In Vivo Testing Services

➢ Syngeneic Tumor Models

All models are well characterized in regards of their response to various immune-checkpoint inhibitors and their profile of tumor-infiltrating immune cells.

Please find the Info Sheets of the individual models on our homepage.

Cell line	Entity	Validated syngeneic models		
		subcutaneous	orthotopic	metastasis
4T1	Breast	✓	✓	✓
B16.F10	Skin	✓	✓	✓
Clone M-3	Skin	✓	-	-
Ct26wt	Colon	✓	✓	-
LL/2	Lung	✓	-	-
MC38-CEA	Colon	✓	-	-
RENCA	Kidney	✓	✓	-

➢ Flow Cytometry

Tumor-infiltrating immune cell subsets can be characterized with our state-of-the-art flow cytometry facility.

Staining for the following immune cell subsets are established: T lymphocytes, dendritic cells, myeloid-derived suppressor cells, macrophages and neutrophils. Please inquire for further immune cell subsets that are of interest to you.

Sorting and capturing of defined cell subsets is possible.

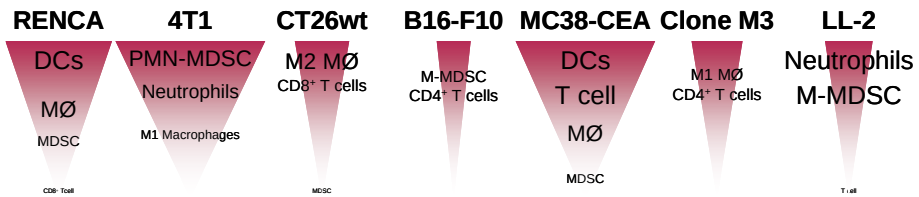

Figure 1. *Graphical presentation of immune cell infiltrates in tumors. The widths of the triangles reflect the quantity of overall leukocyte infiltration and the font sizes reflect the relative quantities of the specific immune cell population. Please see the following pages for more details.*
MØ – Macrophages, DC – dendritic cells, MDCS – myeloid derived suppressor cells.

➢ Cytokine/Chemokine determination

Fresh tissue and blood samples can be investigated for the presence of cytokines, chemokines, growth factors or other biomarkers via multi-plex technology in cooperation with a partner laboratory.

➢ Target Validation

To verify the presence of your target protein in the tumor tissue, we can provide the following tumor materials: Formalin-fixed or cryo-conserved tumor tissue, lysates or RNA of tumors. Target validation can be performed either by yourself or at ProQinase.

➢ Immunohistochemistry

The abundance and location of immune cells in the tumor and stroma tissue can be investigated via immunohistochemistry in cooperation with our partner laboratory who are experts for histology and pathology.

+Please turn over+

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➢ Flow Cytometry of Myeloid-Derived Suppressor Cells and Neutrophils

Myeloid-derived suppressor cells (MDSCs) comprise a heterogeneous cell population and are precursors to dendritic cells, granulocytes, and macrophages. The two subsets, monocytic (M)-MDSCs and polymorphonuclear (PMN)-MDSCs, differ in the factors they release to support angiogenesis, invasion, and metastasis. Both subsets produce anti-inflammatory factors inhibiting T cells and programming macrophages toward the M2 phenotype.

Neutrophils play an ambivalent role comparable to that of macrophages. Depending on their environment, they can exhibit tumor-suppressive or tumor-supporting tendencies via polarization into different phenotypes.

