Establishment of Mouse Prostate Homograft Tumor Models for Efficacy Evaluation of Combinatory Immunotherapies

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INTRODUCTION

Despite the early approval of Sipuleucel-T for metastatic castration-resistant prostate cancer, which is often perceived as a milestone achievement in cancer immunotherapy, subsequent progress in prostate cancer immunotherapy development has been limited. This includes disappointing results with tumor vaccines, and prostate cancer resistance to immune checkpoint inhibitors, such as PD-1 and PD-L1. It is now generally accepted that we need to tackle prostate cancer by combinatorial approaches of chemo-, targeted- and immunotherapies. Highly relevant preclinical models are therefore in great demand for proof of principle efficacy evaluation.

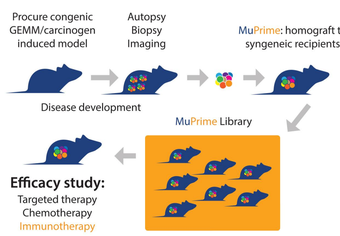
Genetically engineered mouse models (GEMM) recapitulate some aspects of human prostate cancer both histopathologically and molecularly. Among these, PTEN loss of function in prostate epithelium is one of the central events in human prostate cancer. PTEN haploinsufficiency in mice is sufficient to drive mouse prostatic intraepithelial neoplasia (PIN) formation, while loss of both alleles of PTEN in mouse prostate leads to hyperplasia at 4 weeks, PIN at 6 weeks, and fully invasive adenocarcinoma from 12 weeks of age. PTEN null tumors are also resistant to androgen depletion. Concurrently, although KRAS mutations are not often seen in human prostate cancer, activation of the MAPK pathway often happens in advanced tumors. Mutant KRAS or BRAF can robustly promote mouse prostate cancer progression. *Pten null* and *Kras G12D; Pten null* mouse prostate cancer models have been well characterized by a number of labs.

However, parental GEMM models are difficult to use for pharmacological studies due to the spontaneous nature of tumor onset and progression. Compound mutant mice are also costly to breed. We have generated transplantable mouse prostate cancer homograft models (MuPrime™) by passaging the primary tumor subcutaneously in C57BL/6 mice. These mouse tumors featuring *Pbsn-Cre;LSL-Kras$^{G12D/+}$;Pten$^{flox/flox}$* or *Pbsn-Cre;Pten$^{flox/flox}$* retain morphological similarity to moderate to poorly differentiated human prostate cancer. Growth of these mouse prostate tumors is resistant to androgen depletion, but sensitive to treatment with an mTOR inhibitor. They are also moderately responsive to immune checkpoint antibodies, i.e. PD-1 and CTLA-4.

This model, as well as other MuPrime models, serve as valuable tools for evaluating combinatory therapies with chemotherapies, immunomodulators, and immune checkpoint antibodies, etc. before moving to clinical testing.

METHODS

Fig 1. The MuPrime concept and model examples. Mouse tumor homografts offer a murine version of PDX models in immunocompetent hosts, an effective I/O platform



Parental Model	Cancer Type	MuPrime Model
MMTV-PyMT	Breast	mBR6004
APC$^{min/+}$	Skin SCC	mSK6005
Eμ-Myc	B lymphoma	mLY6043
Kras$^{(G12D/+)}$;Trp53$^{-/-}$	Lung	mLU6045
Pdx1-Cre; Kras$^{(G12D/+)}$; Trp53$^{-/-}$ (KPC)	PDAC	mPA6115
Pbsn-Cre; Kras$^{(G12D/+)}$; Pten$^{F/F}$	Prostate	mPR6135 mPR6189 mPR6190
Kras$^{(G12D/+)}$; Pten$^{F/F}$	Lung	mLU6054
Kras$^{(G12D/+)}$; Pten$^{F/F}$	Bladder	mBL6078
Pten$^{F/F}$; Trp53$^{-/-}$	Lung	mLU6073
Trp53$^{-/-}$	Sarcoma	mSA9003
Urethane induced	Lung	mLU6050
AML-ETO9a	AML	mAM9006

RESULTS

Fig 2. Tissue microarray of MuPrime models for target screening

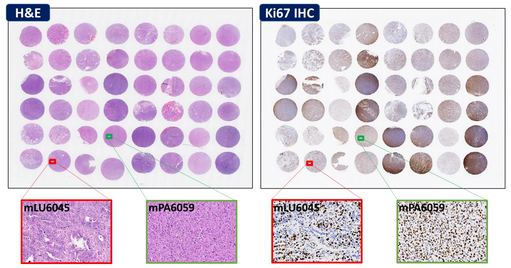


Fig 3. Mutation load of MuPrime tumors is significantly lower than syngeneic cell lines

