Multifaceted approach to discover effective anti-DKK1 antibodies with diverse function and epitope

TWIST BIOSCIENCE | BIOPHARMA SOLUTIONS

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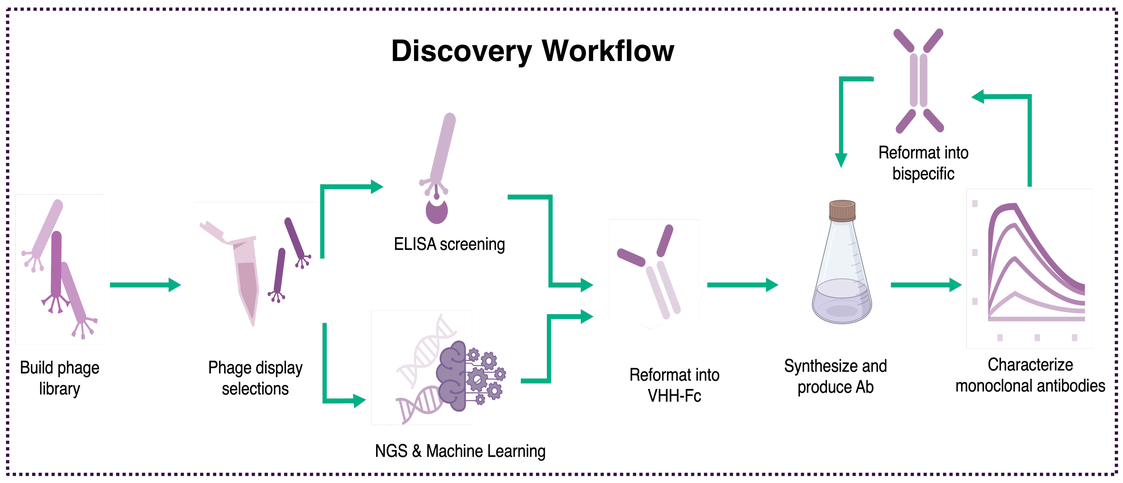
I. INTRODUCTION & ANTIBODY CANDIDATE DISCOVERY

Dickkopf-1 (DKK1) is an endogenous protein known to over-express in tumor micro-environments. Previous studies have shown the anti-tumor effect of anti-DKK1 antibodies in multiple different cancers. Twist Biopharma's multidisciplinary gold-standard platform integrates in vitro, in vivo, and in silico approaches for antibody discovery.

In this campaign, we leveraged Twist's in vitro and in silico approaches, taking advantage of Twist's high-diversity synthetic phage libraries as well as machine-learning strategies to discover potent anti-DKK1 antibodies. Two synthetic humanized VHH libraries were panned using phage display, and phage panning outputs were screened by ELISA to identify potential candidates. Panning outputs were also sequenced by Next Generation Sequencing (NGS)– NGS outputs were leveraged in various machine learning models to predict additional potential candidates. Sequences were reformatted into VHH-Fc, and synthesized and produced with Twist's high throughput synthesis and production platform. Resulting antibodies were evaluated for binding on SPR, as well as further downstream characterization and in vivo studies. Additionally, our modular VHH platform allowed us to rapidly reformat promising mAbs into bispecific antibodies (bsAb). For example, TB725-003 is a VHH-Fc-VHH bsAb derived from SC52-1 + SC52-5, 2 heavy-chain mAbs discovered from our initial phage display campaign.

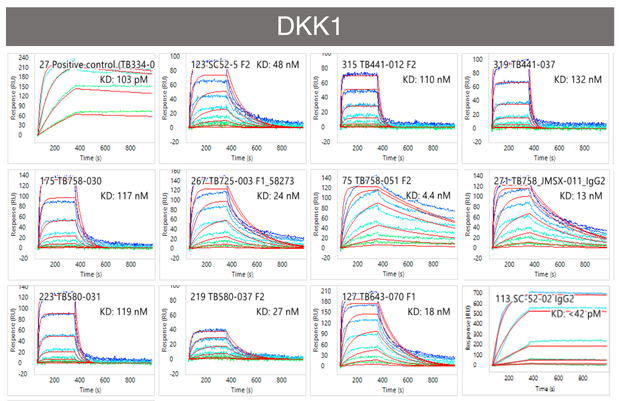
Discovery Method	Campaign ID
In Vitro Phage Display	SC52
NGS-derived Machine Learning	TB441, TB580, TB643, TB758
Bispecific (BsAb)	TB725-3 (SC52-1 + SC52-5)