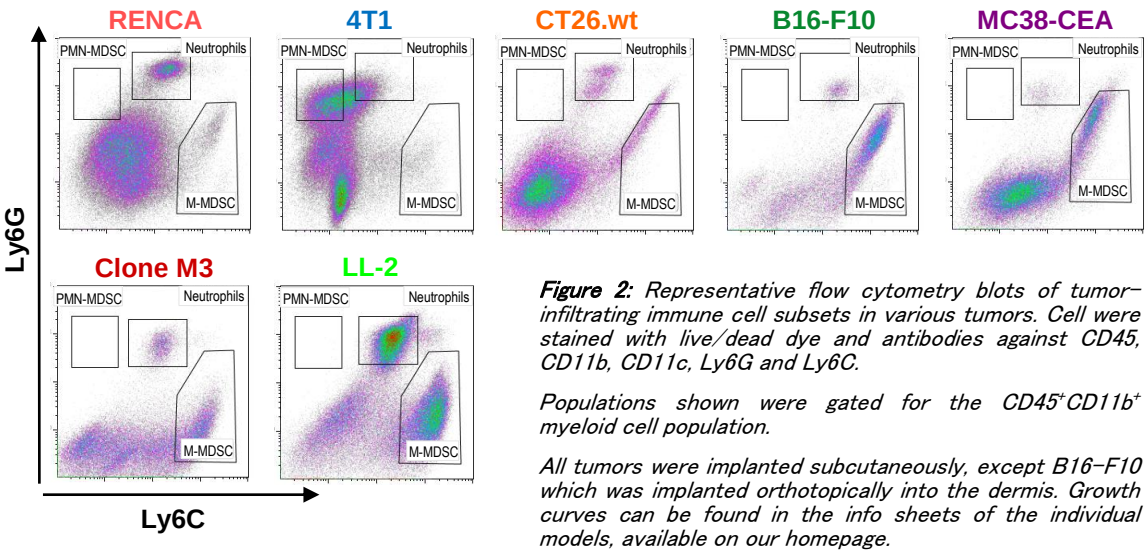

Figure 2: *Representative flow cytometry blots of tumor-infiltrating immune cell subsets in various tumors. Cell were stained with live/dead dye and antibodies against CD45, CD11b, CD11c, Ly6G and Ly6C.*

Populations shown were gated for the $CD45^{+}CD11b^{+}$ myeloid cell population.

All tumors were implanted subcutaneously, except B16-F10 which was implanted orthotopically into the dermis. Growth curves can be found in the info sheets of the individual models, available on our homepage.

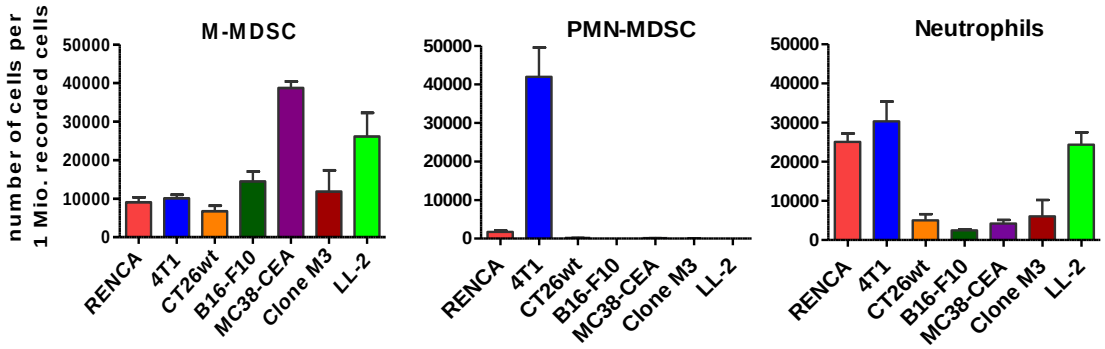

Figure 3: *Comparison of the numbers of M-MDSCs, PMN-MDCSs and neutrophils per 1 million cells of tumor cell suspension. Mean of 3 to 10 tumors, +/- SEM.*

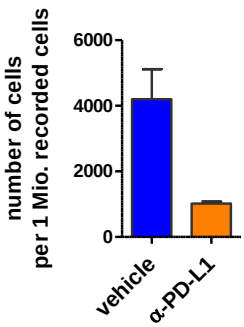

Figure 4: *Treatment with the immune checkpoint inhibitor anti-PD-L1 antibody reduces the number of neutrophils in MC38-CEA tumors.*

A detailed **mode of action** analysis of **immune-checkpoint inhibitor** anti-PD-L1 antibody with CT26.wt tumors is shown in our White Paper available on our homepage!

