A Novel Mouse Skin Squamous Cell Carcinoma Allograft Model For *In Vivo* Pharmacological Analysis Of Immunotherapy

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ABSTRACT

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 around 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 restricted number of available models responsive to current checkpoint inhibitors. Genetically engineered mouse models (GEMMs) of human cancers are effective tools for mechanistic analysis, but they are often not suitable for efficacy studies due to usually unsynchronized tumor progression. Syngeneic allografts of spontaneous mouse tumors derived from GEMMs (MuPrime®) may be used as a new type of immuno-oncology model with the following advantages: 1) their primary nature of "stem cell disease" and relevant tumor microenvironment mirror patient-derived xenograft (PDX) models; 2) the availability of diverse cancer types and oncogenic drivers deriving from a wide range of GEMMs. We set out to build a library of allografts of spontaneous mouse tumors, including those derived from GEMM, in immunocompetent hosts to support pharmacological investigation, particularly of immuno-oncology agents[1].

It is well documented that heterozygous $Apc^{Min/+}$ mice are highly susceptible to developing intestinal adenoma[2,3]. Female $Apc^{Min/+}$ mice also occasionally develop mammary squamous cell carcinomas[4]. Recently, we observed a spontaneous cutaneous tumor on the neck of a C57BL/6J $Apc^{Min/+}$ mouse. Histopathology suggests the tumor is a well-differentiated skin squamous cell carcinoma. We transplanted the tumor subcutaneously into syngeneic C57BL/6J mice. The allografts grow robustly, they are serially transplantable, and maintain the primary tumor histopathology. Transcriptome sequencing revealed that allografts maintain the original Apc^{Min} mutation, express high levels of ErbB2, and present frameshift mutations in both c-Met and EGFR. *In vivo* pharmacological assessment indicates that the allograft tumors respond to 5-FU, paclitaxel, gemcitabine, docetaxel, and cisplatin chemotherapies. They are also sensitive to an anti-mouse CTLA-4 antibody; this response is associated with an increased number of tumor infiltrating lymphocytes, including T cells. Interesting, although the tumor cells express PD-L1, they are not responsive to PD-1 treatment.

In summary, we show that we have established an allograft model suitable for *in vivo* efficacy analysis of immunotherapy using surrogate anti-mouse antibodies.

References

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3. Su, L.K., et al. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science 256, 668-670 (1992).
4. Moser, A.R., Hegge, L.F., Cardiff, R.D. Genetic background affects susceptibility to mammary hyperplasia and carcinomas in $Apc^{Min/+}$ Mice. Cancer Research 61, 3480–3485 (2001).

RESULTS

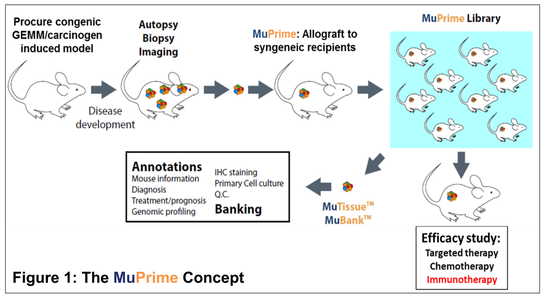


Figure 1: The MuPrime Concept

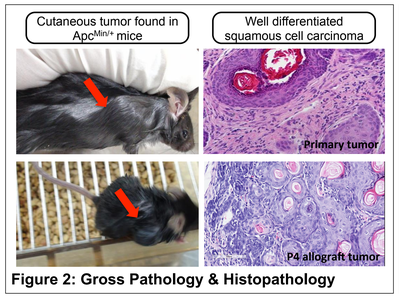


Figure 2: Gross Pathology & Histopathology

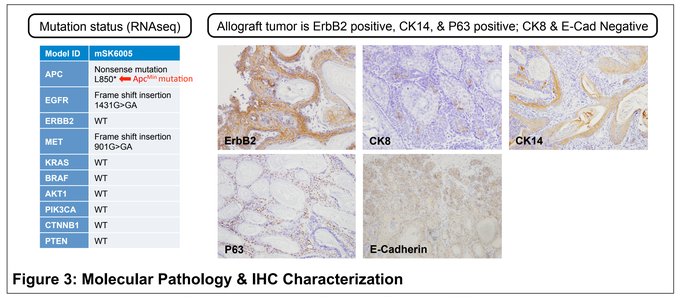


Figure 3: Molecular Pathology & IHC Characterization

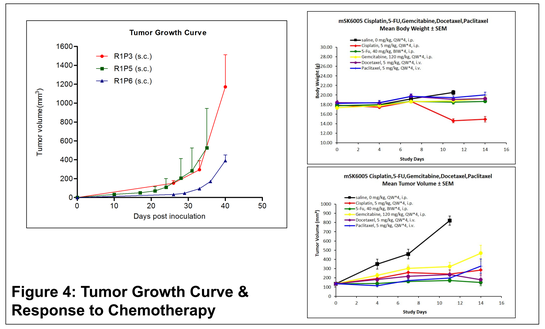
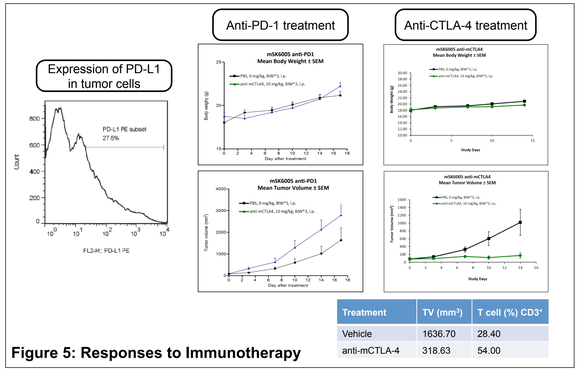


