NBbiolab

Case Study —— Nanobody Development for HER3

Target Background

Protein Type	Type I transmembrane receptor tyrosine kinase (RTK) with defective catalytic activity. Undergoes N glycosylation and proteolytic cleavage (shedding of ECD into circulation).
Family	HER3, also known as ErbB3, is one of the four members of the ERBB/HER receptor tyrosine kinase family.HER3 is most closely related to HER4 and is unique in lacking functional kinase activity among the four.
Expression	Normal tissue: Widely expressed in epithelial, mesenchymal, and neuronal tissues. Tumor tissue: Overexpressed or activated in breast (especially HR+/HER2 low), NSCLC, colorectal, prostate, pancreatic, ovarian, and gastric cancers. Frequently co expressed with HER2.
Function	Lacks robust autophosphorylation but is the preferred dimerization partner for HER2. Promotes cell survival, proliferation, migration, and EMT. In cancer, HER3 signaling compensates for HER2 or EGFR inhibition, driving resistance to anti EGFR/HER2 therapies.
Clinical Role	Validated resistance target and biomarker. Used as a patient-stratification marker in HER2+ and EGFR-mutant NSCLC/breast cancer trials. High HER3 activation predicts poor outcome with EGFR-TKI or HER2-targeted therapy and suggests benefit from HER3-directed intervention.
Therapeutic Modalities	• Anti-HER3 mAbs, ADCs, Bispecific, CAR-T • Mechanism: blocks NRG-1 binding, prevents HER2:HRE3 heterodimerization, induces ADCC or delivers cytotoxic payload.

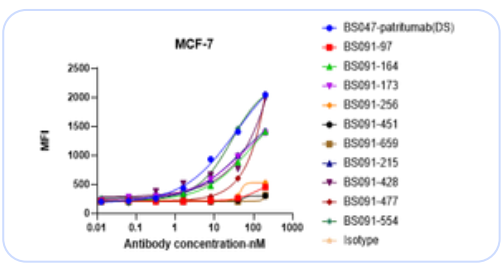
Project Objective & Achievement

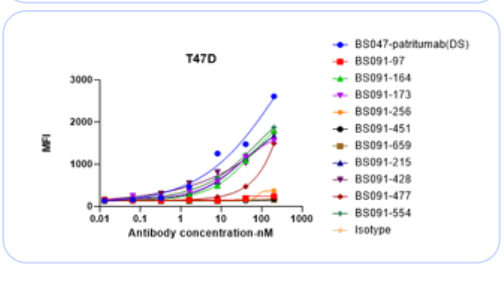
Objective	① Bind HER3-positive tumor cells. ② Exhibit internalization capability.
Immunization	One Alpaca was immunized with recombinant HER3 protein over 49 days (4 SC injections at two-week intervals). Post-immune sera reached an ELISA endpoint titer of 1:128,000 and demonstrated specific binding to HER3-overexpressing cells by FACS, with positive signals maintained at a serum dilution of 1:1,000.
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 to human HER3.
Binder Validation	A total of 180 monoclonal clones were randomly selected and sequenced, yielding 17 unique VHH sequences. Ten clones were validated as HER3-specific binders and showed robust soluble VHH expression with good yield and purity.
Deliverable	10 unique HER3-specific VHHs were successfully discovered, including 3 lead candidates with desirable binding and developability profiles.

