Evaluate in Vivo Efficacy of Anti-Tumor Immuno-Therapeutics Using Mixeno™ Mouse Models

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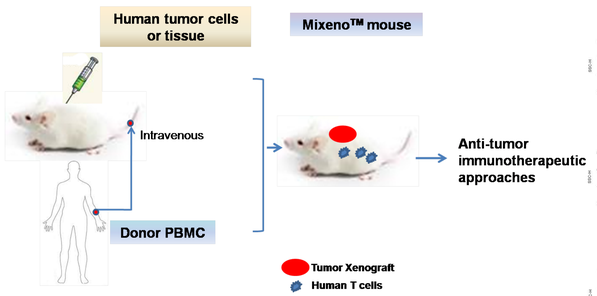
CROWNBIO TRANSLATING BIOLOGY INTO MEDICINE

#2562

Background

The past few years have witnessed a renaissance in the field of cancer immunotherapy, relating largely to the clinical advances associated with the development of immunomodulatory agents, e.g. monoclonal antibodies targeting the immune inhibitory pathways (CTLA-4 and PD-1/PD-L1). Often, the preclinical efficacy assessments are based on the evaluation of surrogate anti-mouse target antibodies using mouse syngenic tumor models, because of the need for T cell activation in an immune competent system. However, this strategy is limited due to the fact that the immune systems in human and mouse are different, and a surrogate molecule needs to be tested in those syngenic models. Here we set out to validate mouse models that harbor human immune cells by pre-engrafting the immuno-deficient mice with human PBMC (the Mixeno™ model), and use them for efficacy evaluation of the humanized anti-PD-1 antibody. PD-L1 high-expression human tumor cell lines are selected for these in vivo models. Based on the preliminary result, the Mixeno™ models are hopefully becoming useful tools in immunotherapeutic antibody development, and may greatly increase the clinical translatability of animal studies.

What is Mixeno™?



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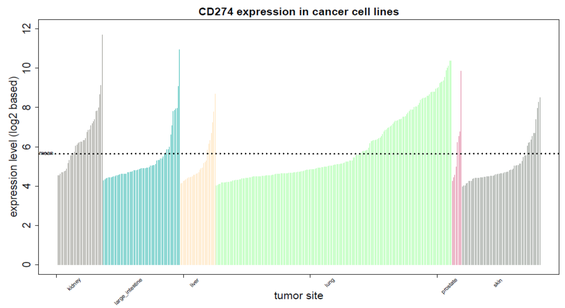
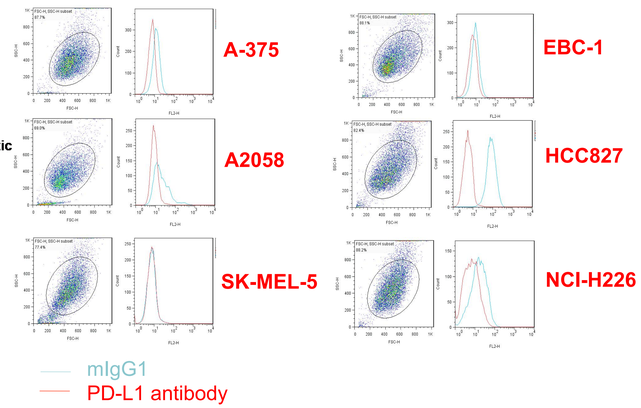
Selecting High PD-L1 Expressing Cell Lines

a) Explore PD-L1(CD274) mRNA Expressing Level by XenoBase™



- **XenoBase®:** http://xenobase.crownbio.com/xenobase/login.aspx
  A free web-based tool developed by CrownBio, combining the publically available profiling data of more than 1000 cell lines, with our proprietary in vivo pharmacology data;
- To select the cell lines for in vivo efficacy evaluation of anti-PD-1 antibody, we screened the cell lines based on PD-L1 expressing level;
- a) 381 cell line originated from skin, lung, kidney, large intestine, liver and prostate are screened for PD-L1 expression, because of the encouraging outcomes by anti-PD-1 antibody clinical trials in these cancer types;
- b) FACS analysis was performed to further determine the surface PD-L1 expression level of 6 cell lines (3 melanoma and 3 NSCLC).

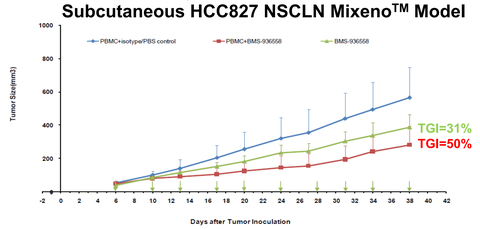
b) FACS Analysis on surface PD-L1 Expression



Cancer Type	Cell Line	CD274(PD-L1) Expression- XenoBase Value	Surface CD274(PD-L1) Expression- FACS Value
Melanoma	A2058	5.0168	35.9
	A-375	4.8704	13.4
	SK-MEL-5	4.2539	8.39
NSCLC	HCC827	8.4718	95.0
	NCI-H226	8.0082	22.3
	EBC-1	7.5264	10.2
RCC	SK-MES-1	7.3042	NA
	Caki-2	7.8034	NA
	786-O	7.2959	NA
Prostate	PC-3	6.7756	NA
	DU 145	6.5257	NA

Efficacy Evaluation in HCC827 MiXeno™ Model

Anti-tumor Activity of BMS-936558 in the Treatment of Subcutaneous HCC827 NSCLN Mixeno™ Model



Available Syngeneic Models in CrownBio

Model Type	Tumor Type	Cell line
Subcutaneous model (14 models)	Breast cancer	4T1, EMT6
	Colon cancer:	CT-26, MC38
	Liver cancer:	H22, Hepa1-6, Hepa1-6-lux
	Lung cancer:	LL/2
	Melanoma:	B16BL6, B16F10, B16-lux
	Pancreatic cancer:	Pan02
	Prostate cancer:	RM-1
	Renal cancer:	Renca
Orthotopic model (1 model)	Breast cancer:	4T1

Three Types of In Vivo Models for Immunotherapy

	Syngeneic Model	BLT (bone marrow, liver, thymus) Model	MiXeno Model (Xenograft in Hu-PBL Model)
Advantages	● Easy to build; ●Complete murine immune system.	● Maximally reconstituted human immune system components	● Partially reconstituted human immune system
Disadvantages	● Different from human immune system; ● Many antibodies lack the cross-reaction with mouse target	● Difficulty in acquisition of human BLT; ● The time to develop a robust human hemato-lymphoid system is relatively long (12-16 weeks).	● Human lymphocytes may not last long in murine environment .

Summary

- XenoBase ® is an integrated tool in searching for gene mutation, amplification, and expression, profile as well as tumor growth information in vivo and response to SOC treatments.
- PD-L1 RNA expression levels of the cell line cohort selected by XenoBase ® are consistent with the surface PD-L1 level examined by FACS analysis.
- High expression of PD-L1 is found in many Melanoma, NSCLC, RCC and prostate cancer cell lines.
- HCC827 cell line was identified as a high PD-L1 expression cell line, and selected to develop in vivo MiXeno™ model for in vivo efficacy evaluation, though correlation between PD-L1 expression level and the efficacy by anti-PD-1 antibody therapy is still not clear.
- BMS-936558 produced 50% tumor growth inhibition in the HCC827 MiXeno™ Model.
- MiXeno™ Models are hopefully becoming the useful tools for in vivo evaluation of immunotherapeutic agents, but more investigation is required to determine many parameters about the status of the human immune components along with the immunotherapeutic treatment.

References

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