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➢ Flow Cytometry of T lymphocytes

T lymphocyte populations are important for immune surveillance. Activated cytotoxic CD8⁺ T cells kill tumor cells and CD4⁺ T cells regulate the adaptive immune response. Regulatory T cells (CD4⁺FoxP3⁺CD25⁺) hold a particularly important role in tumor-specific immune responses through their immune-suppressive phenotype.

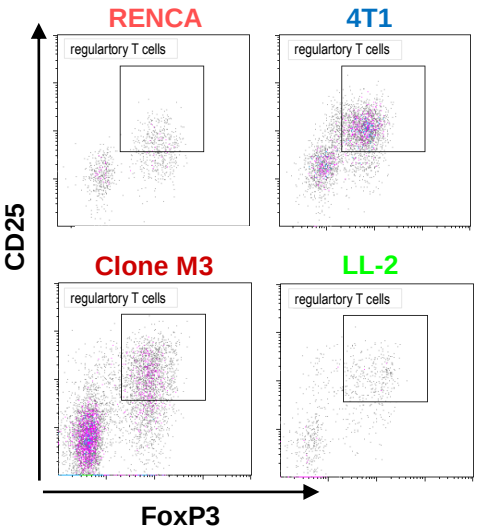

Figure 5: *Representative flow cytometry blots of tumor-infiltrating regulatory T cells in various tumors. Cell were stained with live/dead dye and antibodies against CD45, CD3, CD4, CD44, CD8a, CD25 and FoxP3.*

Populations shown were gated for the CD45⁺CD3⁺CD4⁺ T lymphocyte population.

All tumors were implanted subcutaneously, except B16-F10 which was implanted orthotopically into the dermis. Growth curves can be found in the info sheets of the individual models, available on our homepage.

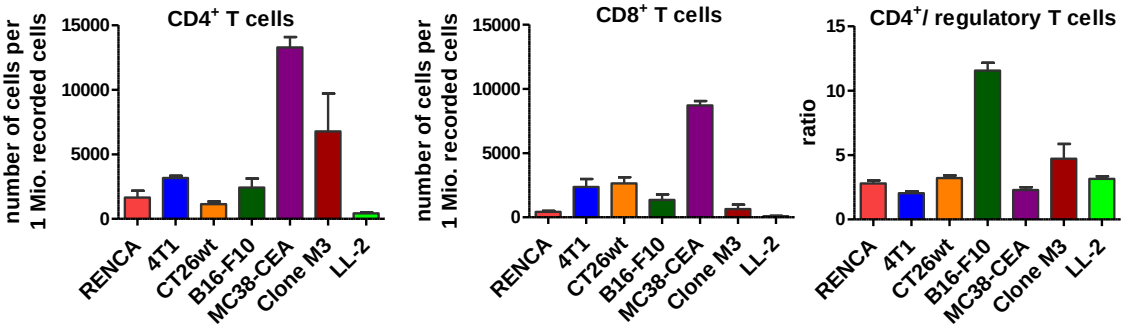

Figure 6: *Comparison of the numbers of CD4⁺ and CD8⁺ T cells per 1 million cells of tumor suspension (left panels) and representation of the ratio of CD4⁺ T cell vs. regulatory T cell (right panel). Mean of 3 to 10 tumors, +/- SEM.*

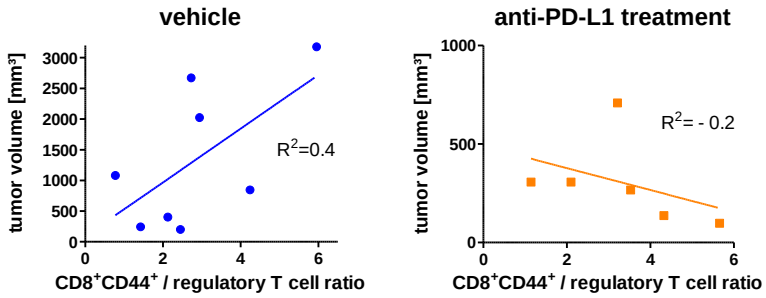

Figure 7: *Correlation blots of tumor sizes versus ratio of cytotoxic CD8⁺/regulatory T cells are shown for CT26.wt tumors with and without anti-PD-L1 treatment. Upon treatment the tumor-killing activities of cytotoxic CD8⁺ T cells become dominant over the tumor-suppressing activities of regulatory T cells.*

A detailed **mode of action** analysis of **immune-checkpoint inhibitor** anti-PD-L1 antibody is shown in our White Paper available on our homepage!

