Discovery of Agonistic 4-1BB-Nectin-4 Bispecific Antibodies Using Twist's Library of Libraries

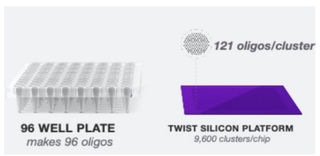
Alyssa Wong, Zhen Han, Tammy Htoy, Kara Chan, Carson Holliday, Paul Vandyke, Linya Wang, Sean M Peterson, Cameron F Hu, Mouna Villalta, Ana Lujan, Tom Z Yuan, Marisa Yang, Fumiko Axelrod, Hoa Giang, and Aaron Sato

Twist Bioscience, South San Francisco, CA, USA

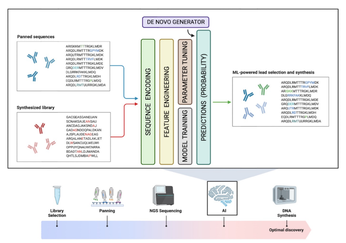
ABSTRACT

Twist Biopharma Solutions is committed to collaborating with our partners to develop efficient antibody therapeutics, leveraging our integrated in vitro and in vivo antibody discovery platforms. In this presentation, we showcase the discovery of anti 4-1BB-Nectin-4 bispecific antibodies (bsAbs) using our library of libraries, followed by high-throughput antibody production (HTIgG), surface plasmon resonance (SPR) characterization, and cell-based assays. 4-1BB has gained significant attention as a versatile immunotherapeutic target due to its widespread expression and the ability to activate diverse signaling pathways critical for robust immune responses. Concurrently, Nectin-4 has emerged as an attractive precision medicine target, being notably overexpressed in various solid tumors, including breast, lung, and ovarian cancers. Our top candidates exhibited robust efficacy in signaling assays and immune cell activation assays. These promising results indicate that these candidates merit further exploration in preclinical investigations and potential development as therapeutic antibodies.

OVERALL ANTIBODY DISCOVERY AND CHARACTERIZATION WORKFLOW

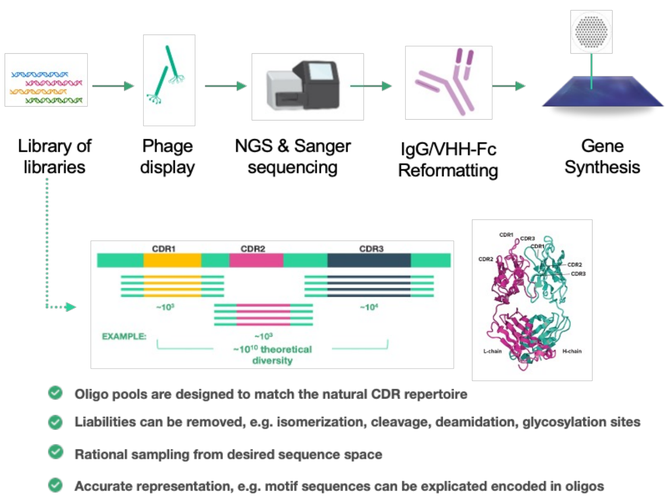


Twist's game changing Silicon based platform can make genes with high throughput, high quality, and low cost.



Neural Net Ranks Leads For Assays

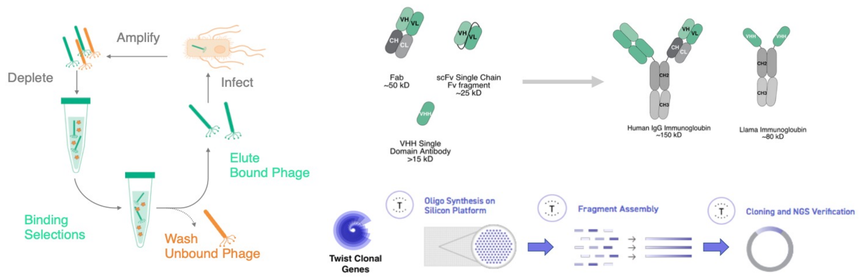
- Twist trains an NLP-based neural net for each panning campaign
- Greatest ML pipelines for *de novo* sequence generation and ranking



