Poster : #95

In life for life

Establishment of a Mouse Breast Cancer Allograft Model for *In Vivo* Pharmacological Analysis of Immunotherapy

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Introduction

The recent clinical success of novel therapeutics blocking the immune checkpoints cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) has fueled an intense interest in immuno-oncology. However the lack of relevant animal models is a major bottleneck for understanding the mechanism of action and evaluating the efficacy of such therapeutics. Syngeneic mouse tumor models, despite being widely used as experimental models for efficacy studies, are limited by the fact that only a restricted number of models is available and their response to the current checkpoint inhibitors is partial. Genetically engineered mouse models (GEMMs) are effective tools for mechanistic analysis, but not suitable for efficacy studies due to unsynchronized tumor progression. Allografts of spontaneous mouse tumors derived from GEMMs (MuPrime™) may be used as a new model for immuno-oncology with the following advantages: 1) their primary nature of “stem cell disease” and relevant tumor microenvironment as seen in patient-derived xenograft (PDX) models; 2) the availability of various cancer types and oncogenic drivers deriving from a wide range of available GEMMs.

Abstract

We created the MuPrime model, mBR6004, by engrafting the breast adenocarcinoma derived from MMTV-PyVT transgenic mice[1] to the syngeneic FVB/N mice. The allografted tumor maintains histopathological features similar to the primary tumor and grows robustly when implanted subcutaneously or orthotopically. Interestingly, we found orthotopic implantation consistently results in lung metastasis. Transcriptome sequencing revealed high levels of HER2 expression in tumor cells, which are negative for ER and PR. When we assessed the responsiveness of the tumor to various treatment, we found it was resistant to Docetaxel. Immune-profiling of the tumor by FACS quantitatively confirmed the presence of tumor-infiltrating immune cells, *e.g*. TIL, CTL, Treg, immune-suppressive macrophages, NK, etc. We have also confirmed that PD-L1 is expressed at relatively low levels in the tumor cell in an inducible manner. Our preliminary data indicated that mBR6004 partially responds to anti-mouse PD-1 and anti-mouse CTLA-4 antibodies when mice are preconditioned. Currently, we are investigating whether these responses are associated with a reduced level of Treg and increased presence of $CD8^+$ TIL in the tumor, and whether combination therapy results in synergistic enhancement of antitumor activity. Together, our data suggest that we have established an allograft model suitable for *in vivo* efficacy analysis of immunotherapy using surrogate anti-mouse antibodies.

