Establishment of a Variety of Primary Mouse Tumor Allografts of Defined Disease Pathways for Evaluating Immunotherapy

Davy Xuesong Ouyang[1*], Li Chen[1], Gavin Jiagui Qu[1], Zhensheng Wang[1], Annie Xiaoyu An[1,2], Likun Zhang[1], Jie Cai[1], and Henry Q.X. Li[1,2]

[1]Crown Bioscience, Inc., 3375 Scott Blvd, Suite 108, Santa Clara, CA 95054, [2]State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

*Presenting author

INTRODUCTION

The lack of relevant animal models is a major bottleneck for developing novel immunotherapies. Syngeneic mouse tumor models, where mouse cancer cell lines were engrafted in mice from the same strain, are the current workhorse. However, these models suffer from several limitations: 1) only a limited number of models are available and responsive to current checkpoint inhibitors; thus for each given strain of mouse, there is little choice of cells/disease types; 2) models don't mimic patient tumors since they are derived from *in vitro* immortalized cell lines[1]; 3) they are not reflective of patient disease pathways thus unfit for common targeted agents, in mono- or combination therapy. We have established allografts of spontaneous mouse tumors (MuPrime™) derived from genetically engineered mouse models (GEMMs) as a new type of immuno-oncology model[2] with the following advantages: 1) MuPrime conserve the primary nature of "stem cell diseases" and relevant tumor microenvironment as seen in patients and patient-derived xenografts (PDX)[1]; 2) MuPrime is a diverse collection of cancer types/strains of mice; 3) MuPrime models carry engineered oncogenic drivers which recapitulate the human diseases, e.g. APC^{Min} mutation, KRAS mutation, P53 loss-of-function, etc.[3,4,5], deriving from a wide range of available GEMMs, suitable for targeted agents. Thus far, we have built a small library of allografts and are testing them to facilitate pharmacological investigation, particularly for immuno-oncology agents.

KRAS G12D mutation and P53 loss-of-function are the most common genetic abnormality found in a variety of cancers, e.g. lung, pancreas, colon, lymphoma, etc. We generated a conditional compound mutant GEMM. By introducing adeno-Cre to different organ sites of this GEMM, we created a series of mouse tumors, including lung, pancreas, colon, and lymphoma, with genetic lesions bearing $KRAS^{G12D/+}/P53^{-/-}$. MuPrime allografts were established by engraftment of these primary tumors to the C57BL/6 syngeneic host. These allograft tumors, while carrying the same mutations, display distinct histopathology: pancreatic tumors exhibit the features commonly found in human PDAC (e.g. glandular tumor structures and exuberant stroma); lung cancer features NSCLC-adenocarcinoma. They also display different tumor immune microenvironments. This set of allografts of different diseases, but with the same oncogenic driver, are ideal models to investigate sensitivity to immunotherapies, as well as to combination therapies targeting MAPK pathway and immune modulation, across a range of tumor types.

METHODS

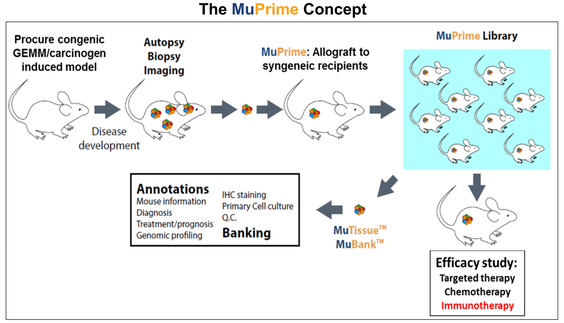
Mouse primary tumors derived from $KRAS^{G12D/+}/P53^{-/-}$ GEMMs were engrafted subcutaneously to syngeneic wild type C57BL/6 mice. Following serial transplantation, cryobanking, histopathology characterization, RNAseq, and immune profiling, well-annotated MuPrime allograft tumors with robust growth were enrolled in benchmark efficacy studies with targeted & immuno-oncology treatments. Treatment started when the mean tumor size reached around 100 mm^3, TGI (%) was used as the endpoint for efficacy evaluation, where TGI (%) =100 x (1-T/C), T and C are the mean tumor volume of the treated and control groups, respectively, on a given day.

