Orthotopic Syngeneic Tumor Models for Preclinical Evaluation of Cancer Immunotherapy Strategies

Abstract No.5176

Juan Zhang, Lan Zhang, Sheng Guo, 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: The recent clinical success of immunotherapy in indications such as melanoma and lung cancer has encouraged researchers to investigate the potential application of this treatment in many more solid and hematologic tumors. Syngeneic tumor models have been widely used to evaluate the efficacy of immunotherapy in several different cancer types. However, most of these experimental models were established as subcutaneous grafts, raising the concern that there may be significant differences in the composition of the immune microenvironment compared to an orthotopic setting. In addition, drug delivery and metabolism may change in orthotopic vs. subcutaneous tumors. To address the unmet need of finding the most translatable syngeneic models for immunotherapy, we set out to validate a panel of orthotopic syngeneic models for the evaluation of anticancer immunotherapeutics.

Results: A large collection of orthotopic syngeneic models covering breast and liver cancer, as well as various hematological malignancies, was established in immunocompetent mice. Orthotopic liver tumors were monitored by ultrasound imaging. Survival curves were taken and the response to anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies was evaluated in these models. The efficacy of these immunotherapeutics in reducing tumor burden was compared between the orthotopic model and its subcutaneous counterpart. The role of natural killer (NK) cells in the development of a panel of systemic hematological tumor models was investigated with NK depleting antibodies.

Conclusion: Despite operational challenges, orthotopic syngeneic models are model systems that more closely resemble the human disease and represent a valuable tool for the evaluation of cancer immunotherapies and their combination strategies.

METHODS

Animals and syngeneic models: Immunocompetent mice (C57BL/6, BALB/c, etc.) were used to generate syngeneic models. Each mouse was inoculated at right lower flank for subcutaneous (s.c.) model establishment, within the mammary fat pad, liver or via intravenous tail vein injection with a predetermined number of cells or tumor fragments.

Therapeutics: Treatment was initiated when the mean tumor size reached 80-120 mm^3 or 7 days post cell injection. Each group contained 6-10 tumor bearing mice.

Endpoints:

1. TGI (%): TGI (%) = 100 x (1-T/C)
2. Survival time: ILS (%) = 100 × [(Median Survival Time of drug treated group/Median Survival Time of vehicle group) – 1] (%)
3. Metastasis evaluation: Metastatic lesions in the intestine and lung were inspected and counted macroscopically.

RESULTS

Cancer Type	Cell lines	aPD-1	aPD-L1	aCTLA-4	RNAseq	TIL Profiling
Breast cancer	EMT6 (s.c.)	✓	✓	✓	ongoing	✓
	MBT2	✓	✓	ongoing		✓
	4T1 (orthotopic)	✓	✓	✓	✓	✓
	4T1 (s.c)	✓	✓	✓	ongoing	
	JC	ongoing				
Colon cancer	Colon 26	ongoing				
	CT-26	✓	✓	✓	✓	✓
	MC38	✓	✓	✓	✓	✓
Kidney cancer	Renca	✓	✓	✓	✓	✓
Fibrosarcoma	WHI-164	ongoing				
Leukemia	L1210	✓	✓	✓	✓	✓
	C1498	ongoing				
Liver cancer	H22	✓	✓	✓	✓	✓
Lung cancer	LL/2	✓	✓	ongoing	✓	✓
	KLN205	✓	✓	✓	✓	✓
Lymphoma	P388D1	✓	✓	✓	ongoing	✓
	A20	✓	✓	✓	✓	✓
	EL-4	✓	✓	✓	✓	✓
	L5178-R	✓	✓	✓	ongoing	✓
	E.G7_ova	ongoing				
Neuroblastoma	Neuro-2a	ongoing				
Mastocytoma	P815	ongoing	✓	ongoing		
Melanoma	S91	ongoing	✓	ongoing	ongoing	ongoing
	B16BL6 subQ	✓	✓	✓	✓	✓
	B16F10	✓	✓	✓	✓	✓
Myeloma	MPC-11	✓	✓	✓	ongoing	✓
Pancreatic cancer	Pan02	✓	✓	✓	✓	
Prostate cancer	RM-1	✓	✓	✓	ongoing	✓
Plasmacytoma	J558	ongoing				