II. ANTIBODY BINDING & EPITOPE CHARACTERIZATION

A. Kinetics and Epitope Binning demonstrates high affinity of antibody candidates and suggests diversity in epitope

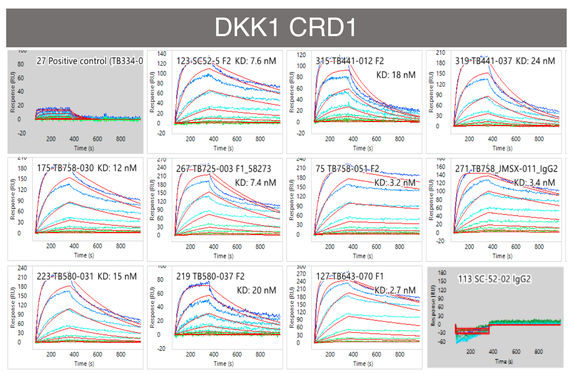
Kinetics for candidates were evaluated through high-throughput SPR capture kinetics, yielding a diversity of affinities. TB334-7 is the positive control mAb. Antibodies selected from the high throughput kinetics experiment were matrixed in a classical epitope binning assay. Red and green cells in the heat map represent competitive and non-competitive blocked analyte/ligand pairs, respectively.

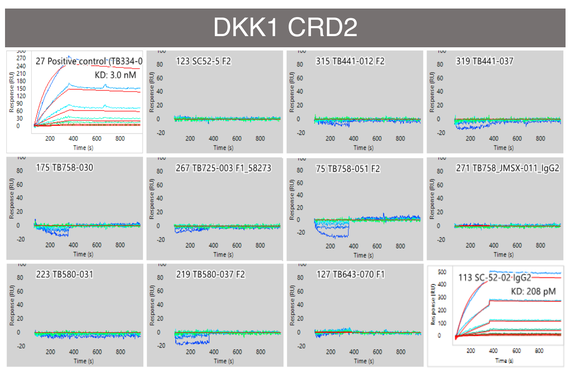


*SC52-2 kinetics were performed in a separate run with antibody directly coupled to biosensor chip

		1	1	2	2	2	2	3	3	4	5
		SC52-2	TB334-7	TB580-037	SC52-5	SC52-11	TB643-070	TB441-037	TB580-031	TB758-030	TB758-051
1	SC52-2		-0.02	1.15	1.24	1.08	1.06	0.98	1.32	1.50	1.87
1	TB334-7	-0.10		1.26	1.35	1.14	1.08	1.07	1.34	1.83	2.27
2	TB580-037	1.15	1.73		-0.18	-0.05	-0.02	0.05	0.09	0.96	1.62
2	SC52-5	1.07	1.71	-0.28		-0.15	-0.08	-0.21	0.08	0.57	1.33
2	SC52-11	1.19	2.02	0.15	0.21		-0.01	0.00	0.06	0.73	1.16
2	TB643-070	1.20	2.27	0.13	0.15	-0.05		0.02	0.04	0.93	1.41
3	TB441-037	0.86	1.48	-0.17	-0.04	-0.16	-0.07		-0.12	0.05	0.28
3	TB580-031	0.40	0.77	-1.67	-0.25	-0.16	-0.22	-0.25		-0.80	-0.37
4	TB758-030	0.97	1.21	0.36	0.52	0.18	0.23	0.28	0.34		0.53
5	TB758-051	0.96	1.61	0.03	0.15	0.13	0.15	0.12	0.16	0.07	

We further investigated the diversity of epitope suggested from the epitope binning assay by exploring binding kinetics of the antibodies to the cysteine-rich N-terminal (CRD1) and C-terminal (CRD2) regions of DKK1. We found that the antibodies that belonged to Bin 1 also showed binding to CRD2, while the antibodies that belonged to bin 2, 3, 4, & 5 showed binding to CRD1.

