MuScreen™: *In Vivo* Screening with a Panel of Well-Characterized Syngeneic Models

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INTRODUCTION

Background: *In vitro* screening is frequently used in oncology to quickly identify responsive cells, models, and pharmacodynamic (PD) effects. However it cannot be applied to immunotherapy because of the lack of a complex host immune system. Alternatively, *in vivo* screening with a panel of immuno-competent models is required to investigate stimulation of the immune system, but it is both resource intensive and cost prohibitive. Syngeneic tumor models are robust model systems for cancer immunotherapy and are amenable to immuno-profiling of both monotherapy and combination studies. Here we describe the use of a panel of syngeneic models in a large scale, staggered screen (MuScreen), which allows cost-effective, *in vivo* drug discovery profiling.

Material and methods: Leveraging our in-house detailed profiling data from our syngeneic models, which include efficacy benchmarking with aPD-1, aPD-L1, and aCTLA-4 antibodies, RNAseq data on tumor samples, and FACS analysis on both baseline and treated tumor samples, CrownBio recently launched a new service platform: MuScreen. MuScreen consists of a 3 month screening run on either 12 or 20 well-characterized syngeneic models. Both PD and efficacy can be evaluated in the same run, which provides results from large data sets facilitating go or no-go decisions. Test agents from multiple clients are pooled together in each run (sharing vehicle and other common groups) providing a significant reduction in the number of animals used and in the associated costs.

Results: CrownBio has established the largest collection of syngeneic models with well-characterized immunotherapy data. In the first MuScreen run, we have generated new data on common immuno-oncology (IO) agents, e.g. aPD-1 antibodies, including FACS analysis, IHC, and efficacy data (shown here).

Conclusions: MuScreen is the first *in vivo* screening tool to profile cancer immunotherapeutics across adaptive and innate immune cell lineages. It provides detailed response data from a panel of syngeneic models, enabling decision making in a cost-effective and timely manner.

METHODS

Syngeneic model establishment and treatment: A predetermined number of cells suspended in 0.1ml PBS were inoculated within the right flank of immunocompetent mice (C57BL/6, BALB/c, etc.). Each experimental group contained 6-10 staged tumor bearing mice.

Endpoints:

1. TGI(%): TGI(%)=100x(1-T/C); represented as the median TGI of multiple historical studies.
2. Untreated tumors at 250-350mm^3 were collected for RNAseq analysis; RNAseq data is available via MuBase® at **mubase.crownbio.com.**
3. Efficacy/PD studies testing checkpoint inhibitors:
   - Hepa 1-6 murine liver cancer model (efficacy study): treatment initiated ~ 100mm^3.
   - H22 murine liver cancer model (efficacy study): treatment initiated ~ 100mm^3; tumors were collected on Day 18 (3 days post the 4th dose of aPD-1 antibody).
   - EMT-6 murine breast cancer model (PD study): treatment started at around 280mm^3; tumors were collected on Day 18 (7 days post the 2nd dose of aPD-1 antibody).

RESULTS

Figure 1. Efficacy Evaluation of Immune Checkpoint Inhibitors in the Treatment of Murine Syngeneic Models.

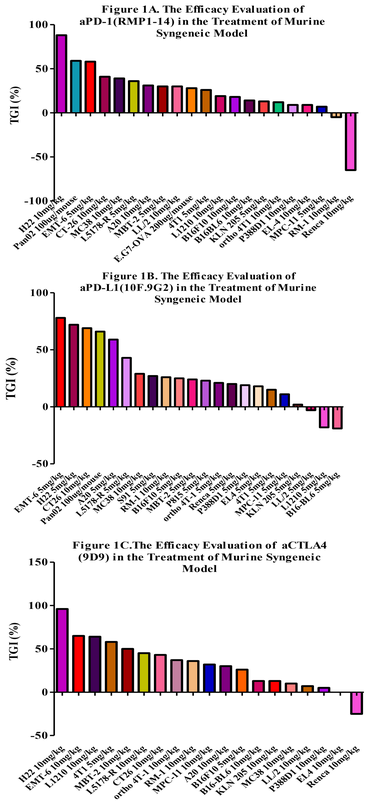


Table 1. Validated Immuno-Oncology Markers (FACS/IHC).

