, blood sampling, immune cell frequency determination in the tumor and lymphatic tissues by flow cytometry, paraffin embedding of tumor tissue, histological & pathological analysis, cytokine determination, provision of tumor tissue for target validation

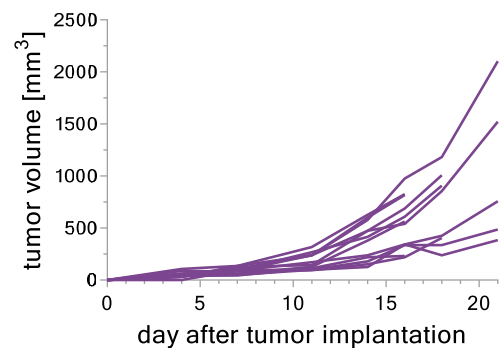


Figure 1: Growth of CT26wt tumors *in vivo*. Tumor volume, mean values +/- SEM. Termination on day 21.

➤ Study example – 5-FU

In the study shown here, mice bearing CT26wt tumors were treated with 5-FU or vehicle. Treatment started after randomization when tumor volumes had reached a size of approximately 70-100 mm³.

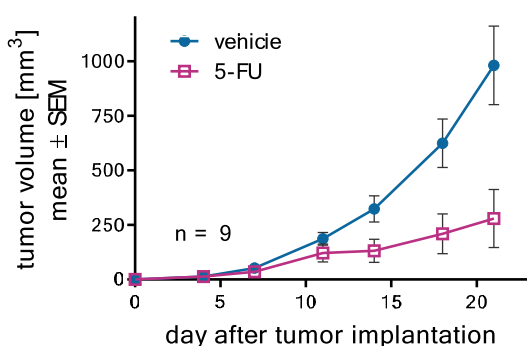


Figure 2: CT26wt tumors were treated with a 5-FU. Tumor growth was monitored by calipering.

➤ please turn over <

➤ Study example – Immune checkpoint inhibition

Treatment of CT26wt tumor-bearing mice with checkpoint inhibitors anti-PD-L1, anti-PD-1 and anti-CTLA-4 antibody results in tumor growth inhibition.

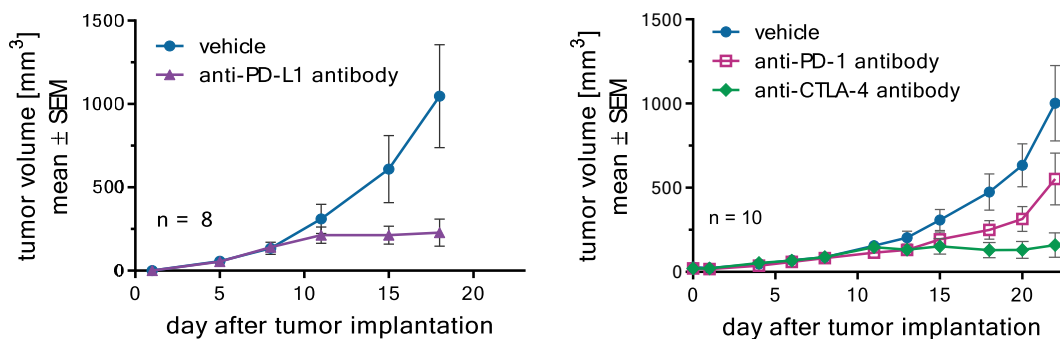


Figure 3: CT26wt tumors were treated with anti-PD-1 and anti-CTLA-4 antibodies starting at day 8 and anti-PD-L1 antibodies starting at day 5. Tumor volume, mean values +/- SEM

➤ Study example – Flow cytometry analysis

The mode of action of immuno-modulating therapies can be investigated via detection of changes in the frequency of tumor-infiltrating immune cells.

The frequencies of a variety of immune cell populations in subcutaneous CT26wt tumors is shown on the right.

For flow cytometry analysis we offer a 17-marker staining panel including

Live/dead dye, CD3, CD4, CD8α, CD45, CD25, CD11b, Ly6C, Ly6G, F4/80, CD11c, MHC class II, CD206, CD335, CD49b, B220 and FoxP3

for investigation of

T cells (CD8+, CD4+, Treg), B cells, NK cells, Macrophages (M1, M2), dendritic cells, Neutrophils/Granulocytes, M-MDSCs

Please find more information on the flow cytometry info sheet.

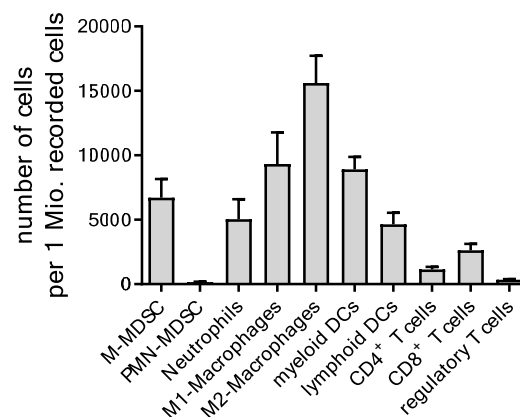


Figure 4: Distribution of immune cells infiltrating CT26wt tumors per 1 million tumor suspension cells.