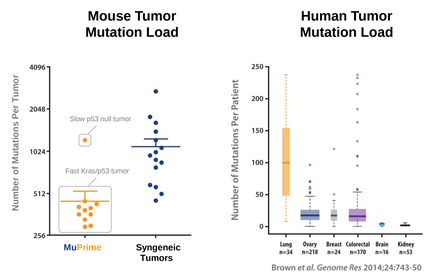


Fig 4. Histopathology and IHC characterization of MuPrime prostate models

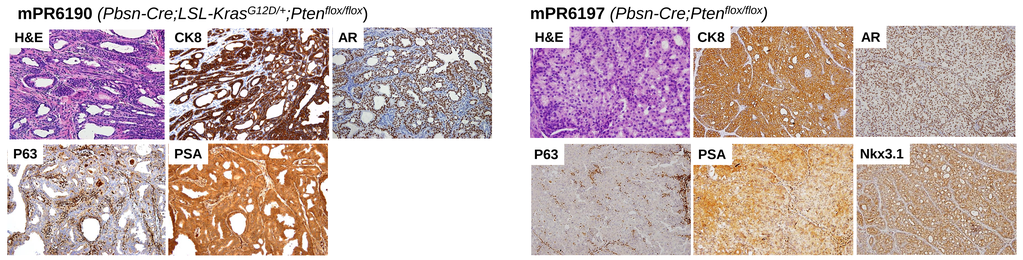


Fig 5. Immunoprofiling of MuPrime prostate models
Subcutaneously grafted tumor: mPR6135 *(Pbsn-Cre;LSL-Kras$^{G12D/+}$;Pten$^{flox/flox}$)*

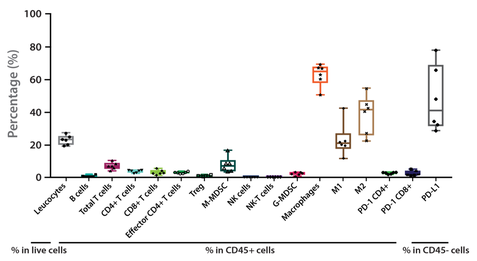


Fig 6. *Pbsn-Cre;LSL-Kras$^{G12D/+}$;Pten$^{flox/flox}$* MuPrime prostate models are resistant to hormonal treatments

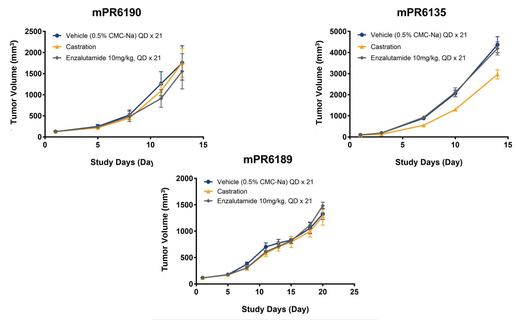


Fig 7. *Pbsn-Cre;LSL-Kras$^{G12D/+}$;Pten$^{flox/flox}$* MuPrime prostate model response to chemotherapy and targeted agents

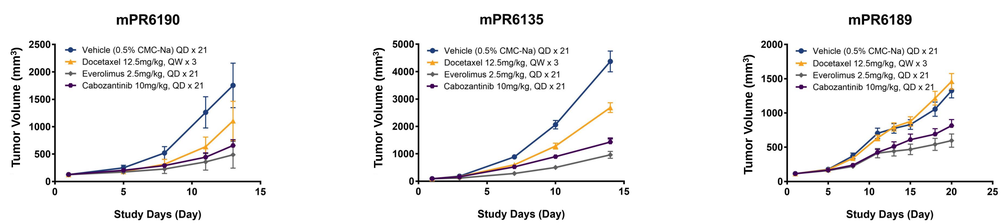
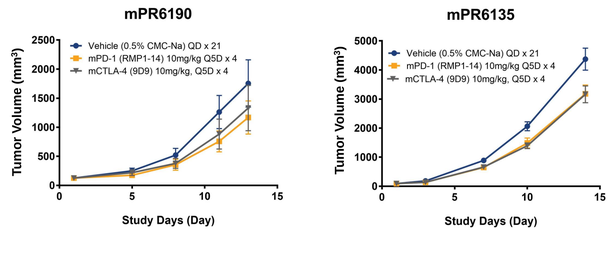
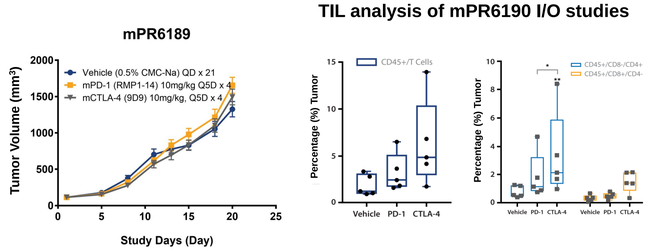


Fig 8. *Pbsn-Cre;LSL-Kras$^{G12D/+}$;Pten$^{flox/flox}$* MuPrime prostate models respond poorly to I/O treatments



TIL analysis of mPR6190 I/O studies



SUMMARY

- We have established and characterized a series of MuPrime homograft tumor models, carrying various mutations highly relevant to human cancer, representing a highly valuable model platform for cancer immunotherapy
- Human prostate cancer, due to its hostile tumor microenvironment, responds poorly to immuno-oncology treatments
- We have developed MuPrime prostate models based on two types of mutations, *Pbsn-Cre;LSL-Kras$^{G12D/+}$;Pten$^{flox/flox}$* or *Pbsn-Cre;Pten$^{flox/flox}$*. These models recapitulate human PCa histopathology and disease biomarker expression
- MuPrime tumor homograft prostate models show high levels of M2 macrophage infiltration in the tumors
- MuPrime tumor homograft prostate models are resistant to hormonal therapies, modestly responsive to SoC agent docetaxel, but generally non-responsive to I/O treatments. They serve as good tool models for testing combinatory therapies

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