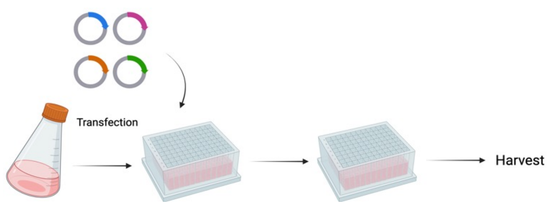
HTIgG PRODUCTION

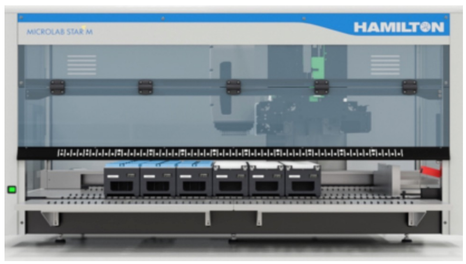
I. Reformatting & Clonal Gene Synthesis

Top binders from phage selections are reformatted to full length IgGs where their codons are optimized for mammalian expression and cloned into custom or catalog mammalian expression vectors.



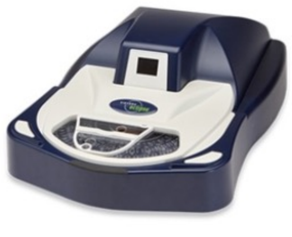
II. HT Antibody Production (HTIgG)





The reformatted IgG DNAs are transiently transfected into HEK293 cells. The supernatant is harvested after 4 days of culture and purified on Hamilton using Protein A columns.

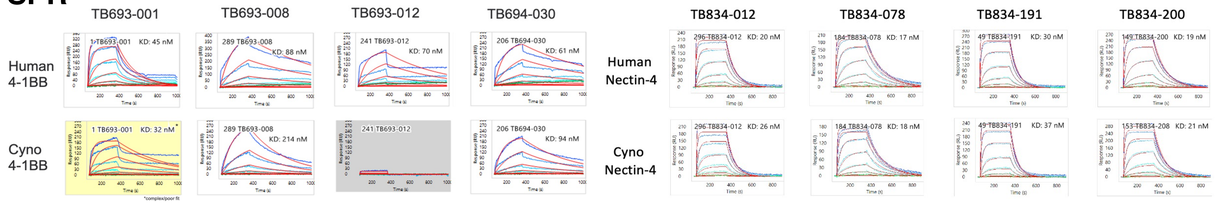
III. Antibody QC Panel



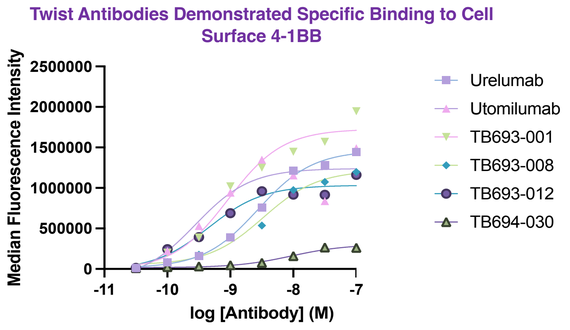
The purified IgGs quants, purity, thermostability, and endotoxin levels are measured by Lunatic, CE-SDS Labchip, HPLC, UNCLE, and Sievers Eclipse, respectively.