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➢ Flow Cytometry of Macrophages

Macrophages play a versatile role in tumors; they can directly attack tumor cells, or they may support tumor growth by promoting angiogenesis and facilitating invasion and metastasis. They are capable of influencing the tumor environment by both activation and suppression. Two well-described subsets of macrophages are the "good" M1 macrophages and the "bad" M2 macrophages.

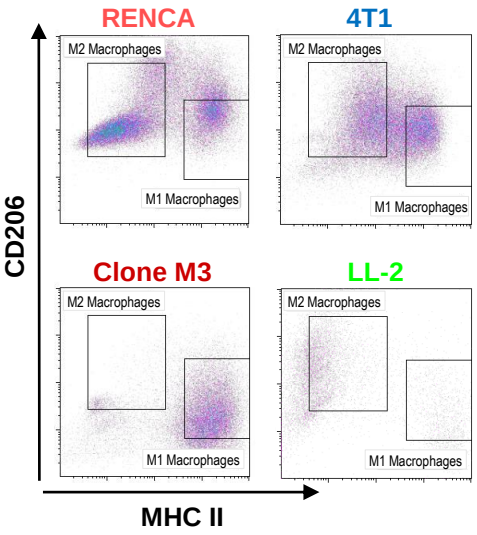

Figure 8: *Representative flow cytometry blots of tumor-infiltrating macrophages in various tumors stained with live/dead dye and antibodies against CD45, CD11b, F4/80, MHCII and CD206.*

The population shown was gated for CD45⁺CD11b⁺F4/80⁺.

All tumors were implanted subcutaneously, except B16-F10 which was implanted orthotopically into the dermis. Growth curves can be found in the info sheets of the individual models, available on our homepage.

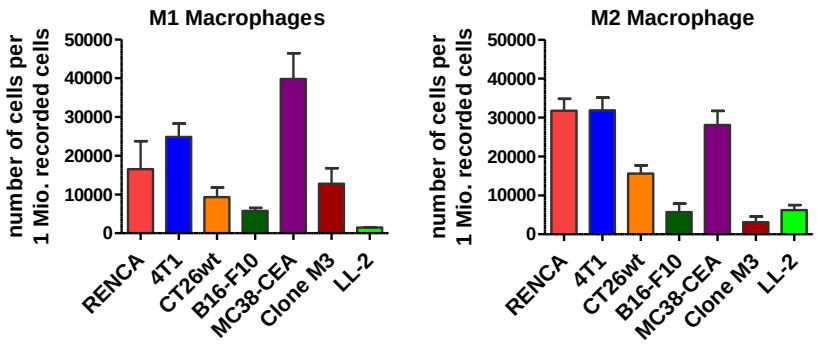

Figure 9: *Comparison of the numbers of M1 and M2 macrophages per 1 million cells of tumor cell suspension.*
Mean of 3 to 10 tumors, +/- SEM.

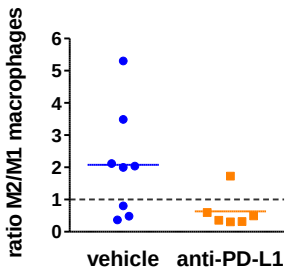

Figure 10: *Macrophages show different phenotypes in CT26.wt tumors treated with immune checkpoint inhibitor.*
In vehicle treated tumors the "bad" M2 phenotype is prevalent whereas in anti-PD-L1 treated tumors the majority of macrophages belong to the tumor-suppressing M1 phenotype.

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