NBbiolab

Case Study —— Nanobody Development for PD-L1

Target Background

Protein Type	Type I transmembrane immunoglobulin superfamily (IgSF) glycoprotein. Undergoes N-linked glycosylation; soluble PD-L1 (sPD-L1) can be detected in serum.
Family	B7/CD28 co-stimulatory/co-inhibitory molecule family (IgSF).
Expression	Normal tissue: Inducible on resting T, B, dendritic cells, macrophages, and low baseline on endothelial/epithelial cells. Tumor tissue:Aberrantly overexpressed on tumor cells and infiltrating immune cells across multiple solid tumors and Hodgkin lymphoma; often induced by IFN-γ in the TME. Also detected on some hematologic malignancies.
Function	Engagement of PD-1 on activated T cells inhibits TCR-mediated signaling, reduces IL-2 production, and promotes T-cell exhaustion. Tumor-cell PD-L1 expression is a major adaptive immune-resistance mechanism. Reverse signaling via PD-L1 may modulate tumor cell survival and proliferation.
Clinical Role	Validated predictive biomarker for anti–PD-1/PD-L1 therapy . Used for patient selection in NSCLC, TNBC, HNSCC, urothelial carcinoma, etc. sPD-L1 levels correlate with disease burden and prognosis.
Therapeutic Modalities	• Anti–PD-L1 mAbs, Bispecifics, ADCs, CAR-T • Mechanism: Blockade of the PD-1/PD-L1 interaction restores T-cell activation, proliferation, and antitumor immunity.

Project Objective & Achievement

Objective	① Cross-reactivity to both human and cynomolgus PD-L1. ② Bind PD-L1-positive tumor cells. ③ Exhibit internalization capability.
Immunization	One Alpaca was immunized with recombinant PD-L1 protein over 49 days (4 SC injections at two-week intervals). Post-immune sera reached an ELISA endpoint titer of 1:128,000.
Library Construction	VHH genes were amplified from immunized alpaca PBL-derived cDNA and cloned into a yeast display vector, yielding a library with >1 × 10^8 independent clones and 100% insert-positive rate.
Library Screening	Sequential MACS enrichment and FACS sorting were performed to isolate specific binders with cross-reactivity to both human and cynomolgus PD-L1.
Binder Validation	A total of 180 monoclonal clones were randomly selected and sequenced, yielding 10 unique VHH sequences. All clones were validated as PD-L1-specific binders and showed robust soluble VHH expression with good yield and purity.
Deliverable	10 unique PD-L1-specific VHHs were successfully discovered.

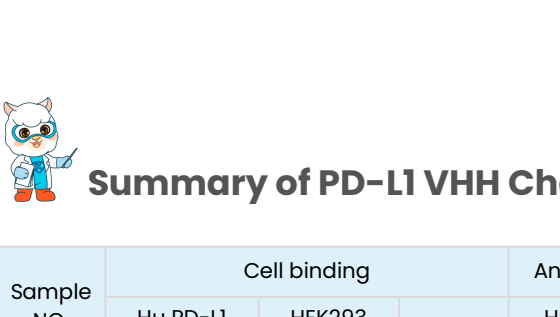
Sequence Properties of 10 unique PD-L1-specific VHHs

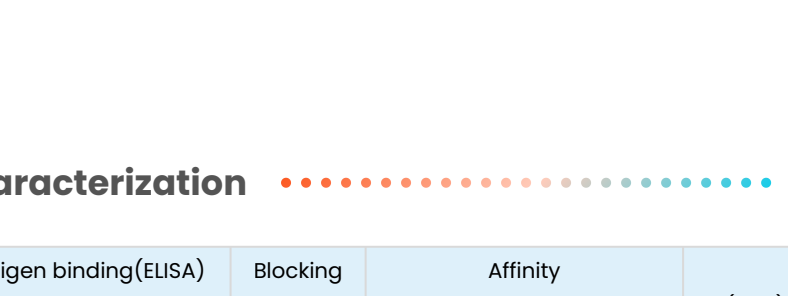
ID	Length	pI	kDa	Isomerization	Cys	Deamidation	Glycosylation
BS072-2	116	8.27	12.68	1	2	0	0
BS072-4	124	8.3	13.31	0	2	0	2
BS072-8	124	8.75	13.33	0	2	0	0
BS072-9	120	4.76	13.03	1	2	0	0
BS072-12	124	9.14	13.24	0	2	0	0
BS072-17	115	8.79	12.46	1	2	0	0
BS072-18	125	5.34	13.89	0	2	0	0
BS072-19	115	9.36	12.48	0	2	0	0
BS072-20	124	8.76	13.74	0	2	0	0
BS072-21	123	9.27	13.53	1	2	0	1

Summary of PD-L1 VHH Characterization

Sample NO.	Cell binding			Antigen binding(ELISA)		Blocking	Affinity		HIC(min)
	Hu PD-L1 cell(EC50 nM)	HEK293 (50μg/mL)	NCI-H292	Hu PD-L1(EC50 nM)	Cynp PD-L1(EC50 nM)	ELISA-IC50(nM)	Hu PD-L1(KD)	Cynp PD-L1(KD)	
KN035	0.4294	no binding	0.004071	0.05	0.06	0.6377	5.85E-09	5.63E-09	22.779
Anti-P1-HL43	N/A	N/A	0.06759	N/A	N/A	5.759			N/A
BS072-2	3.012	no binding	0.07468	0.32	0.49	3.679	1.25E-09	1.13E-09	22.066
BS072-4	0.639	no binding	0.01991	0.44	0.37	2.205	2.78E-09	1.81E-09	21.614
BS072-8	2.015	no binding	0.04575	0.21	0.19	2.774	8.47E-09	5.95E-09	21.824
BS072-9	1.193	no binding	0.07283	0.6	1.03	2.185	4.5E-09	3.89E-09	19.655
BS072-12	1.174	no binding	0.03176	0.11	0.1	2.127	5.58E-09	5.34E-09	28.383
BS072-17	0.649	no binding	0.09009	0.14	0.17	1.936	7.74E-10	1.29E-09	17.398
BS072-18	4.781	no binding	25.58	0.18	0.2	7.244	1.42E-09	6.43E-10	23.126
BS072-19	8.47	no binding	0.1825	10.6	10.51	3.567	1.11E-08	3.38E-09	21.942
BS072-20	10.73	no binding	0.0388	0.11	0.11	2.655	4.77E-09	2.66E-09	23.889
BS072-21	0.7399	no binding	0.05321	0.19	1.1	9.253	3.19E-09	2.09E-09	22.701

Internalization Capability





Conclusion & Key Highlights

Validated Immunization

ELISA assay confirmed recognition of both human and cynomolgus PD-L1.

High-Quality Library

>1 × 10^8 diversity, 100% insert-positive rate, and no duplicate sequences detected.

High-Fidelity Screening

MACS and FACS enabled efficient enrichment of human/cyno cross-reactive PD-L1 binders.

Robust Validation

Multi-step validation ensured reliable identification of target-specific VHHs.

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