Validation of Anti-human PD1 and PD-L1 Antibodies in MiXeno™ Mouse Models

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Poster : 3235

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Introduction

As evidenced by strong clinical data from antibodies targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death protein-1 (PD-1), and its ligand PD-L1, cancer immunotherapy is now a major breakthrough in cancer treatment. Meanwhile, new immunotherapeutic agents with various targets and mechanisms, as well as new drug combinations are under active preclinical development. However, reliable humanized animal models are of increasing importance as more programs are reaching the in vivo stage, where direct evaluation of therapeutics with anti-human targets are necessary. We have previously reported our efforts in generating mouse MiXeno models that harbor human immune cells by engrafting immuno-deficient mice with human PBMC (the MiXeno model) to evaluate anti-PD-1 antibody. In the current study, we took an effort to generate the MiXeno models by different routes of T cell transfer (i.v. or s.c.) and testing the models with anti-human PD-1 or PD-L1 antibodies by using different animal strains (NOD/SCID vs. NPG). Our studies demonstrated MiXeno model is simple and fast, and may become valuable tools for in vivo evaluation of immunotherapeutic agents.

What is MiXeno?

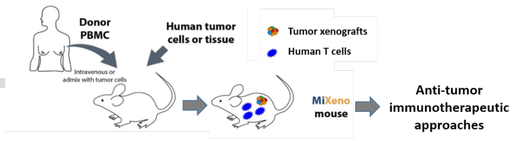

Figure 1. The concept of MiXeno Model

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Results

Figure 2. Explore PD-L1(CD274) mRNA expressing level by XenoBase®

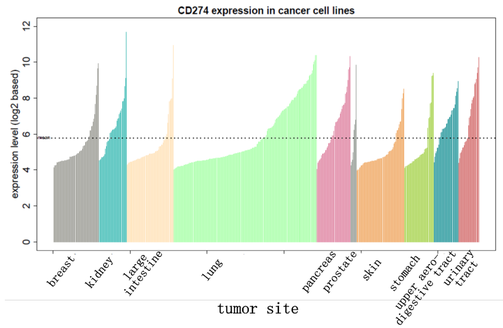


Table 1. FACS analysis on surface PD-L1 expression

No.	Cancer type	Cell line	PD-L1 Level (XenoBase)	PD-L1 level (MFI)
1	Melanoma	SK-MEL-28	5.5561	7
2		A2058	5.0168	35.9
3		A375	4.8704	13.4
4	NSCLC	NCI-H292	9.2971	136.8
5		HCC827	8.4718	95
6		NCI-H358	7.5277	150.9
7		SK-MES-1	7.3042	157.8
8		NCI-H1975	6.8753	134.8
9		NCI-H2228	6.3227	131.2
10		NCI-H1650	5.8356	31.4
11	TNBC	MDA-MB-231	8.1606	283.1
12	Kidney Cancer	786-O	7.2959	43.7
13		A498	5.1619	19.5
14	H&N	SCC-4	7.3154	26.7
15	Bladder Cancer	5637	9.0472	NA
16		RT-112	4.9792	NA

● **XenoBase:**
http://xenobase.crownbio.com/xenobase/login.aspx
A free web-based tool developed by Crown Bioscience Inc., combining the publically available gene expression/ mutation/SNP data of more than 1000 cell lines, with our proprietary RNAseq and in vivo pharmacology data;

● PD-L1 expression level was screened in XenoBase (Figure 2) and determined by FACS analysis (Table 1).

Results

Figure 3. Efficacy evaluation of anti-PD-1 antibody in HCC827 NSCLC MiXeno model

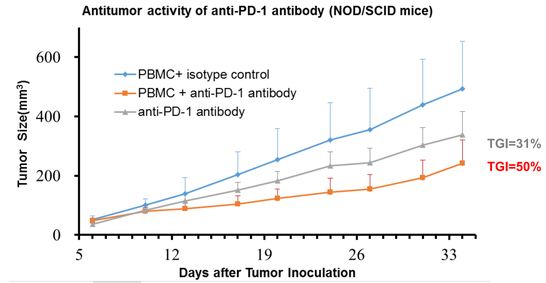
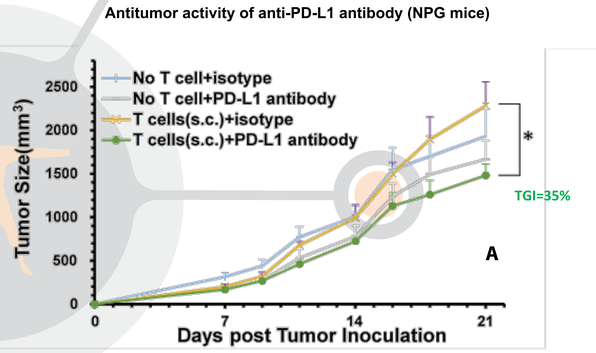
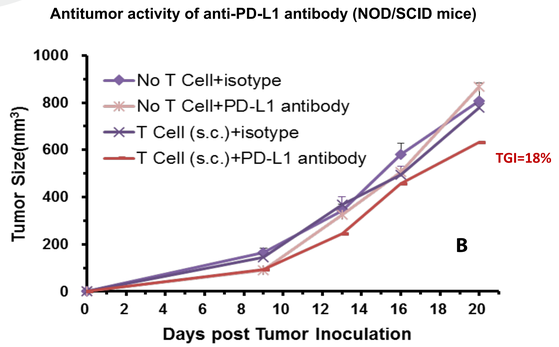


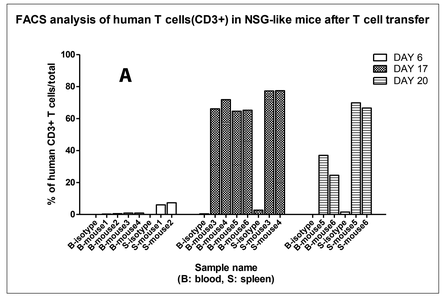
Figure 4. Efficacy evaluation of anti-PD-L1 antibody in A375 Melanoma MiXeno model

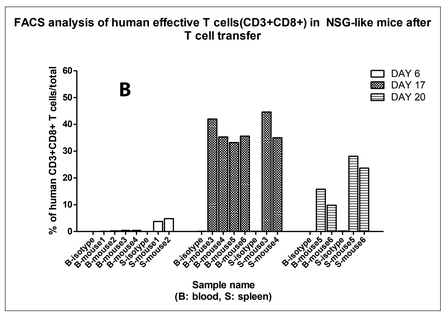




Results

Figure 5. Adoptive T Cell Transfer in NPG (NOD-Prkdcscid Il2rgnull) Mice





Conclusions & Future Studies

- The tested human anti-PD-1 antibody produced 50% tumor growth inhibition in the HCC827 NSCLC MiXeno Model;
- The tested human anti-PD-L1 antibody produced 35% tumor growth inhibition (p=0.031) in the A375 Melanoma MiXeno Model that was established in NPG mice.
- The same anti-PD-L1 antibody didn't achieve significant anti-tumor activity in the A375 MiXeno Model established in NOD/SCID mice, demonstrating the advantage of NPG mice in MiXeno system.
- High T cell reconstitution via i.v. transfer has been validated in the immuno-deficient NPG mice.
- More MiXeno models are to be validated; the molecular markers that might help on model selection for different types of immuno-modulators will be explored.