Immune cells		Human	Mouse
B cell		CD19 or CD20	CD45R/B220
T Cell	Total T Cell	**CD3**	**CD3**
	Helper T Cell	**CD4**	**CD4**
	Cytotoxic T Cell	**CD8**	**CD8**, CD69
	T_{reg}	CD25, **FOXP3**	CD25, **FOXP3**
Dendritic Cell		CD11c, CD123	CD11c, CD123
NK Cell		**CD56**	**CD335**, CD69
Macrophage		CD14, CD33, **CD68**	CD11b, F4/80, CD206, MHCII
Monocyte		CD14, CD33	CD11b
Neutrophil			**Ly-6G/C**
MDSC		CD11b, CD33	**CD11b**, **Ly-6G/C**, Gr-1
Checkpoint		**PD-L1**, **PD-1**	**PD-L1**, PD-1

Note: validated by FACS and IHC

Table 2. Syngeneic Models with RNAseq Data (19 models)

Cancer Type	Model
Bladder	MBT-2
Breast	4T1 (ortho), EMT-6 (s.c.)
Colon	CT-26, MC38
Kidney	Renca
Leukemia	L1210
Liver	H22
Lung	KLN205, LL/2
Lymphoma	A20, EL4, L5178-R, P338D1
Melanoma	B16-BL6 ,B16-F10
Myeloma	MPC-11
Pancreatic	Pan02
Prostate	RM-1

Figure 2. Newly Validated Murine Syngeneic Models

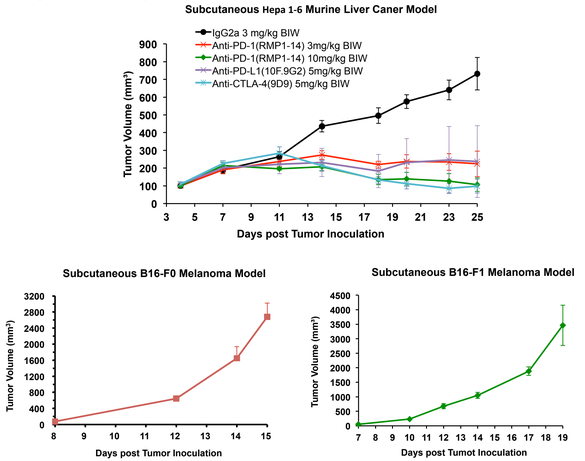
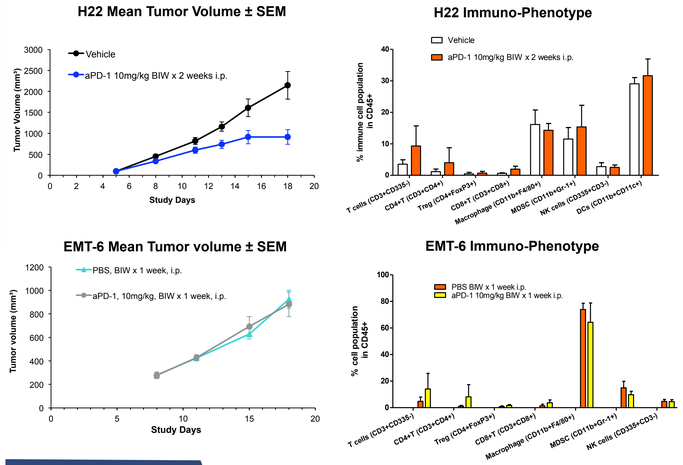


Figure 3. Efficacy/PD Evaluation of aPD-1 Antibody in Subcutaneous H22 and EMT-6 Murine Syngeneic Models and Immunophenotyping by FACS (data generated in the first MuScreen campaign).



SUMMARY

- **MuScreen** is a cost-effective and time efficient *in vivo* screening platform using a panel of syngeneic models to evaluate efficacy and explore potential mechanisms of action by immunotherapeutics.
- Hepa 1-6 is sensitive to the three common IO checkpoint targets and represents an ideal liver cancer model to test novel IO monotherapies.
- Immuno-phenotyping in a large *in vivo* PD screen (**MuScreen**) enables confirmation of immune cell lineage modulation, selection of appropriate syngeneic models for follow-up studies, and greater understanding of mechanisms of immunosuppression.