



Poster :

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# Immunological characterization: Allograft of Primary Murine Breast Cancer (MuPrime™) versus Murine Breast Cancer Cell-Derived Syngeneic Tumors

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## Introduction

Thanks to the recent success in clinical trials, immunotherapy has become the main focus of anticancer drug development. Murine cancer models played an important role in understanding tumor immunology and developing effective immunotherapy strategies. In this study, we characterized by immunohistochemistry (IHC) the tumor microenvironment, including its general morphology, stroma, immune cells recruitment, and checkpoint markers in an allograft of spontaneous breast tumors, MuPrime™ mBR6004, and in 3 syngeneic breast cancer models, including EMT-6, 4T1 and JIMT1. FFPE tissues of tumors and plasma from the mBR6004 model treated with anti-PD1 or anti-CTLA4 were analyzed with immunohistochemistry and Luminex multiplex assay, to observe the change of immune cells and cytokines after the immunotherapy. We hope to develop a systematic biomarker approach helping selection of murine breast cancer models in cancer immunotherapy drug development.

## Results

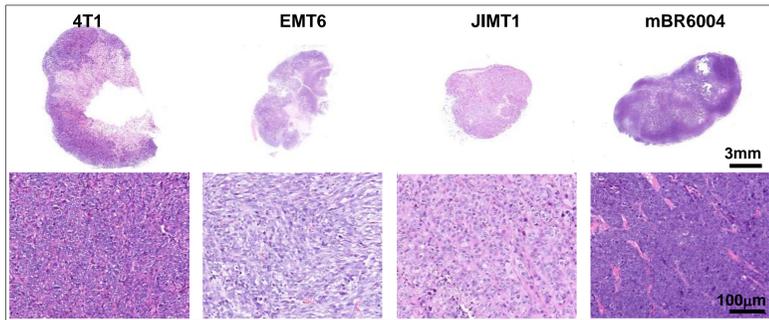


Figure 1. Tumor Pathology

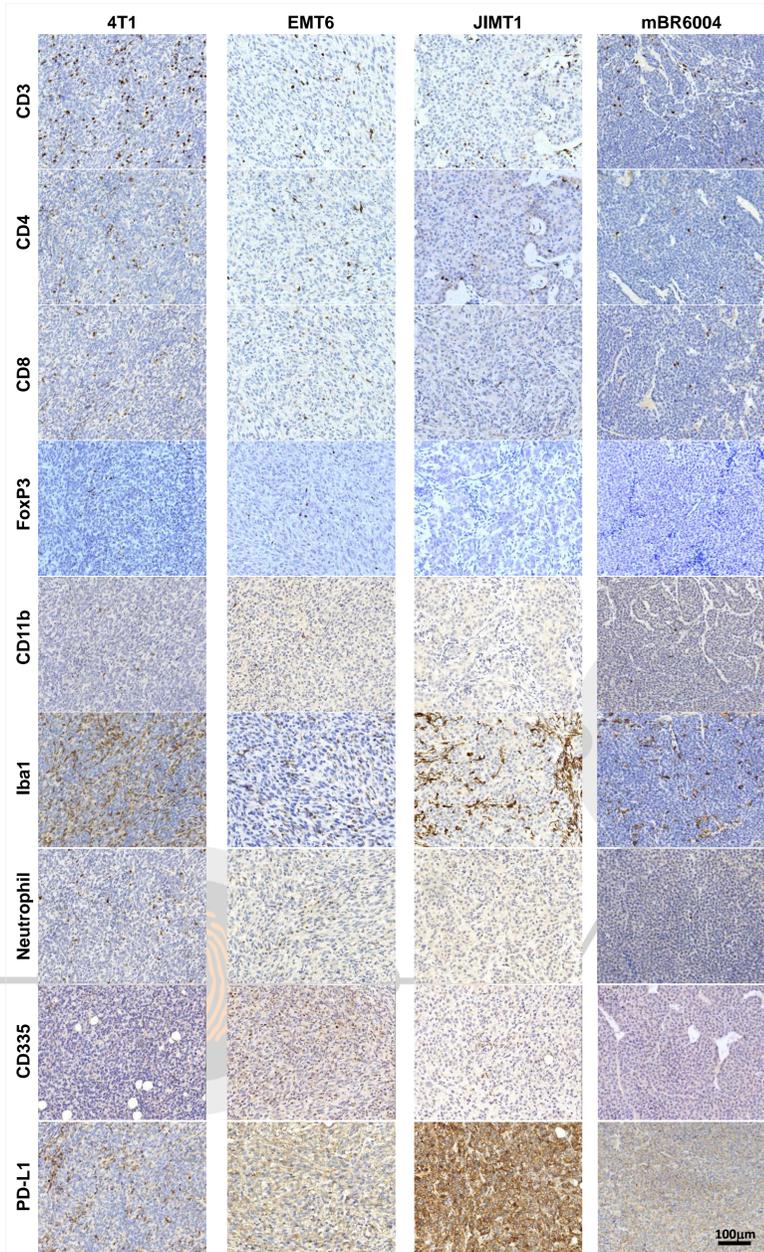


Figure 2. IHC staining of immune markers

