NBbiolab

Case Study | Nanobody Development for TROP2

Target Background

Protein Type	Type I transmembrane cell-surface glycoprotein
Family	TACSTD (Tumor-Associated Calcium Signal Transducer) family. Shares homology with EpCAM (TACSTD1/TROP1)~49% similarity
Expression	Normal tissue: Low or focal expression in renewing epithelia (skin, esophagus, cervix, respiratory/GI tract); minimal in most vital organs. Tumor tissue: Widely overexpressed in ≥80% of epithelial malignancies.
Function	Physiologically involved in embryogenesis, tissue regeneration, and stem cell self-renewal. In cancer, TROP2 promotes proliferation and survival; its overexpression correlates with poor prognosis and increased metastatic potential.
Clinical Role	Validated target for solid tumor ADC therapy, particularly in TNBC, HR+/HER2- breast cancer, and metastatic urothelial carcinoma.
Therapeutic Modalities	Primarily Antibody–Drug Conjugates (ADCs) Other modalities (bispecifics, CAR-T, radioligand) in early development.

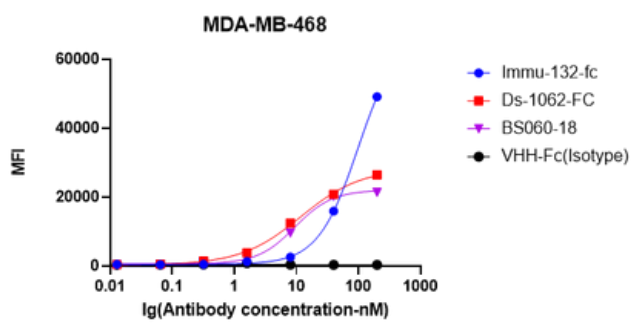
Project Objective & Achievement

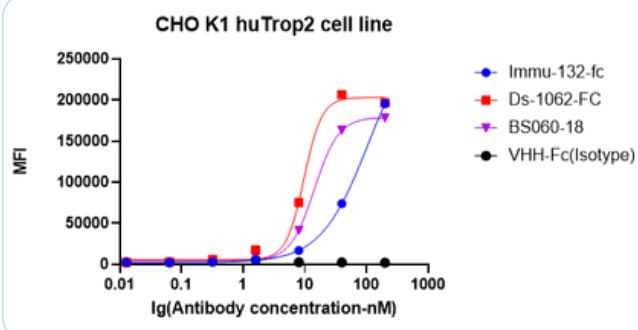
Objective	① Cross-reactivity to both human and cynomolgus TROP2. ② Bind TROP2-positive tumor cells. ③ Exhibit internalization capability.
Immunization	One llama was immunized with recombinant TROP2 protein over 49 days (4 SC injections at two-week intervals). Post-immune sera reached an ELISA endpoint titer of 1:128,000 and demonstrated specific binding to TROP2-overexpressing cells by FACS, with positive signals maintained at a serum dilution of 1:1,000.
Library Construction	VHH repertoires were amplified from immunized llama PBL-derived cDNA and cloned into a yeast display vector, generating an immune yeast display library with >1 × 10_8 diversity 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 TROP2.
Binder Validation	A total of 180 monoclonal clones were randomly selected and sequenced, yielding 20 unique VHH sequences. All were confirmed as TROP2-specific binders.
Deliverable	20 unique TROP2-specific VHHs were successfully discovered, including 2 lead candidates with desirable binding and developability profiles.

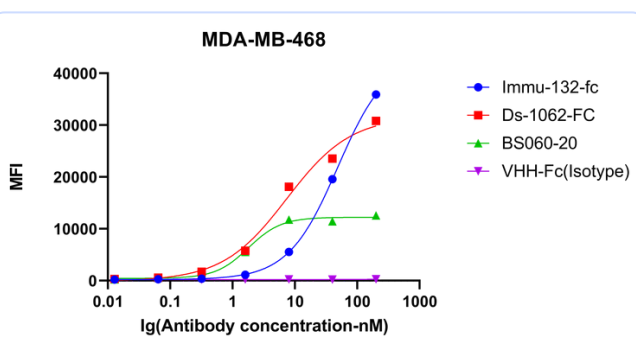
In Silico Analysis and Quality Properties of 2 lead candidates

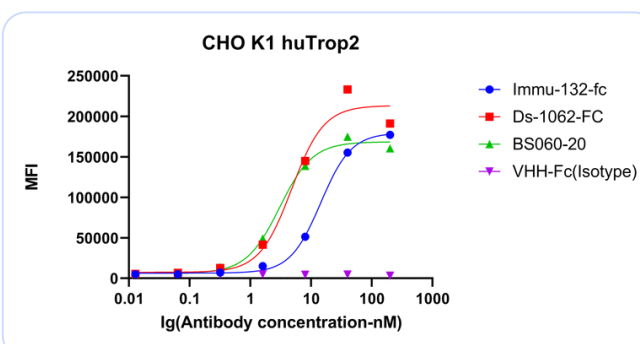
Name	Length	Molecular_Weight (kDa)	pI	Cysteine_Count	Hydrolysis	Isomerization	Glycos-ylation	Deamidation
BS060-18	120	13.23	4.89	2	N/A	73:DN(5.0%)	N/A	59:NY(15.0%), 74:NA(10.0%), 84:NS(30.0%), 108:NS(30.0%)
BS060-20	124	13.47	8.65	2	N/A	62:DS(25.0%), 66:DR(10.0%) 73:DN(5.0%)	N/A	74:NA(10.0%), 84:NS(30.0%)

Cell-based Binding Activity









Affinity Measurement

TROP2 ECD Species	Loading Sample ID	KD (M)	ka (1/Ms)	kdis (1/s)	Full R^2
Human	Immu-132-fc	0.0000000124	112000	0.00139	0.4332
	Ds-1062-FC	0.0000000135	123000	0.00166	0.9785
	BS060-18	0.00000000391	182000	0.000711	0.9922
	BS060-20	0.000000019	84100	0.00159	0.9642
Cynomolgus monkey	Immu-132-fc	0.0000000107	103000	0.0011	0.9113
	Ds-1062-FC	0.0000000215	170000	0.00366	0.9888
	BS060-18	0.00000000658	184000	0.00121	0.9834
	BS060-20	0.0000000939	221000	0.0208	0.8923

Conclusion & Key Highlights

Validated Immunization

ELISA and cell-based assays confirmed recognition of both recombinant and cell-surface TROP2.

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 TROP2 binders.

Robust Validation

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

www.nbbiolab.reanodb2b.cn

Contact us