Results

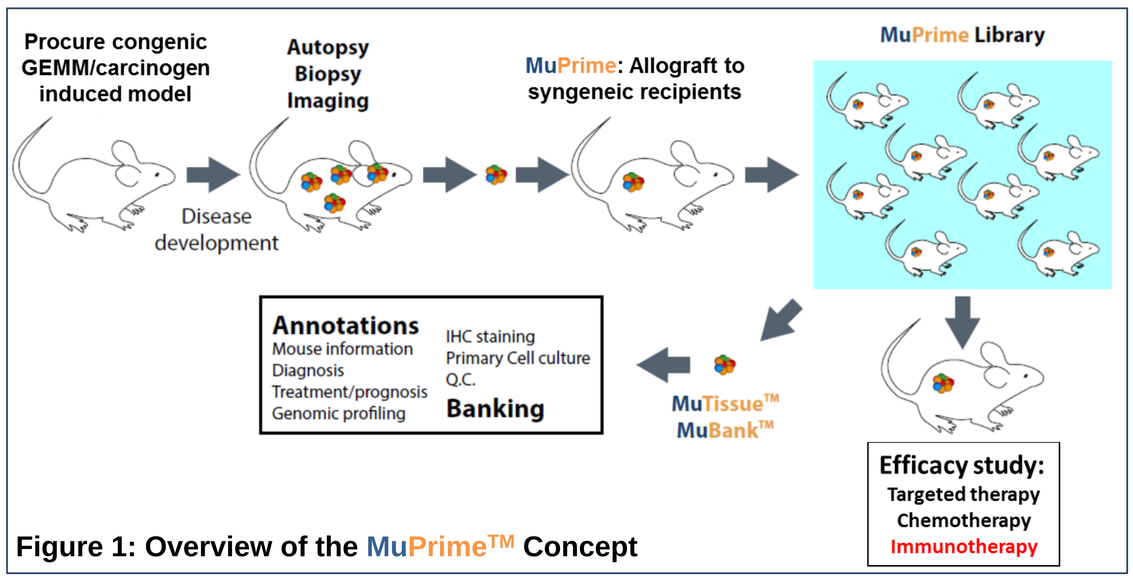


Figure 1: Overview of the MuPrime™ Concept

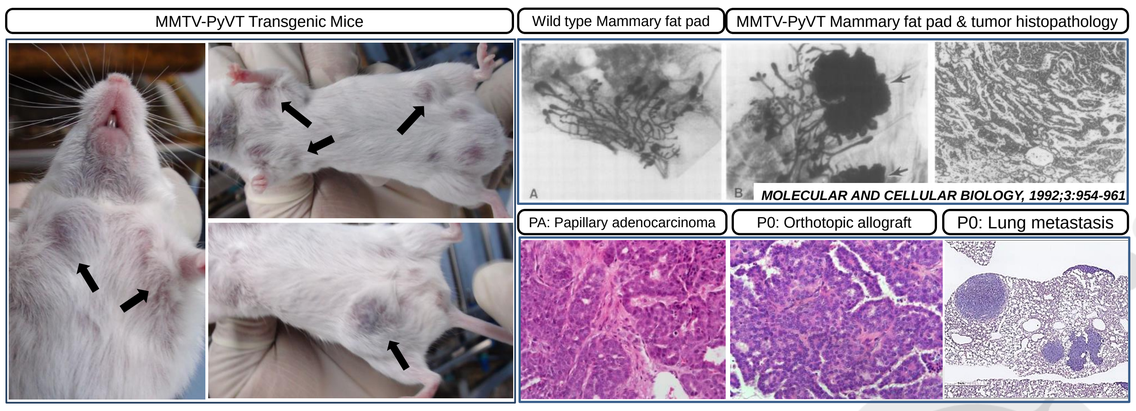


Figure 2: Gross Pathology & Histopathology

Model ID	mBR6004
EGFR	Frame shift insertion 1431G->GA
ERBB2	A130T
MET	I851M
KRAS	WT
BRAF	WT
AKT1	WT
PIK3CA	WT
CTNNB1	WT
PTEN	WT

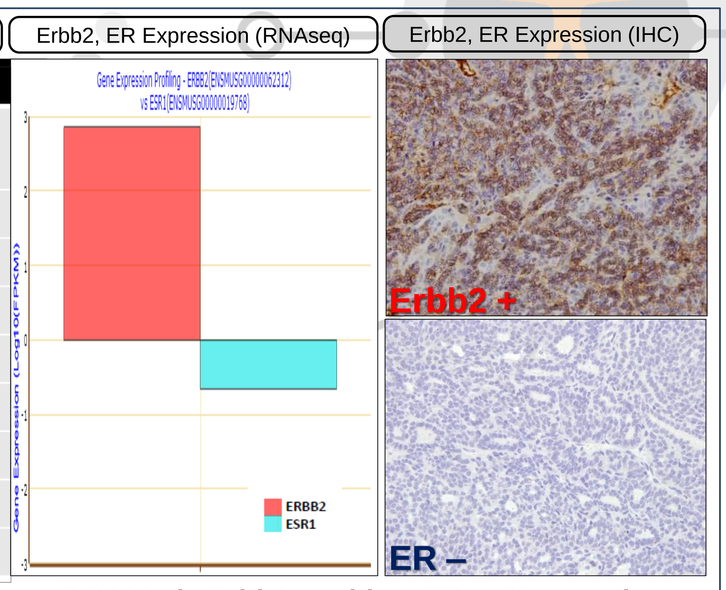


Figure 3: Molecular Pathology. mBR6004 is Erbb2 positive, ER & PR negative.