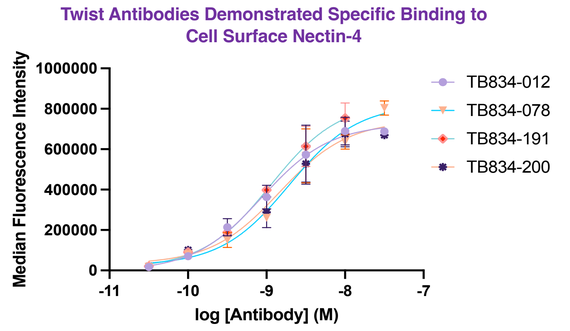
MAJOR RESULTS

I. Affinity Measurement of Top 4-1BB and Nectin-4 Antibody Candidates with Carterra SPR



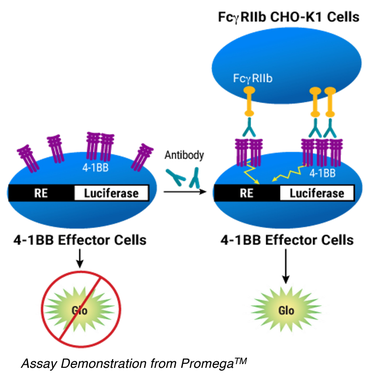
Anti 4-1BB antibodies are discovered from Twist's library of libraries using phage display, followed by clonal ELISA and sanger sequencing. The antibodies were then expressed with Twist High throughput IgG Production (HTIgG) service, followed by Carterra SPR characterization. Notably, these antibodies demonstrated nanomolar affinities and exhibited cross-reactivity towards both human and cynomolgus monkey 4-1BB and Nectin-4, respectively, which were selected for further characterization with cell-based assays.

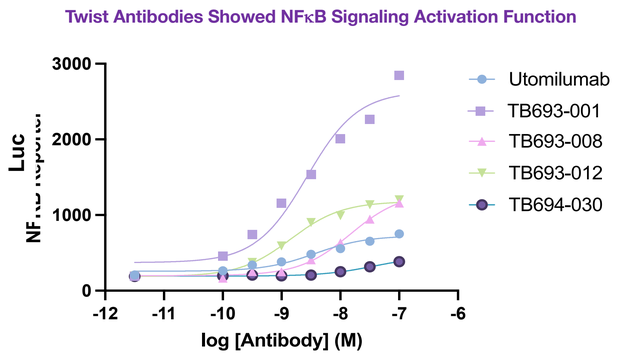




4-1BB and Nectin-4 antibody candidates were tested with cell binding assays using human 4-1BB and Nectin-4 overexpressing HEK293 cells, respectively. Antibodies with top affinity showed dose dependent binding to cell surface antigens.

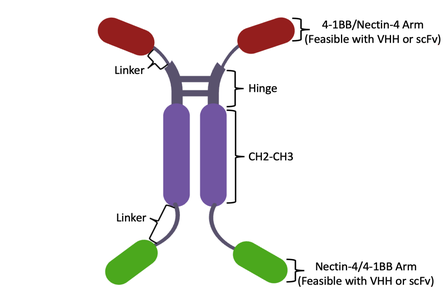
II. 4-1BB Antibody Candidates Trigger In Vitro NF-κB Activation