In Silico Analysis and Quality Properties of 10 unique HER3-specific VHHs

Name	Length	Molecular_ Weight(kDa)	pI(VHH)	Cysteine _Count	Hydrolysis	Isomerization	Glycosylation	Deamidation
BS091-97	119	12.98	6.77	2	54:NG(35.0%)	16:DS(25.0%), 31:DY(10.0%), 62:DS(25.0%)	N/A	54:NG(45.0%), 84:NS(30.0%)
BS091-164	127	13.53	9.39	2	52:TS(12.0%)	62:DS(25.0%), 73:DN(5.0%)	N/A	74:NA(10.0%), 84:NS(30.0%)
BS091-173	119	12.68	9.04	2	N/A	73:DN(5.0%), 102:DS(25.0%)	N/A	62:NS(30.0%), 74:NA(10.0%), 84:NS(30.0%)
BS091-256	116	12.53	8.62	2	N/A	72:DN(5.0%), 104:DY(10.0%)	N/A	58:NA(10.0%), 61:NS(30.0%), 73:NA(10.0%), 83:NS(30.0%)
BS091-451	116	12.35	9.47	2	N/A	62:DS(25.0%), 73:DN(5.0%)	N/A	59:NY(15.0%), 74:NA(10.0%), 84:NS(30.0%)
BS091-659	117	12.87	8.65	2	53:NG(35.0%)	61:DS(25.0%), 72:DN(5.0%)	N/A	53:NG(45.0%), 73:NA(10.0%)
BS091-human-HER3-215	116	12.54	9.34	2	N/A	N/A	N/A	83:NS(30.0%)
BS091-human-HER3-428	122	13.48	5.73	2	54:NG(35.0%), 58:TS(12.0%), 74:NP(30.0%)	62:DS(25.0%), 73:DN(5.0%), 84:DS(25.0%), 108:DY(10.0%), 110:DY(10.0%)	N/A	31:NY(15.0%), 54:NG(45.0%)
BS091-human-HER3-477	124	13.66	7.99	2	104:TS(12.0%)	16:DS(25.0%), 62:DS(25.0%), 73:DD(30.0%)	N/A	N/A
BS091-human-HER3-554	118	12.81	9.43	2	52:TS(12.0%)	32:DD(30.0%), 72:DN(5.0%)	N/A	61:NS(30.0%), 73:NA(10.0%), 83:NS(30.0%)

Cell-based Binding Activity

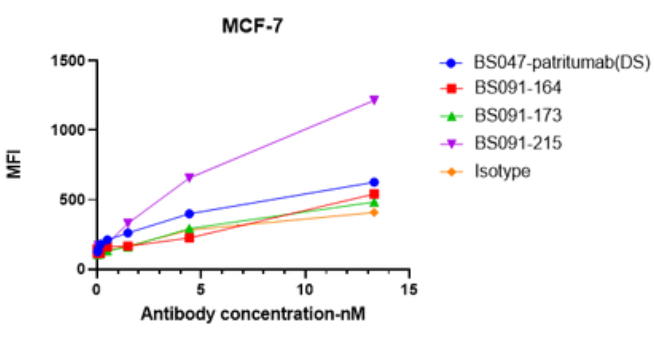




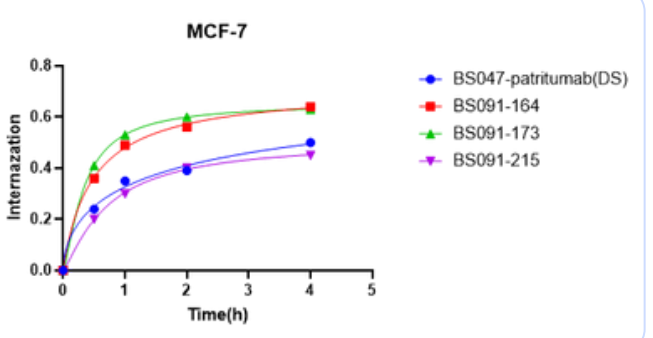
Sample	EC50-MCF-7(nM)	EC50-T47D(nM)
BS047-patritumab(DS)	35.83	298.6
BS091-97	122.7	39.85
BS091-164	93.09	69.87
BS091-173	56.6	26.13
BS091-256	45.69	no binding
BS091-451	no binding	no binding
BS091-659	no binding	no binding
BS091-215	45.23	35.85
BS091-428	~	14806
BS091-477	206370	3326709
BS091-554	27.92	77.08
Isotype	~	~

Internalization Capability

Internalization Assay (pH-Sensitive Dye-Based Method)



Internalization Assay (Temperature-Shift Method)



Conclusion & Key Highlights

Validated Immunization

ELISA and cell-based assays confirmed recognition of both recombinant and cell-surface HER3.

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 HER3 binders.

Robust Validation

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

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