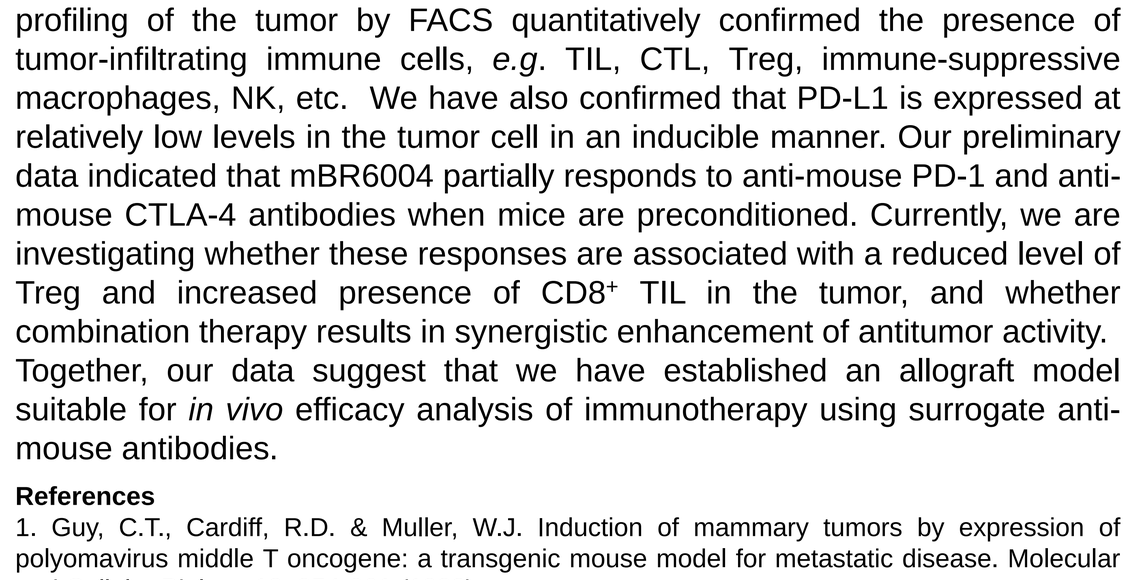
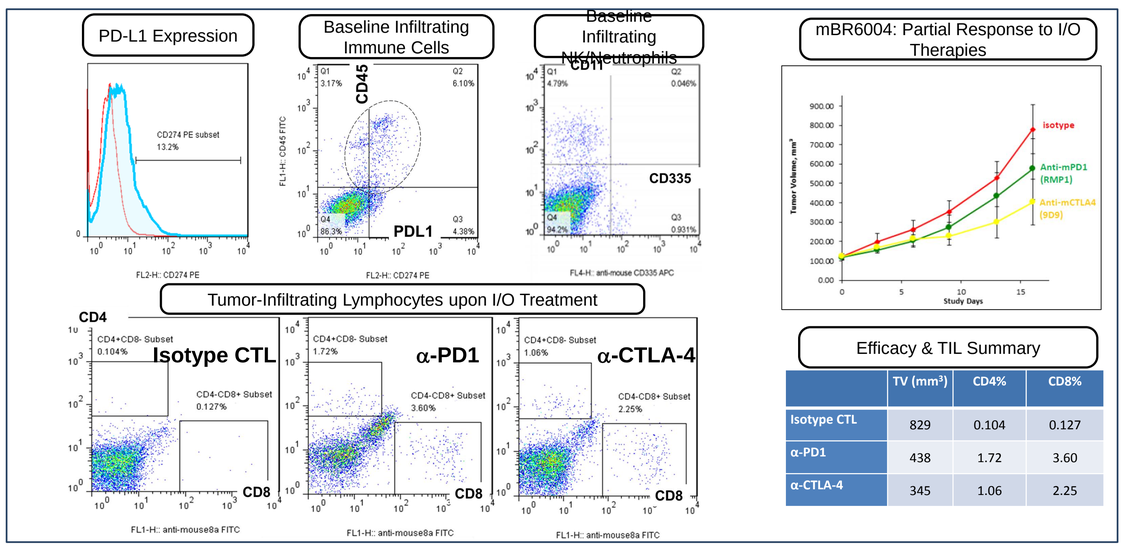


Figure 4: mBR6004 Growth Curve & SOC. The mBR6004 model is Resistant to Docetaxel.



	TV (mm^3)	CD4%	CD8%
Isotype CTL	829	0.104	0.127
α-PD1	438	1.72	3.60
α-CTLA-4	345	1.06	2.25

Figure 5: Immune Profile of mBR6004 and its Response to α-PD1, α-CTLA4 mAbs

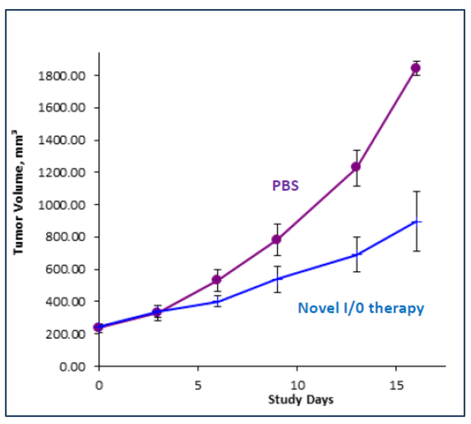


Figure 6: Partial Response to a Novel I/O Agent

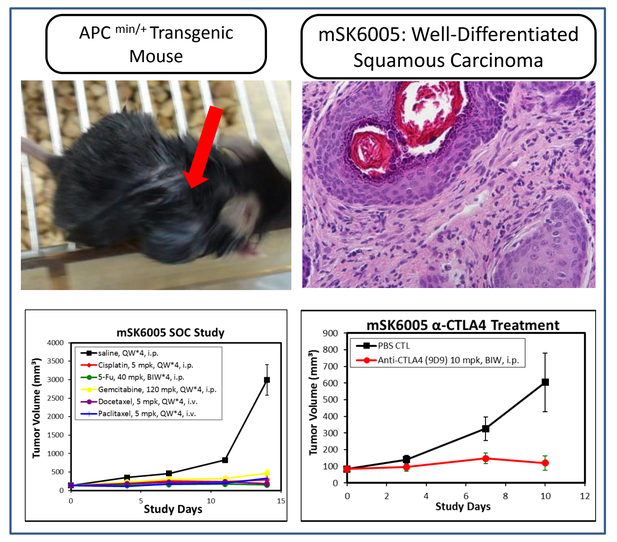


Figure 7: mSK6005 Skin Squamous Carcinoma MuPrime Model

Conclusions

We successfully established a MuPrime allograft models for immuno-oncology research using spontaneous murine tumors derived from a genetically engineered model.

- The mBR6004 model is derived from MMTV-PyVT transgenic mice with FVB/N background.
- The mBR6004 model responds to both anti-mPD1 and anti-mCTLA-4 antibodies
- These responses correlate with increased TIL.
- More MuPrime models are currently being built and validated (*e.g.* mSK6005).

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References

1. Guy, C.T., Cardiff, R.D. & Muller, W.J. Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Molecular and Cellular Biology 12, 954-961 (1992).