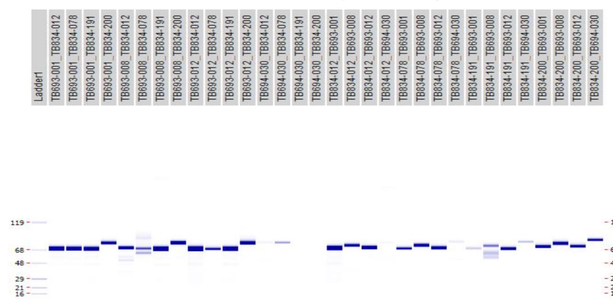


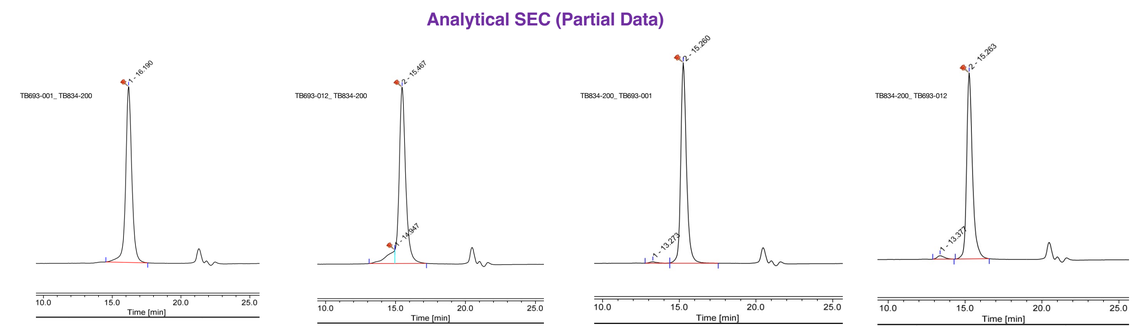


Anti 4-1BB candidates with top binding affinities were selected for further characterization with NFκB signaling activation assay. Twist Antibodies demonstrated NFκB activation activity similar to or stronger than the clinical control antibody Utomilumab when tested using an assay kit from *Promega™*.

III. Design, Expression, and QC of "2+2" 4-1BB-Nectin-4 Bispecific Antibodies

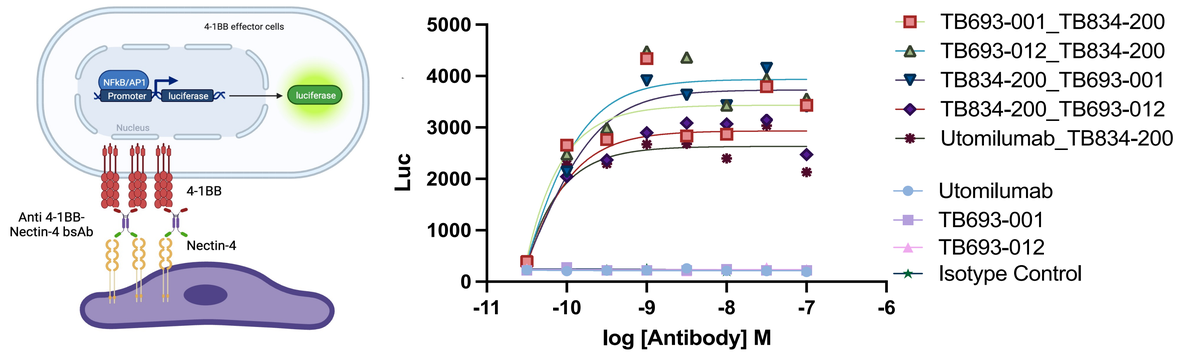






Top 4-1BB and Nectin-4 antibody candidates were selected and formatted to "2+2" bispecific antibodies. Following Twist's HTIgG 8 mL antibody expression, they were checked with CE-SDS and analytical SEC for initial developability/QC characterization. Antibody candidates with high purity, % of monomer, and minimal heterogeneous post translational modifications are selected to move forward with further assays.

IV. 4-1BB-Nectin-4 Bispecific Antibodies Candidates Show Dual Binding And NF-κB Signaling Activation Function In A Two-Cell Assay



In the two-cell assay setup, HEK293 cells over expressing Nectin-4 were initially treated with 4-1BB-Nectin-4 bsAbs. Non-bound antibodies were removed via thorough washing. Subsequently, 4-1BB effector cells were introduced and allowed to interact with the Nectin-4-expressing cells, followed by a subsequent measurement of luminescence signal. Twist's bsAbs exhibited similar to stronger level of signaling activation activity in comparison to Utomilumab when using the same bsAb format.

SUMMARY

Twist Biopharma Solutions (TBS) is committed to collaborating with our partners in the pursuit of antibody therapeutics. Our approach leverages an integrated platform that encompasses synthetic libraries, immunized libraries, AI/ML (Artificial Intelligence/Machine Learning), and high-throughput IgG Production (HTIgG). Herein, we have showcased how TBS harnesses our synthetic Libraries of Libraries (LOL) to discovery and engineer anti-4-1BB-Nectin-4 bispecific antibodies that exhibit both a high level of target-specific affinity and potent signaling activation functions.