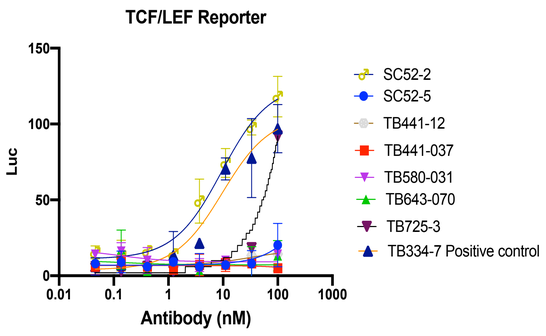


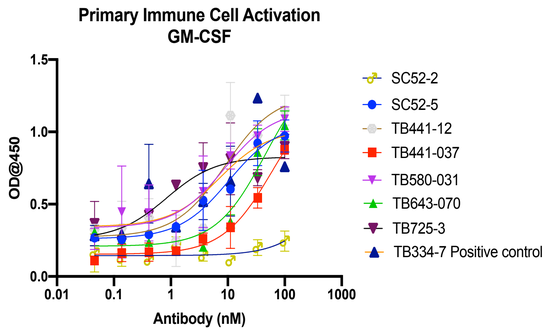


B. Wnt signaling and Immune response in in-vitro cellular assays

A Wnt TCF/LEF reporter assay assessed whether our antibodies blocked DKK1 binding and initiated upregulation of TCF/LEF signaling, and induced restoration of Wnt signaling. Interestingly, the antibodies that showed restoration of Wnt signaling included SC52-2 and TB334-7-- the same antibodies that bound to DKK1 CRD2.

A primary immune cell activation assay assessed whether our antibodies exhibited NK cell activation. DKK1 leads to immune suppression like T cell inactivation and NK cell clearance. For this in-vitro primary immune cell activation assay, human PBMCs were treated with immune simulator, mWnt3a, hDKK1, and DKK1 mAbs. Cytokine release of GM-CSF, a marker for NK cell activation, was measured by ELISA. Antibodies that showed stronger NK cell activation were also antibodies that bound to DKK1 CRD1, apart from TB334-7 which binds CRD2 and still showed NK cell activation.





C. Epitope Mapping via Hydroxyl Radical Footprinting confirms potential epitopes

Potential epitopes were identified utilizing a Plasma Induced Modification of Biomolecules Hydroxyl Radical Footprinting (PLIMB-HRF) method of epitope mapping. First, DKK1 was allowed to bind antibody candidates. Exposed residues were labeled with hydroxyl groups, while protected residues were not. The sample was then digested and analyzed via LC-MS/MS to identify modification. By comparing the Log2 fold modification of residues in a DKK1 + candidate antibody complex, compared to unbound DKK1 control, we identified critical epitopes for antibody binding.

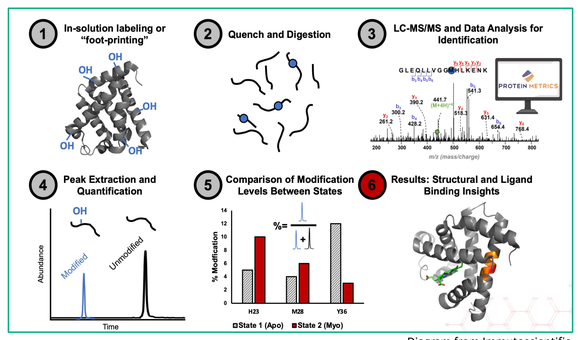
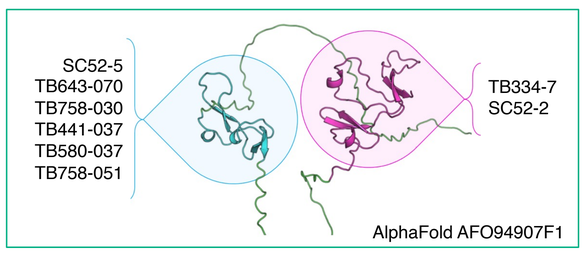
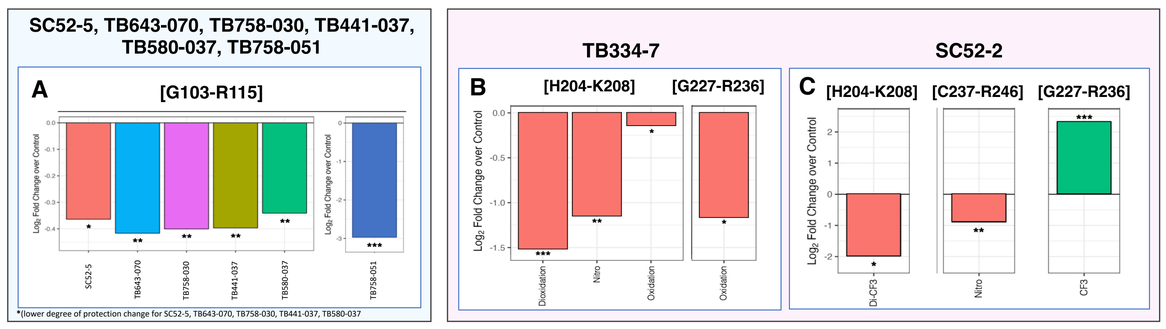


Diagram from Immutoscientific



8 candidate antibodies were mapped with the PLIMB-HRF approach. SC52-5, TB643-070, TB758-030, TB441-037, TB580-037, and TB758-051 showed binding to the N-terminal cysteine rich domain (CRD1, shown in blue), while TB334-7 and SC52-2 showed binding to a distinctly different region, the C-terminal cysteine rich domain (CRD2, shown in pink).