Cancer Type	Cell lines	aPD-1	aPD-L1	aCTLA-4	RNAseq	TIL Profiling
Breast cancer	EMT-6 (orthotopic)	ongoing				
	4T1 (orthotopic)	✓	✓	✓	✓	ongoing
Liver cancer	H22 (orthotopic)	ongoing				
Multiple Myeloma	MPC-11 (systemic)	✓	✓	✓	NA	NA
Lymphoma	A20 (systemic)	✓	✓	✓	NA	NA
	EL4 (systemic)	✓	✓	✓	NA	NA
	L5178-R (systemic)	✓	✓	✓	NA	NA
	P388D1 (systemic)	✓	✓	✓	NA	NA
	E.G7-OVA (systemic)	✓	✓	✓	NA	NA
Leukemia	L1210 (systemic)	✓	✓	✓	NA	NA
	C1498 (systemic)	✓	✓	✓	NA	NA

Table 1 (left): CrownBio Validated Subcutaneous Syngeneic Models

Table 2 (above): Validated Orthotopic/Systemic Syngeneic Models

Syngeneic models under development: ATDC5, 2PK-3, B16-F0, B16-F1, BaF3, BCL1 clone 5B1b, C127I, CMT-93, GL261 Red-Fluc, MFC, N1E-115, NFS-60, P3X63-Ag8, RAG, RAW 264.7, WEHI-3, and EHS.

Figure 1: 4T1 Breast Cancer Model Response to Anti-CTLA-4

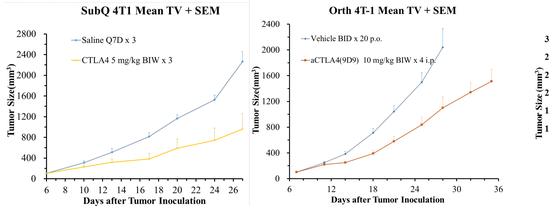


Figure 2: MPC-11 Plasmacytoma Model Response to Anti-PD-1, Anti-PD-L1 and Anti-CTLA-4

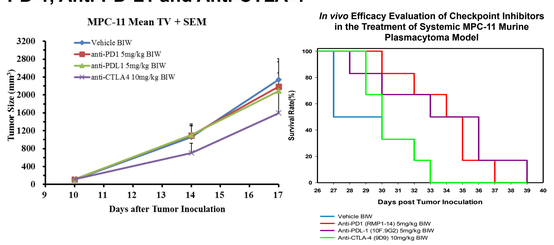


Table 3: Metastasis Evaluation in the Subcutaneous and Orthotopic 4T1 Model

Site	Treatment	Incidence of intestine matastasis	No. of metastatic lesions/intestine	Incidence of lung matastasis	No. of metastatic lesions/lung
SubQ 4T1	No treatment At day 25	8/10	4±1	3/10	1±0
Orth 4T1	Vehicle Control At day 28	9/10	4±1	1/10	0±0
Orth 4T1	aCTLA4 (9D9) (10 mg/kg) At day 35	9/9	4±0	5/9	2±1

Table 4: Comparison of Antibody Efficacy

Treatment	SubQ		Systemic	
	TGI %	p Value	ILS%	p Value
Anti-PD-1	7	0.835	26.9	0.012
Anti-PD-L1	11	0.691	23.1	0.040
Anti-CTLA4	32	0.339	11.5	0.685

Figure 3: The Impact of NK Depletion on the Survival of Disseminated Hematologic Syngeneic Models

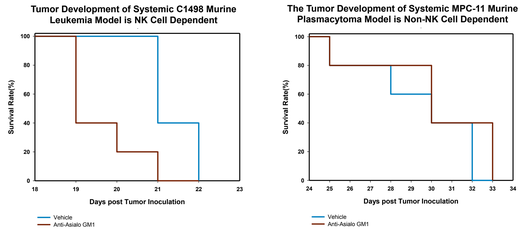
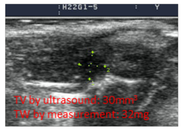
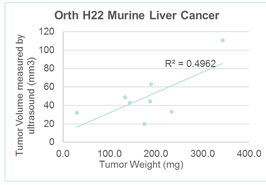


Figure 4: Ultrasound Imaging from the Orthotopic H22 Model to Evaluate Tumor Burden





SUMMARY

- The antitumor activity of anti-CTLA-4 is comparable in the s.c. and orthotopic settings for the 4T1 syngeneic model; both s.c and orthotopic implantation developed intestine and lung metastases;
- Anti-PD-1 and anti-PD-L1 have superior efficacy in the systemic MPC-11 model than in the s.c. MPC-11 model;
- Among the nine disseminated hematologic syngeneic models, C1498 is identified as a NK dependent tumor;
- Orthotopic H22 syngeneic liver cancer model growth was evaluated using ultrasound imaging, which may become helpful in facilitating randomized grouping and monitoring tumor growth and efficacy.