NBbiolab

Case Study —— Nanobody Development for CD20

Target Background

Protein Type	Type III tetraspanning membrane phosphoprotein, nonglycosylated, non-secreted, does not undergo significant internalization upon antibody binding, and is not shed into circulation. Exists as homo-oligomers (dimers/tetramers) on the plasma membrane.
Expression	Normal tissue: Restricted to B-lymphocytes from late pro-B through mature naïve and memory B cells; absent on hematopoietic stem cells, early pro-B cells, and terminally differentiated plasma cells. Pathologic: Expressed on >95% of B-cell non-Hodgkin lymphomas (NHL), DLBCL, FL, MCL, and CLL cells, as well as some B-ALL; also detected on rare melanoma cancer stem cells.
Function	Functions as a store-operated Ca^{2+} channel component / regulator of BCR-coupled calcium influx, modulating B-cell activation, proliferation, and differentiation.
Clinical Role	A validated diagnostic and therapeutic target for B-cell malignancies (NHL, CLL, ALL) and B-cell–mediated autoimmune diseases (multiple sclerosis, rheumatoid arthritis, pemphigus vulgaris, lupus nephritis).
Therapeutic Modalities	• Anti-CD20 mAbs, Radiolabeled mAbs, Bispecific, CAR-T • Mechanisms: CDC, ADCC/ADCP, direct apoptosis, B-cell depletion.

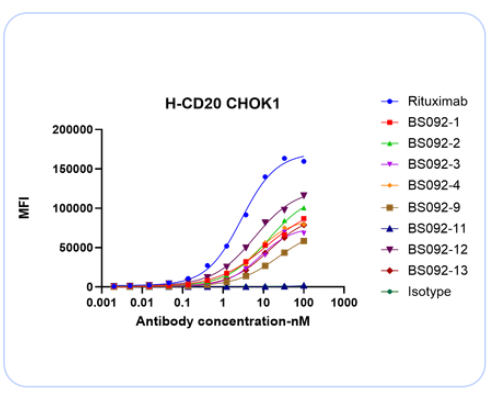
Project Objective & Achievement

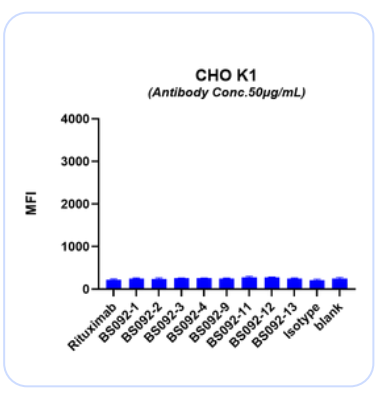
Objective	① Cross-reactivity to both human and cynomolgus CD20. ② Bind CD20-positive tumor cells.
Immunization	One alpaca was immunized with CD20-overexpressing cells for 49 days (4 SC injections at two-week intervals). Serum titers reached 1:1,000, with FACS signals >3-fold over the negative control.
Library Construction	VHH genes were amplified from immunized alpaca PBL-derived cDNA and cloned into a phagemid vector, yielding a library with >1 × 10^9 independent clones and 100% insert-positive rate.
Library Screening	Three rounds of cell-based biopanning were performed using counter-selection and CD20 positive selection, resulting in progressive enrichment of target-specific binders.
Binder Validation	From 270 screened clones, 9 unique VHH sequences were identified, of which 8 were confirmed as CD20-specific binders with favorable sequence properties.
Deliverable	A total of 8 unique CD20-specific VHHs were discovered.

In Silico Analysis and Quality Properties of 8 candidates

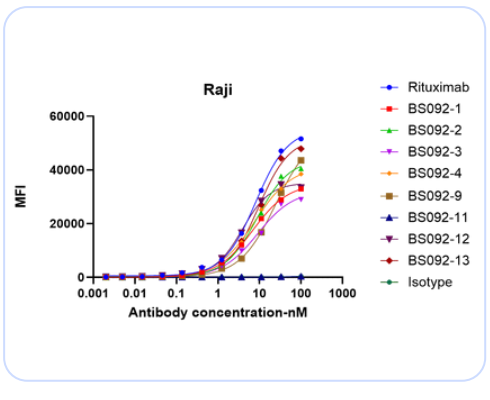
ID	Length	Molecular_Weight(kDa)	pI	Cysteine_Count	Hydrolysis	Isomerization	Glycosylation	Deamidation
BS092-1	116	12.43	8.04	2	N/A	59:DY(10.0%), 73:DN(5.0%)	N/A	74:NA(10.0%), 84:NS(30.0%), 97:NA(10.0%)
BS092-2	116	12.48	7.95	2	N/A	59:DY(10.0%), 73:DN(5.0%), 103:DS(25.0%)	N/A	74:NA(10.0%), 84:NS(30.0%), 97:NA(10.0%)
BS092-3	122	13.15	8.03	2	107:NP(30.0%)	61:DS(25.0%), 72:DN(5.0%)	N/A	32:NA(10.0%), 73:NA(10.0%), 83:NS(30.0%)
BS092-4	122	13.22	8.64	2	107:NP(30.0%)	61:DS(25.0%), 72:DN(5.0%)	N/A	32:NA(10.0%), 73:NA(10.0%), 83:NS(30.0%)
BS092-9	121	12.92	8.66	2	N/A	62:DS(25.0%), 73:DN(5.0%)	N/A	74:NA(10.0%), 84:NS(30.0%)
BS092-11	121	13.07	6.91	2	N/A	54:DG(35.0%), 61:DS(25.0%), 72:DN(5.0%), 98:DR(10.0%)	N/A	73:NA(10.0%), 83:NS(30.0%)
BS092-12	118	12.75	8.64	2	N/A	61:DS(25.0%), 72:DN(5.0%), 101:DR(10.0%)	N/A	73:NA(10.0%), 83:NS(30.0%), 96:NA(10.0%)
BS092-13	114	12.29	8.66	2	N/A	61:DS(25.0%), 72:DN(5.0%)	N/A	83:NS(30.0%)