Figure 4: Tumor Growth Curve & Response to Chemotherapy



Treatment	TV (mm³)	T cell (%) CD3⁺
Vehicle	1636.70	28.40
anti-mCTLA-4	318.63	54.00

Figure 5: Responses to Immunotherapy

Table 1: MuPrime Pipeline. Parental GEMMs available for some models

MuPrime ID	Cancer Type	Mutations/Carcinogen	Strain Background	Tumor Progression	MuPrime Setup	MuPrime Validation	Efficacy Ready
Traditional GEMM-Derived MuPrime							
mPR6003	Prostate	TRAMP (Pbsn-SV40T TG	C57BL/6			Ongoing	Q3, 2016
mBR6004	Breast	MMTV-PyVT TG	FVB/N				Ready
mSK6005	Skin squamous cell carcinoma	$Apc^{Min/+}$	C57BL/6				Ready
mLY6043	B cell lymphoma	IgH-Myc TG (Eμ Myc)	C57BL/6				Ready
mSA9003	Sarcoma	$P53^{-/-}$	C57BL/6				Ready
mLY6041	B cell lymphoma	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
mLUD42 mLUD44 mLUD45	Lung	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
mCR6046	Colon	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
mLI6047	Liver	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
	Pancreatic	KRAS (G12D); $P53^{-/-}$	C57BL/6		Ongoing		Q2, 2016
	Prostate	KRAS (G12D); $Pten^{Flox/Flox}$	C57BL/6	Ongoing			Q4, 2016
	Ovarian	KRAS (G12D); $Pten^{Flox/Flox}$	C57BL/6	Ongoing			Q3, 2016
	Bladder	KRAS (G12D); $Pten^{Flox/Flox}$	C57BL/6	Ongoing			Q3, 2016
	Lung	KRAS (G12D); $Pten^{Flox/Flox}$	C57BL/6		Ongoing		Q2, 2016
	Glioblastoma	KRAS s (G12D); $Pten^{Flox/Flox}$	C57BL/6	Ongoing			Q3, 2016
	Bladder	$PTEN^{Flox/Flox}$; $P53^{-/-}$	C57BL/6	Ongoing			Q2, 2016
	Prostate	$PTEN^{Flox/Flox}$; $P53^{-/-}$	C57BL/6	Ongoing			Q4, 2016
	Pancreatic	KRAS (G12D); $P16^{-/-}$	C57BL/6	Ongoing			Q3, 2016
	Lung	KRAS (G12D); $P16^{-/-}$	C57BL/6	Ongoing			Q3, 2016
MuPrime from Spontaneous/Carcinogen-Induced Mouse Tumors or Pseudo-GEMM							
mNE6014	Neuroendocrine tumor	N/A	NSG (NOD)				Ready
m6032	Cutaneous tumor	N/A	BALB/c nude (BALB/c)				Ready
mLE6026	Leukemia	N/A	NSG (NOD)			Ongoing	
	NASH-HCC	Streptozotocin and high-fat diet	C57BL/6	Ongoing			Q2, 2016
	HCC	Diethylnitrosamine and phenobarbital	C3H/He	Ongoing			Q4, 2016
	Lung	Urethane	BALB/c; C3H/He; A/J			Ongoing	Q2, 2016
	AML	NRAS (G12D); AML1-ETO*	C57BL/6			Ongoing	Q2, 2016
	AML	NRAS (G12D); MLL-ENL*	C57BL/6			Ongoing	Q2, 2016
MuPrime Derived from Non-Germline GEMM using CRISPR-Cas9 *in Vivo* Gene Editing							
	Bladder	P53 cKO; PTEN cKO	C57BL/6	Ongoing			
	Breast	P53 cKO; BRCA1 cKO; MMTV-Cre	C57BL/6	Ongoing			
	Breast	P53 cKO; SMAD4 cKO; CDH1 cKO; MMTV-Cre	C57BL/6	Ongoing			
	Gastric	P53 cKO; SMAD4 cKO; CDH1 cKO	C57BL/6	Ongoing			
	Lung	KRAS (G12D); P53 cKO; LKB1 cKO	C57BL/6	Ongoing			
	Pancreatic	KRAS (G12D); P53 (R172H); Pdx1-Cre	C57BL/6	Ongoing			Q3, 2016
	Pancreatic	KRAS (G12D); SMAD5 cKO; Pdx1-Cre	C57BL/6	Ongoing			
	Prostate	BRAF (V600E); PTEN cKO; Pbsn-Cre	C57BL/6	Ongoing			Q4, 2016
	Prostate	PTEN cKO; Smad4 cKO; Pbsn-Cre	C57BL/6	Ongoing			Q4, 2016
	Ovarian	KRAS (G12D); PTEN cKO	C57BL/6	Ongoing			
	Medullablastoma	PTCH1 cKO; P53 cKO	C57BL/6	Ongoing			Q3, 2016

SUMMARY

- ❑ **We observed a skin squamous carcinoma which developed in a C57BL/6 $Apc^{Min/+}$ mouse.**
- ❑ **We have established & characterized the mSK6005 MuPrime allograft model, and utilized this model for *in vivo* pharmacological studies.**
- ❑ **mSK6005 responds well to I/O therapeutics, i.e. anti-mCTLA4 antibody, but not anti-mPD1 antibody.**
- ❑ **The response is correlated with increased tumor infiltrating T cells.**
- ❑ **To date, 12 MuPrime models have been validated for immuno-oncology efficacy studies.**

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