Anti-PD-1, Anti-PD-L1 and Anti-CTLA-4 checkpoint inhibitor treatment leads to different responses in syngeneic tumor models

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ProQinase TARGETING CANCER

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Introduction

Checkpoint inhibitor treatment has already become a common therapy of various cancer types. Still there is a growing need for well-characterized preclinical mouse models, as clinical data indicate that patients only partially respond to this regiment. We examined the efficacy of anti-CTLA-4, anti-PD-1 and anti-PD-L1 therapy on our syngeneic tumor model platform (see Fig. 1) Although humanized mouse models may provide a starting point, syngeneic mice models appear to be the better method of choice, reflecting the whole spectrum of a functional immune system. Here, we report on the establishment of syngeneic mouse models for breast, colon, kidney, lung and skin cancer both for subcutaneous and orthotopic implantation including flow cytometry analysis of tumor cell populations.

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	✓	✓	-

Fig.1: Syngeneic tumor model platform established at ProQinase.

Re-challenge experiment

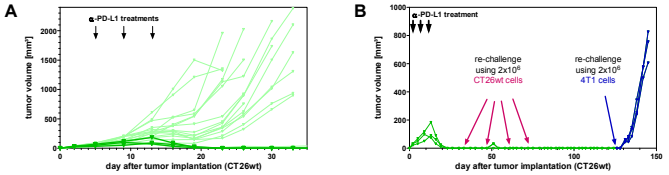


Fig. 3: Mice implanted with CT26wt tumor cells were treated with 10 mg/kg anti-PD-L1 antibody three times. Tumor growth was monitored (A). Three CT26wt tumors showed a complete regression after therapy and were re-challenged with 2 x 10^6 CT26wt tumor cells at Day 33, 47, 61 and 75 (B). No re-growth of CT26wt tumors was observed (B). Finally, unrelated 2 x 10^6 4T1 tumor cells having the same MHC background were implanted at Day 126. 4T1 tumor growth appeared similar to primary increase in immunologically naïve mice (Fig. 2). Single tumor growth curves are shown.