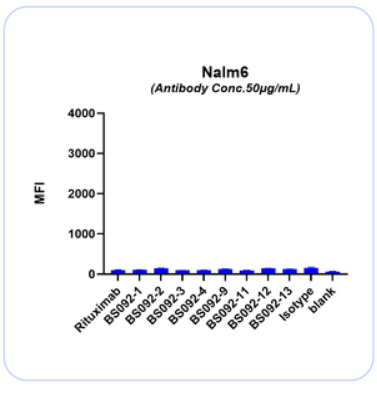
Cell-based Binding Activity



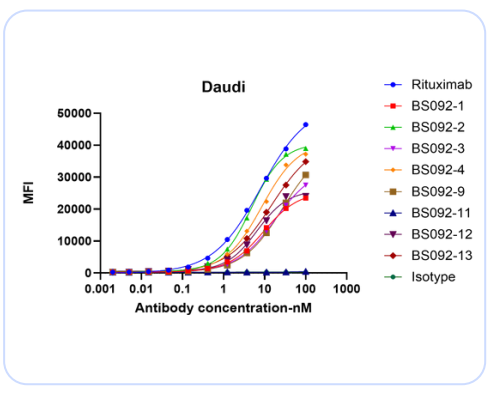


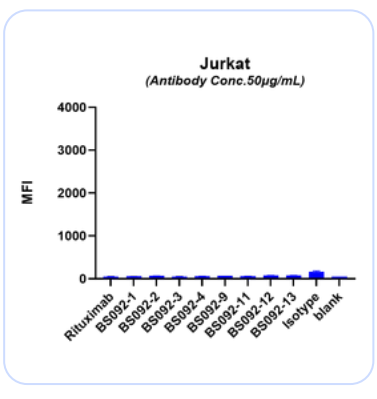
Sample	EC50-H-CD20 CHOK1(nM)
Rituximab	2.875
BS092-1	12.3
BS092-2	13.34
BS092-3	8.176
BS092-4	6.551
BS092-9	23.1
BS092-11	555.1
BS092-12	6.068
BS092-13	13.8
Isotype	~



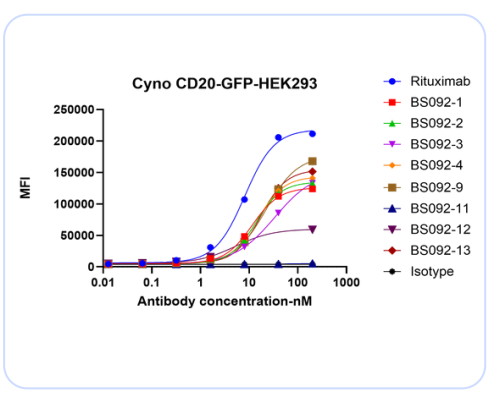


Sample	EC50-Raji(nM)
Rituximab	8.072
BS092-1	6.921
BS092-2	8.8
BS092-3	9.026
BS092-4	7.686
BS092-9	24.64
BS092-11	60.48
BS092-12	3.878
BS092-13	9.482
Isotype	~





Sample	EC50-Daudi(nM)
Rituximab	7.962
BS092-1	9.386
BS092-2	4.843
BS092-3	18.08
BS092-4	8.561
BS092-9	27.65
BS092-11	36.41
BS092-12	6.601
BS092-13	14.61
Isotype	~



Sample	EC50-Cyno CD20-GFP-HEK293(nM)
Rituximab	8.141
BS092-1	11.43
BS092-2	13.69
BS092-3	33.6
BS092-4	14.79
BS092-9	22.2
BS092-11	No binding
BS092-12	5.252
BS092-13	17.1
Isotype	~

All sequences can bind to to Cyno CD20-GFP-HEK293 cells except BS092-11.

Conclusion & Key Highlights

Cell-Based Immunization

Effective for generating immune libraries against challenging multi-pass membrane proteins.

High-Quality Library

10^9 diversity, 100% insert-positive rate, and no sequence redundancy detected.

High-Fidelity Screening

Cell-based biopanning with counter-selection enabled efficient enrichment of CD20-specific binders.

Two-Step Validation

Monoclonal phage FACS and recombinant antibody validation ensured high-confidence hit identification.

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