RNAseq and FACS Profiling of Syngeneic Mouse Models Treated with Immune Checkpoint Inhibitors Enables Biomarker Discovery and Model Selection for Cancer Immunotherapy

Abstract No. 5177

Lan Zhang, Juan Zhang, Sheng Guo, Wubin Qian, Zhensheng Wang, Zhongliang Li, Meng Qiao, Qian Shi*. *Crown Bioscience, Inc., Taicang, Jiangsu Province, China*

*:corresponding author Dr. Qian Shi, shiqian@crownbio.com

INTRODUCTION

Background: Syngeneic tumor models have long been used in cancer research. Recently, the clinical success of anti-CTLA-4 and anti-PD-1 antibodies has resulted in increased interest in the use of syngeneic models to evaluate cancer immunotherapeutics. Furthermore, as researchers discovered that chemo, radio, and targeted therapies may interact or change the tumor immune environment, they are looking for suitable models to evaluate combinations of these agents with immunotherapy. More importantly, it is still unknown why some patients respond to certain immunotherapies while others do not. We set out to utilize syngeneic models to address these open questions.

Results: CrownBio has established the largest collection of syngeneic models with well characterized immunotherapy data. Our models display a variety of responses to anti-PD-1, PD-L1, and CTLA-4 antibodies, ranging from close to 100% tumor growth inhibition to promoting tumor growth upon treatment. Most recently, we have generated detailed maps of the expression and mutational profiles of our models. Mutational analysis indicated that a number of syngeneic models harbor mutations (available via mubase.crownbio.com), which may be exploited in combination studies with targeted agents and immunotherapy. Chemo in combination with immunotherapy and immunotherapy/immunotherapy combination regimens tested in these models suggested potential strategies that may be successful in the clinic. Analysis of the RNAseq data indicated markers that may be useful to predict immunotherapy response.

Conclusions: These data will enable selection of models for chemo or targeted therapies in combination with immunotherapy. In addition, predictive biomarkers obtained from the analysis may be useful in understanding patient response in the clinic.

METHODS

Syngeneic model establishment and treatment: A predetermined number of cells suspended in 0.1 ml PBS were inoculated within the right flank of immunocompetent mice (C57BL/6, BALB/c, etc.). Treatment started when the mean tumor size reached 50-120 mm^3. Each experimental group contained 6-10 tumor bearing mice.

Endpoints:

1. TGI (%): TGI (%) =100 x (1-T/C); data represented as the median TGI of a number of historical studies;
2. PD-L1/PD-L2 expression was examined by FACS using the murine tumor cell lines;
3. Immunophenotyping of EMT-6 tumors by FACS: tumors were collected on Day 24 (3 days post the 5th dose);
4. Untreated tumors at 250-350 mm^3 were collected for RNAseq analysis;
5. Cytokine analysis: serum samples were collected at 3 days post the 5th dose in MC38, 3 days post the 3rd dose in B16-F10, and 2 days post the 3rd dose in A20 study and analyzed by luminex.
6. IHC/IF analysis was performed using the tumor samples (data not shown).

RESULTS

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

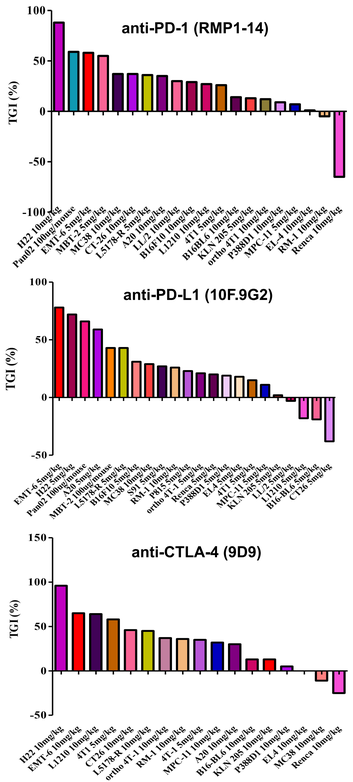


Figure 2. PD-L1/PD-L2 Expression.

