Immunization is Critical to PD-1/PD-L1 Response in a Mouse Breast Cancer Allograft

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INTRODUCTION

Immuno-oncology is an area under intense investigation due to the successful development of several checkpoint inhibitors. However, many key questions remain to be addressed, such as: how to predict response, what factors influence response (i.e. host factors, the specific nature of a tumor, or both), and how to trigger response in the non-responsive population. Recent studies have flagged several different aspects as possible determinants of response to immunotherapy including: the presence of a specific tumor neoantigen, the tumor neoantigen load, the composition of the tumor microenvironment – specifically the presence of tumor infiltrating T cells (TIL) – and even the type of host microbiota.

To gain a better understanding of how the interaction between the tumor and the host may influence response to immunotherapy, animal models with competent immunity are needed. The currently available, most commonly used animal models for immuno-oncology are allografts of immortalized mouse cancer cell line (syngeneic) or allografts of primary mouse tumors within a host with a fully functional immune system. Here, by grafting a primary mouse tumor into a immunocompetent host from the same mouse strain we investigated whether vaccination could enhance the tumor response rate to current immunotherapy.

METHODS

We have created allografts of primary mouse tumors, including the breast adenocarcinoma mBR6004 originating from *MMTV-PyVT* transgenic mice, as a new type of experimental model for immuno-oncology, complementing syngeneic cell line derived models. The mBR6004 model was generated by orthotopic tumor implantation into the mammary fat pad of syngeneic *FVB/N* female mice. The formation of lung metastases were evaluated using light microscopy at study termination. A variety of chemotherapeutics were tested in this model and immunophenotyping of the allograft by flow cytometry was performed, which identified PD-L1 (CD274) and tumor-infiltrating immune cells, e.g. TIL, CTL, T_{reg}, immunosuppressive macrophages, and NK. Treatment with checkpoint inhibitors (anti-PD-1/PD-L1 and anti-CTLA-4) was also evaluated *in vivo* in this model. Having observed a poor response to PD-1/PD-L1 we speculated this could result from low numbers of TILs and we reasoned that an immunization step with mBR6004 tumor lysates two weeks prior to tumor implantation could prime the antitumor immunity and increased TILs. Our findings are presented here.

RESULTS

The mBR6004 allograft grows robustly, and maintains the original GEMM tumor histopathology. mBR6004 overexpresses HER2, but not ER/PR (**Figure 1**). Orthotopic implantation results in lung metastasis at a late stage. The model is responsive to 5-FU, and partially to paclitaxel and doxorubicin. mBR6004 expresses PD-L1 (CD274) at a low/medium level (**Figure 2**), and has a moderate presence of tumor-infiltrating immune cells, e.g. TIL, CTL, T_{reg}, immunosuppressive macrophages, and NK. Without prior immunization, mBR6004 responds poorly to anti-mouse-PD-1 or PD-L1 antibodies, and the treatment is associated with no apparent increase in the number of TIL (**Figure 3**). The model is partially responsive to anti-mouse-CTLA-4 antibodies, which correlates with an increased number of CD8+ TIL. However, when tumor lysate immunization is performed 2 weeks before tumor implantation, the number of CD8+ TIL increases significantly along with the response to PD-1/PD-L1 blockage by antibodies (**Figure 4**). The immunization step has negligible effects on the engraftment rate (100% take rate for all animals) and on the growth kinetics of the engraftments.

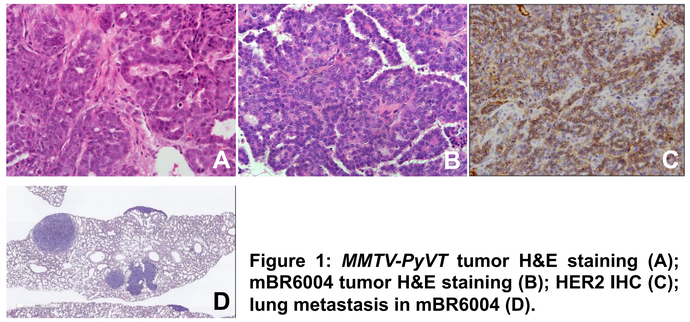

Figure 1: *MMTV-PyVT* tumor H&E staining (A); mBR6004 tumor H&E staining (B); HER2 IHC (C); lung metastasis in mBR6004 (D).

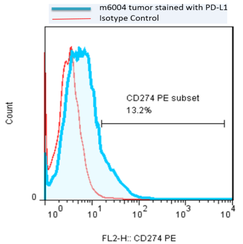

Figure 2: PD-L1 expression in the mBR6004 model.

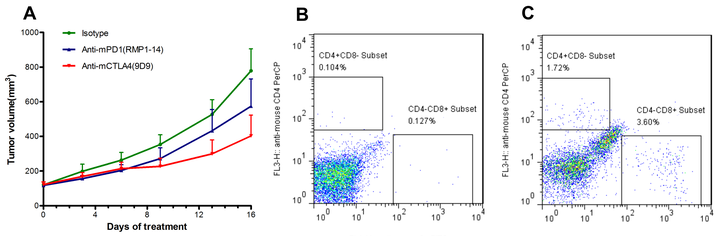

Figure 3: Inhibition of PD-1 in the mBR6004 allograft does not significantly impact tumor growth (A); CD8+ TILs in tumors treated with isotype control antibody (B); moderate increase of CD8+ TILs in anti-PD-1 treated tumors (C).

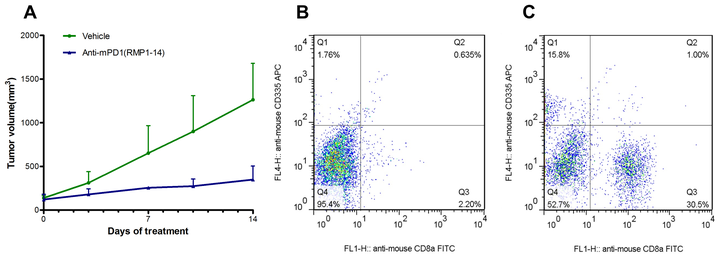

Figure 4: Immunization with tumor lysates 2 weeks prior tumor implantation enhances mBR6004 response to anti-PD-1 (A); CD8+ TILs in isotype control treated animals (B); highly increased CD8+ TILs in anti-PD-1 treated animals after immunization (C).

SUMMARY

1. **We established the mBR6004 breast cancer model and demonstrate it conserves original histopathology, expressing HER2 and PD-L1, growing robustly, and metastasizing to the lung. The model responds to some standard of care agents (SoC);**
2. **mBR6004 is poorly responsive to PD-1/PD-L1 blockage, but partially responds to an anti-CTLA-4 antibody;**
3. **Immunization makes the tumor responsive to PD-1/PD-L1 blockage, suggesting the host immune activation is critical to obtain a response to checkpoint inhibitors;**
4. **Our results demonstrate the benefit of using mBR6004 as an experimental model for immunotherapy.**