

### ➤ SubQperior mouse tumor models

Imagine a syngeneic model with no tumor ulceration, 100% take rate and homogeneous tumor growth. Not possible?

We have developed our tumor models with an implantation method overcoming common problems researchers experience with subcutaneous tumor models. The solution is simple: change the injection site from subcutaneous to mammary fat pad and experience an impressive difference: beautiful growth curves and with the ease of calipering tumor size. SubQperior = superior to subcutaneous.

### ➤ 4T1 cells (CPQ-272)

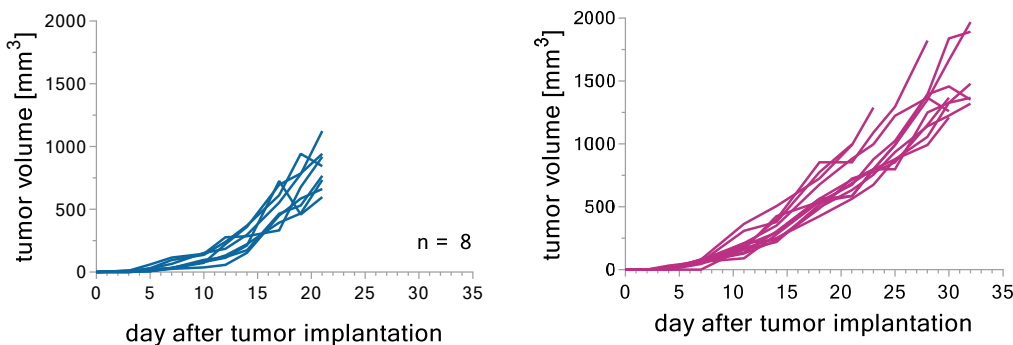
Origin: breast / mouse BALB/c  
Description: mammary gland tumor cell line

### ➤ SubQperior and orthotopic breast cancer model

4T1 origin is a breast tumor. Therefore subQperior is a special case: it is orthotopic. The method for tumor cell implantation into the mammary fat pad is the same procedure as orthotopic implantation of breast cancer cells. We offer implantation of luciferase-expressing and non-luciferase-expressing tumor cells. In addition, cell line 4T1-M3 is used for investigation of metastasis.

### ➤ Study outline

Comparison of 4T1 tumor growth characteristic after subcutaneous vs. subQperior implantation shows larger tumor volumes and longer treatment window for subQperior tumors.



**Figure 1:** Growth of not luciferase-expressing 4T1 tumors in vivo after subcutaneous (abrogated at day 21) and subQperior implantation.

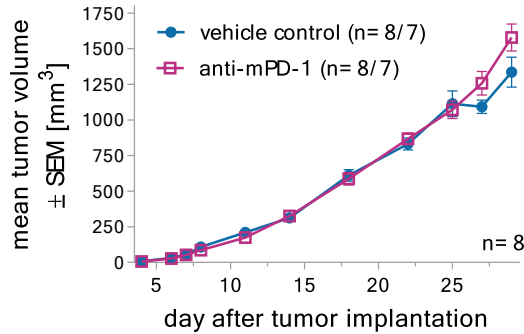
### ➤ Quality assurance

- Routine authentication of tumor cell lines by STR profiling
- Mycoplasma testing of implanted tumor cells by PCR just prior to implantation
- Routine health monitoring of sentinel animals (according to FELASA guide lines)
- Animal work according to the 5R rules (reduce, refine, replace, responsible, remember)

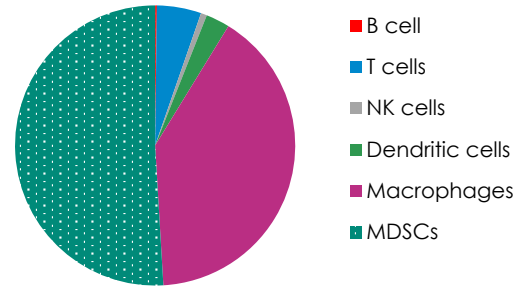
Note: Graphs depicted are derived from study examples. Each study is a biological system of its own and subject to intrinsic variation.

➤ Study example – Immune Checkpoint Inhibitors

Mice bearing 4T1 cells (not luciferase-expressing) implanted in the mammary fat pad were treated with anti-mPD-1. Treatment started after randomization when tumor volumes are approximately 50 mm<sup>3</sup>.



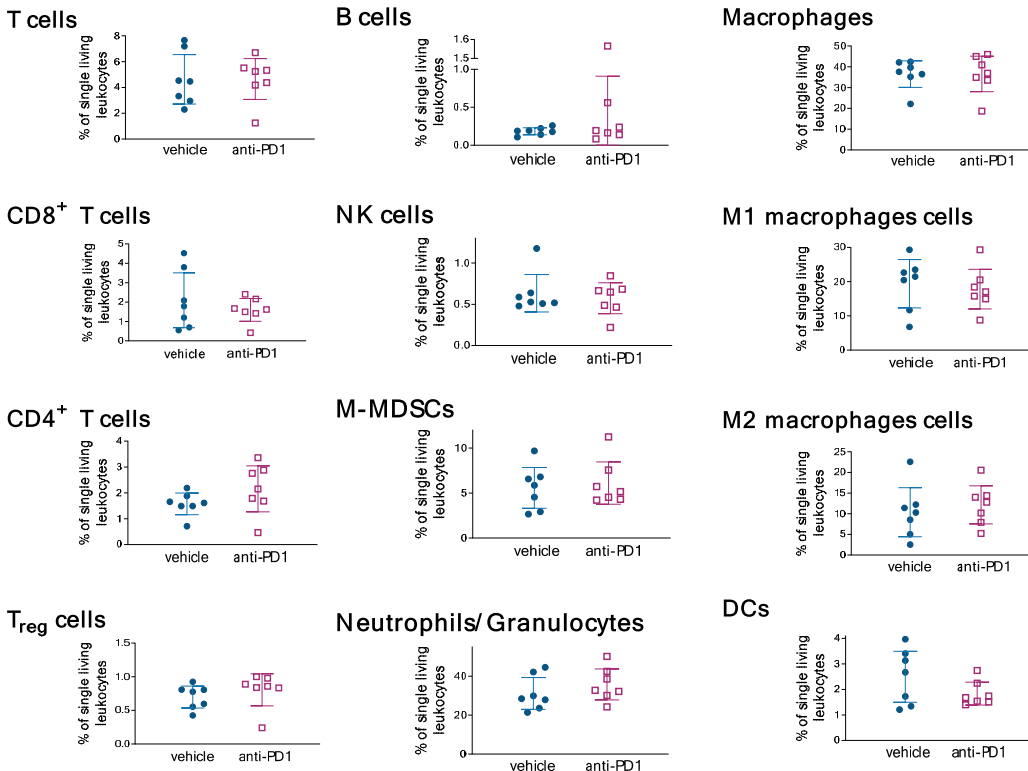
**Figure 2:** 4T1 tumors were treated with 10 mg/kg anti-mPD-1. Tumor growth was monitored by caliper.



**Figure 3:** Flow cytometry analysis of 4T1 tumor tissue showing the relative distribution of the major immune cell populations.

➤ Immune cell populations infiltrating 4T1 tumors

At study endpoint, primary tumor tissues were processed and analyzed by flow cytometry for determination of T cell, B cell, macrophage, NK cell, dendritic cell and myeloid cell populations.



**Figure 4:** Flow cytometry analysis of 4T1 primary tumor tissue is depicted as percentage of living immune cell tumor infiltrate. Data are displayed both as bar graph and single points with their corresponding mean + 95% CI.