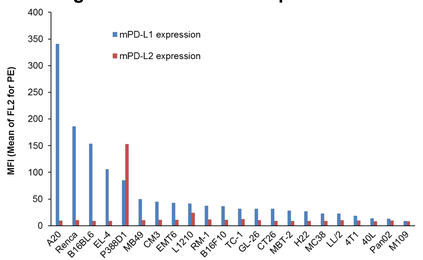


Figure 4. Combination Therapy in MC38 Model.

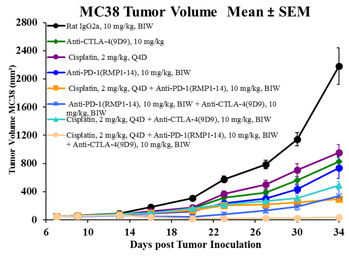


Table 1. Validated Immuno-oncology Markers (FACS/IHC/IF).

Immune cells		Human	Mouse
B cell		CD19, CD20	CD45R/B220
T Cell	Total T Cell	CD3	CD3
	Helper T Cell	CD4	CD4
	Cytotoxic T Cell	CD8	CD8
	T reg	CD25+FoxP3	CD25+FoxP3
Dendritic Cell		CD11c, CD123	CD11c, CD123
NK Cell		CD56	CD335
Macrophage/Monocyte		CD14, CD33, CD68	CD11b+F4/80
Neutrophil			Ly-G/C
MDSC		CD11b+CD33+	CD11b+Gr-1
Check-point		PD-L1	

Figure 3. EMT-6 Model Response to Immune Checkpoint Inhibitor Antibody Monotherapy (top); EMT-6 Model Immunophenotyping (bottom).

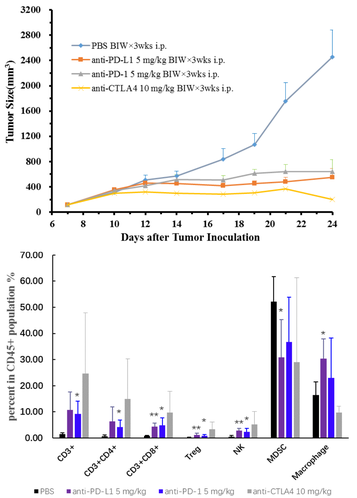


Figure 5. Prediction of Efficacy with Gene Expression Signatures.

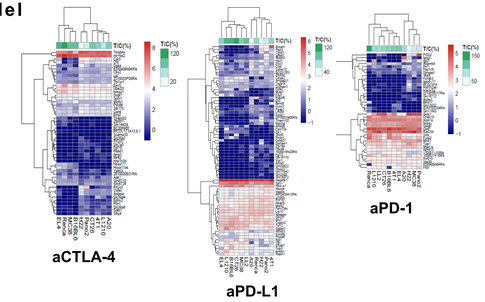
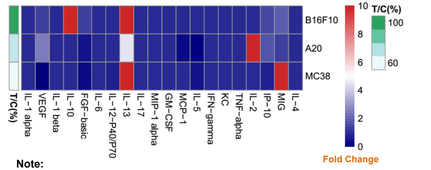


Figure 6. Cytokine Assays after Treatment



Note:
1. B16-F10: treated with 10 mg/kg anti-PD-L1; MC38: treated with 5 mg/kg anti-PD-1; A20: treated with 10 mg/kg anti-PD-1.
2. Fold change is represented as the ratio between the cytokine concentration in the treatment arm/cytokine concentration in the vehicle arm. Ratio=1 indicates no change; ratio=0 (min. value) indicates the cytokine is undetectable **after** treatment; ratio=10 (arbitrarily defined as the max. value) indicates the cytokine is undetectable **before** treatment.

SUMMARY

- FACS and cytokine analysis are useful tools in illustrating the involvement of various immune cell populations/cytokines in the antitumor effect of different types of therapeutics;
- Combination of anti-PD-1 with anti-CTLA-4 or with chemotherapy (cisplatin) demonstrated an additive/synergistic effect in the MC38 model, suggesting potential strategies that may be successful in the clinic;
- Gene profiling data will guide informative selection of models and rational design of combinatory immunotherapeutics;
- **MuScreen™**, an *in vivo* screening platform using a panel of syngeneic models is now available at CrownBio.