## Methods

**Animal study.** A mBR6004 tumor fragment (2-3 mm in diameter) was inoculated in the mammary fat pad of FVB/N female mice for tumor development. Tumor size were monitored twice weekly by caliper 2D measurement, and the volume was calculated using the formula:  $TV(mm^3) = 0.5a \times b^2$ , where a and b are the long and short diameters of the tumor, respectively. Treatment started when the average tumor size reached about 120 mm<sup>3</sup> (for the first 3 groups) or 240 mm<sup>3</sup> (for the last 2 groups). Tumor size was used to calculate tumor growth inhibition (TGI) by the formula:  $\%TGI = (1 - (Ti - T0) / (Vi - V0)) \times 100$ , where the changes of mean tumor volume from treatment initiation (day i vs day 0) in the treated (T) and control (V) groups are compared.

**Pathology and Immunohistochemistry.** 4 µm FFPE sections of MT-6, 4T1, JIMT1 syngeneic and mBR6004 models with/without treatments were prepared and stained with H&E and IHCs as shown in the Results section. All IHCs were run on Bond RX autostainer (Leica). Images were captured using the Nanozoomer H2.0 whole-slide imaging system (Hamamatsu).

**Cytokine analysis.** Plasma samples from the mBR6004 model collected after treatments were diluted 1:2 then analyzed with Mouse Cytokine/Chemokine Magnetic Bead Panel kit (MCYTMAg-70K-PX32, Millipore). The assay was run on Lunimex 200 (Millipore). Data were analyzed with MILLIPLEX Analyst 5.1 software.

