NBbiolab

Case Study —— Nanobody Development for GPRC5D

Target Background

Protein Type	A 7-transmembrane cell-surface receptor of the G protein-coupled receptor family.
Family	Class C G protein-coupled receptor family, GPRC5 subfamily.Shares homology with GPRC5A, GPRC5B, and GPRC5C.
Expression	Normal tissue: Highly restricted, expressed in hair follicles, nail matrix, and filiform papillae of the tongue. Tumor: Strongly overexpressed on the surface of malignant plasma cells in >65% of multiple myeloma (MM) cases; expression is independent of and retained after BCMA downregulation.
Function	Physiological role not fully elucidated. In MM, its precise oncogenic contribution is unclear; its stable surface presentation and lack of shedding make it an ideal immunotherapy target.
Clinical Role	A validated companion diagnostic marker and therapeutic target for relapsed/refractory MM, particularly in BCMA-escape patients. High and selective expression on MM cells supports its use as a tumor-associated antigen for T-cell redirecting therapies.
Therapeutic Modalities	• GPRC5D×CD3 Bispecific Antibodies • GPRC5D-directed CAR-T cells

Project Objective & Achievement

Objective	Develop GPRC5D -specific VHHs via alpaca immunization using GPRC5D OE cell line as immunogen.
Immunization	One alpaca was immunized with GPRC5D-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 repertoires were amplified from immunized alpaca PBMCs and cloned into a phagemid vector, generating an immune library with >1 × 10^9 diversity and 100% insert-positive rate.
Library Screening	Three rounds of cell-based biopanning were performed using counter-selection and CLDN18.2 positive selection, resulting in progressive enrichment of target-specific binders.
Binder Validation	From 270 screened clones, 14 unique VHH sequences were identified, of which 13 were confirmed as GPRC5D-specific binders with favorable sequence properties.
Deliverable	A total of 14 unique GPRC5D-specific VHHs were discovered.

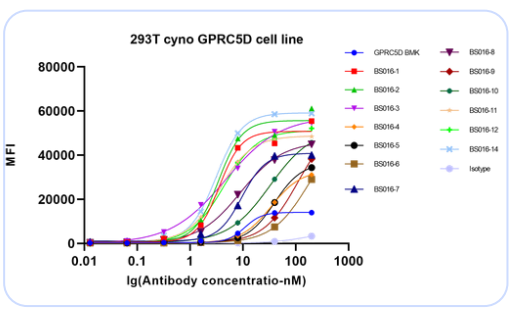
Milestone Overview

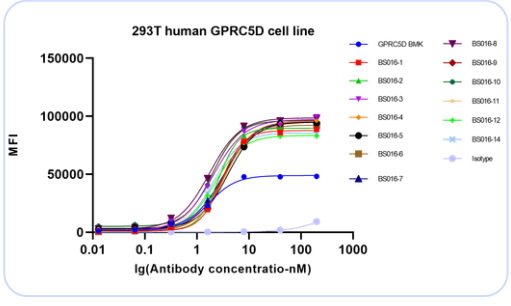
Anti-GPRC5D VHH Sequence Properties

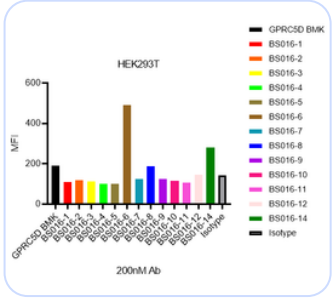
Table 1. Summary of Anti-GPRC5D VHH Sequences

ID	Length	Molecular weight (KDa)	pI (VHH)	Extinction Coefficients (reduced)	Extinction Coefficients (cystine bridges)
BS016-1	114	12.22	9.07	1.6314	1.6416
BS016-2	114	12.32	9.36	2.3065	2.3166
BS016-3	116	13.02	9.23	1.8753	1.8849
BS016-4	125	13.63	7	1.8662	1.8754
BS016-5	125	13.61	9.04	1.8698	1.879
BS016-6	125	13.66	8.66	1.8621	1.8712
BS016-7	125	13.56	8.68	1.8757	1.8849
BS016-8	125	13.6	9.1	1.761	1.7702
BS016-9	125	13.57	8.7	1.7652	1.7744
BS016-10	125	13.63	9.07	1.7571	1.7663
BS016-11	125	13.67	8.06	1.8607	1.8699
BS016-12	127	13.93	8.98	2.328	2.337
BS016-13	117	12.8	5.04	1.7905	1.8002
BS016-14	124	13.64	9.23	1.5712	1.5804

Binder validation







Conclusion & Key Highligts

Cell-Based Immunization

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

High-Quality Immune 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 GPRC5D-specific binders.

Two-Step Validation

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

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