References

1. Guo, S., Qian, W., Cai, J., Zhang, L., Wery, J. P., Li, H. Q. Molecular pathology of patient, patient derived xenografts and cancer cell line *Cancer Research*, 2016. Aug 15;76(16): 4619-4626.
2. Wang, Z., An, A. X., Liu, J., Qu, G. J., Zhang, L., Cai, J., Chen, B., Ouyang, D. X., Wery, J. P., Li, H., Q. Response to checkpoint inhibition by GEMM breast cancer allograft. *[abstract]. In: Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics;* 2015 Nov 5-9; Boston, MA. Philadelphia (PA): AACR; Molecular Cancer Therapeutics 2015;14(12 Suppl 2). Abstract nr B97.
3. Moser, A.R., Pitot, H.C., Dove, W.F. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* **247**, 322-324 (1990).
4. 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).
5. Qu., G. J., An, A. X., Liu, J., Ouyang, D. X., Zhang, L., Cai, J., Wery, J. P., Li, H., Q. A novel mouse skin squamous cell carcinoma allograft model for *in vivo* pharmacological analysis of immunotherapy. *[abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research;* 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA): AACR; 2016. Abstract nr 4043

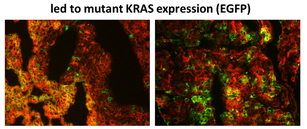
RESULTS

The MuPrime Concept



Benchmark Efficacy Evaluation with Targeted Agents & I/O Treatment

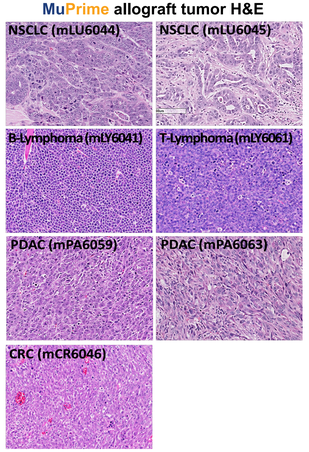
Adeno-Cre local delivery to KRAS LSL-K12D/P53$^{-/-}$ mice led to mutant KRAS expression (EGFP)



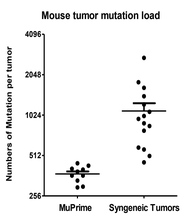
Lung: Intranasal delivery

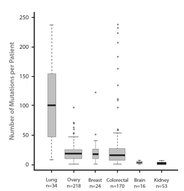
Pancreas: retrograde intra common bile ductal (ICBD) injection

MuPrime allograft tumor H&E



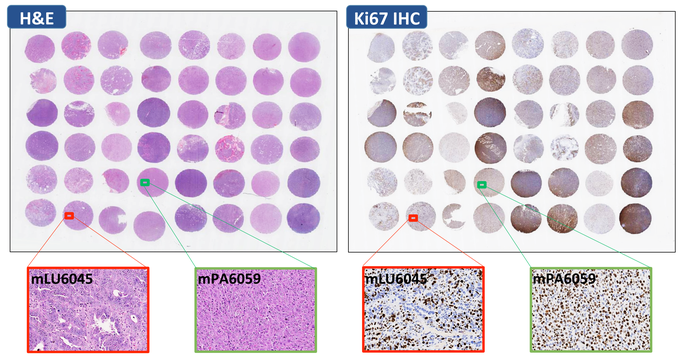
Mutation Load in Mouse & Human Tumors





Adapted from:
Brown SD et al., 2014. *Genome Research* 24:743-750

MuPrime Tissue Microarray



MuPrime Tumor Immune Profiling (percentage)

Model ID	CD45+	CD3+ T	CD4+ T	CD8+ T	MDSC	Macrophage	DC	B	NK	PD-L1
mLY6041	*55.48*	*2.6*	*0.41*	*0.75*	*0.42*	*0.96*	*8.11*	*46.95*	*0.29*	*12.23*
mLY6061	*81.6*	*64.88*	*65.29*	*61.26*	*0.47*	*0.34*	*4.3*	*4.26*	*0.4*	*6.09*
mLU6044	*14.12*	*1.31*	*0.39*	*0.46*	*11.59*	*0.87*	*4*	*0.18*	*0.31*	*17.4*
mPA6059	*8.83*	*0.23*	*0.09*	*0.16*	*3.64*	*3.23*	*0.17*	*0.09*	*0.03*	*17.06*
mCR6046	*14.28*	*0.02*	*0*	*0*	*5.93*	*8.57*	*0.21*	*0.18*	*0.15*	*10.4*

SUMMARY

- We have established & characterized a series of KRAS mutated, P53 loss-of-function MuPrime allograft tumor models, including lung, pancreas, colon, & lymphoma.
- Although carrying same driver mutations & having similar overall mutation load, these allograft tumors display distinct histopathological features & immune microenvironment.
- These KRAS-driven allograft tumors showed different responses to the MEK inhibitor trametinib, i.e., lung & colon cancers are more sensitive than pancreatic cancers; while both B- & T-lymphomas were non-responders.
- KRAS-driven tumors, expressing low- to moderate levels of PD-L1, are in general not responsive to PD-1 or CTLA-4 treatment. Combination of I/O with targeted treatment does not show beneficial effects either.