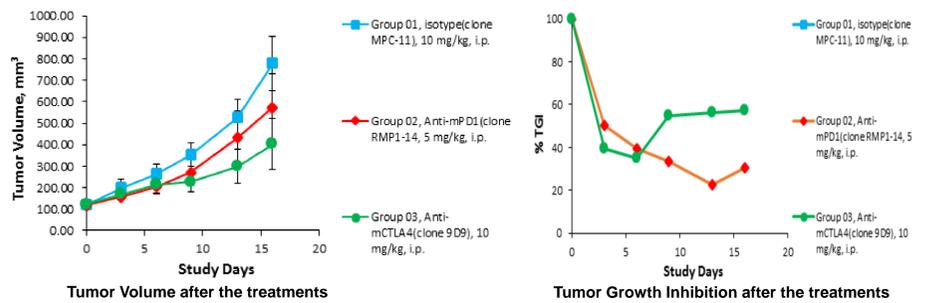


Figure 3. Response of mBR6004 to anti-PD1 and anti-CTLA4 mAb

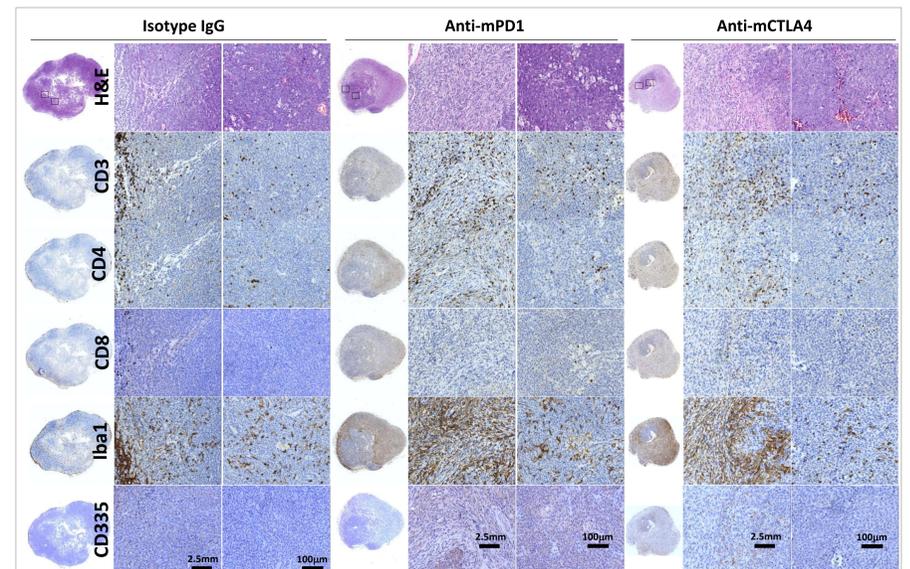


Figure 4. IHC characterization of immune cells in mBR6004 after anti-PD1 and anti-CTLA4 treatment

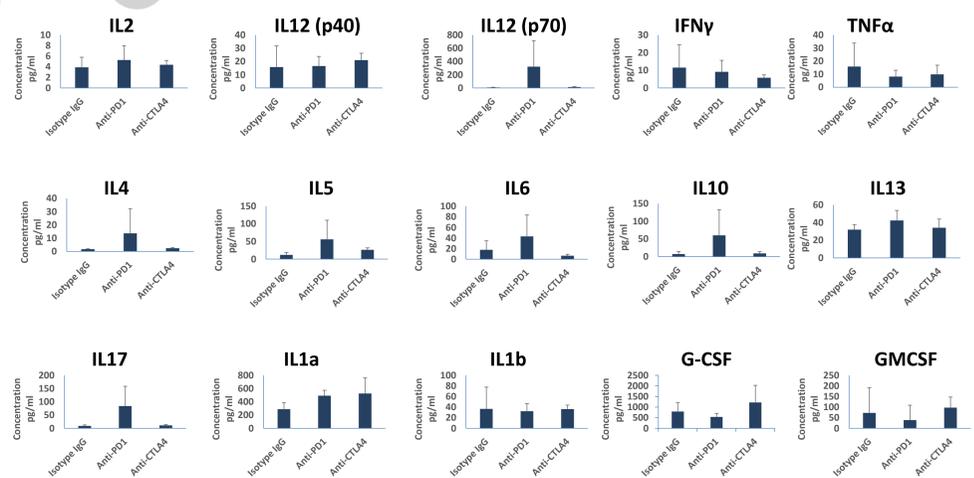


Figure 5. Cytokine analysis of mBR6004 after anti-PD1 and anti-CTLA4 treatment

## Conclusions

1. The mBR6004 model has a higher stroma component and displays a lower level of necrosis compared to syngeneic models. Most tumors in mBR6004 are well differentiated with rich stroma.
2. Tumors from 4T1 and EMT6 models are made of compact, homogeneous cells from the same type. It is possible to identify some differentiated structures in the JIMT1 model.
3. Immune cells are found at high density at the peripheral capsule of the syngeneic and mBR6004 models. Tumor infiltrating T cells (CD3, CD4 and CD8+ cells) are found at low density in both syngeneics and mBR6004, and are more abundant around the vessels. Recruitment of T<sub>reg</sub> (FoxP3+), MDSC (CD11B), Neutrophils and NK (CD335) at the tumor is less often observed in all models while Iba1+ macrophages are frequently encountered at high density. PD-L1 is strongly expressed in JIMT1, moderately in mBR6004, weakly in EMT6, while in the 4T1 the stroma seems to be the only positive component.
4. Tumor growth can be inhibited to some extent after 16 days of treatment with anti-PD1 and anti-CTLA4 mAb in the mBR6004 model.
5. In the mBR6004 model treated with anti-PD1 or anti-CTLA4 mAb, tumors assume a shell-like morphology with a outer layer and core area. Cells in the outer layer are fibroblast-like. T cells (CD3, CD4, CD8, not FoxP3), macrophages and NK cells were more abundant in the outer layer.
6. There is an increasing trend of Th2 related cytokines in the anti-PD1 treated group.