Immune checkpoint inhibitor efficacy studies

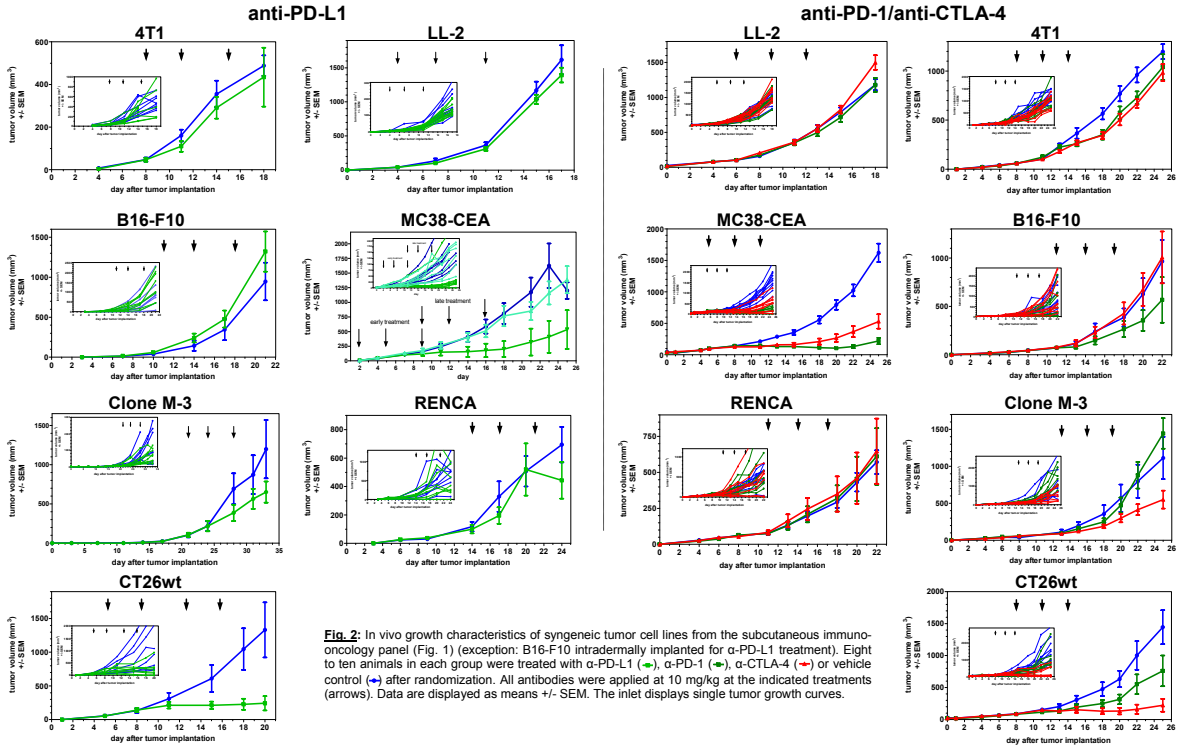


Fig. 2: In vivo growth characteristics of syngeneic tumor cell lines from the subcutaneous immuno-oncology panel (Fig. 1) (exception: B16-F10 intradermally implanted for α-PD-L1 treatment). Eight to ten animals in each group were treated with α-PD-L1 (), α-PD-1 (), α-CTLA-4 () or vehicle control () after randomization. All antibodies were applied at 10 mg/kg at the indicated treatments (arrows). Data are displayed as means +/- SEM. The inlet displays single tumor growth curves.

Flow cytometry analysis

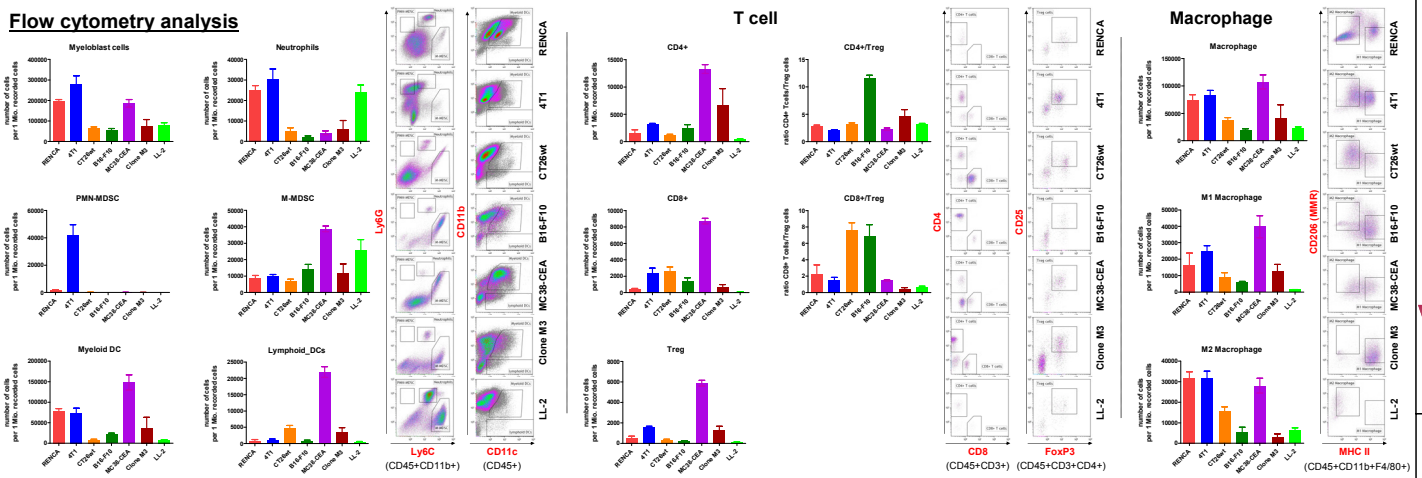
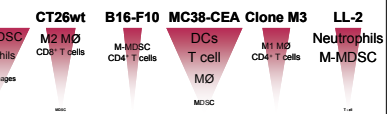


Fig. 4: Cells isolated from solid tumors of the vehicle group (Fig. 2) were stained for MDSC, T cell and Macrophage markers and analyzed by flow cytometry. The graphs depict the number of cells per 1 mio. recorded cells in the indicated tumors. Example flow images are shown for each tumor with additional markers in parenthesis below.

Summary

- Heterogeneous responses to immune checkpoint inhibitor treatment (α-PD-L1, α-PD-1, and α-CTLA-4)
- CT26wt and MC38-CEA tumor model respond best to immune checkpoint inhibitors
- Re-challenging of complete responder animals demonstrate tumor specific immunity (MC38-CEA, RENCA not shown)
- Immune cell populations differ between the models



(triangle width reflects leukocyte tumor infiltration quantity with potentially important immune populations given)

The presented syngeneic models with their difference in immune status are a versatile tool to study the effect of immune checkpoint inhibitors alone or in combination with other drugs