The CRD1-binding antibodies exhibited a potential hotspot for an epitope region at [G103-R115] (Fig. A). CRD2-binding antibody TB334-7 exhibited a potential epitope at [H204-K208] and [G227-R236] (Fig. B). Interestingly, CRD2-binding antibody SC52-2 also exhibited candidate epitopes at [H204-K208] and [C237-R246]; however, also exhibited deprotection at [G227-R236] rather than protection (Fig. C). The unique epitopes identified by PLIMB-HRF provide potential explanations for the diversity in binding characteristics and function identified with kinetics, binning, and cell-based assays.

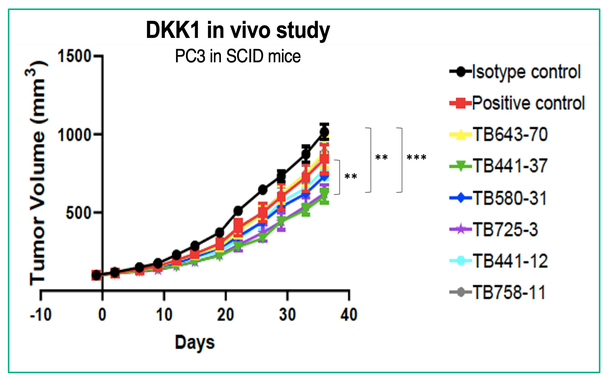


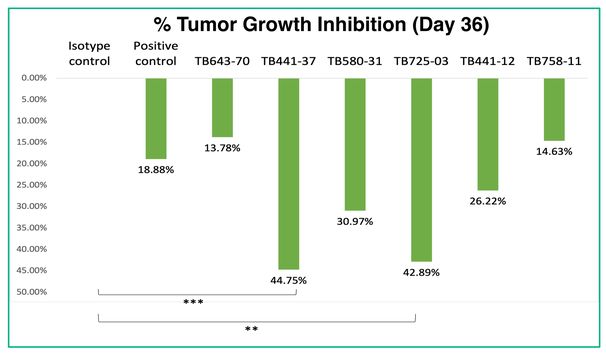
III. IN VIVO STUDIES

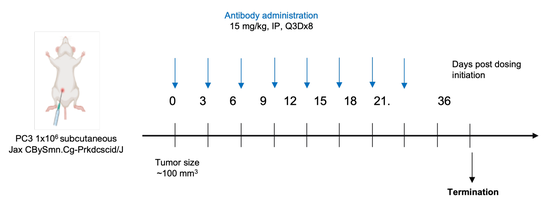
A. Antibodies exhibit strong potency in tumor regression

Homozygous SCID mice were inoculated with PC3 cells. Starting at a tumor volume of ~100mm^3, mice were dosed with 10mg/kg intraperitoneal injections once every 3 days for 8 cycles– tumor sizes were measured 3 times a week.

We find that treatment with TB725-003 and TB441-037 significantly suppresses tumor growth. By day 36, we observed a 42.89% and 44.75% inhibition of tumor growth (TB725-003 bsAb and TB441-037 mAb, respectively), compared to a 18.88% inhibition by the positive control.







IV. SUMMARY

Through Surface Plasmon Resonance (SPR) assays, epitope mapping approaches via Plasma Induced Modification of Biomolecules Hydroxyl Radical Footprinting (PLIMB-HRF), and cell-based functional assays, we demonstrated our candidates to exhibit strong affinity, varied epitope, and diversity in function. Further, multiple of our antibody candidates exhibited significant suppression to tumor growth in in-vivo studies– suggesting a promising future for these candidates. Not only did Twist's robust in-vitro phage display approach provide a strong foundation for this project's antibody discovery (SC52 campaign), additional machine learning (TB441, TB580, TB643, TB758 campaign) and downstream bispecific engineering (TB725 campaign) enabled us to iterate and improve our antibodies, resulting in promising antibody candidates with potential therapeutic activity.