Antibody discovery for GPCR C5aR1

An immunized and synthetic immune phage library approach

Monoclonal antibodies are an increasingly important source for novel therapeutics, however, bringing one to market is far from trivial. Twist Biopharma Solutions is helping researchers improve their odds of success in antibody discovery and optimization by combining immunization and synthetic approaches. This allows for the rapid identification of lead candidates with favorable pharmacokinetic profiles which can be rapidly optimized and humanized. This study illustrates the power of Twists ability to generate robust immune libraries leveraging both in vivo and in vitro workflows.

IMMUNE VS SYNTHETIC IMMUNE PHAGE ANTIBODY LIBRARIES

Display-based antibody lead identification is typically done using either immune or naive antibody libraries. An immune library is derived from the B-cells of an animal that has been immunized with a target antigen. Such a process produces antibodies that have already undergone natural *in vivo* affinity maturation, meaning they're likely to have a high affinity for their target along with favorable pharmacokinetic profiles. One complexity of this approach is that lead candidates' sequences will need to be humanized which can potentially impact both specificity and affinity.

On the other hand, synthetic immune phage libraries are comprised of DNA sequences inspired by natural sources—such as immune libraries. Unlike an immune library, synthetic immune phage libraries will generate novel sequence combinations based on the construction method. Importantly, these libraries have a much greater, and precisely controlled, antibody diversity, providing researchers with a greater pool of potential sequences to screen. Additionally, these antibodies can be synthesized in developable human frameworks, eliminating the need for humanization. A limiting factor of synthetic immune phage libraries is that the antibodies may require careful optimization to improve both pharmacological and pharmacokinetic properties.

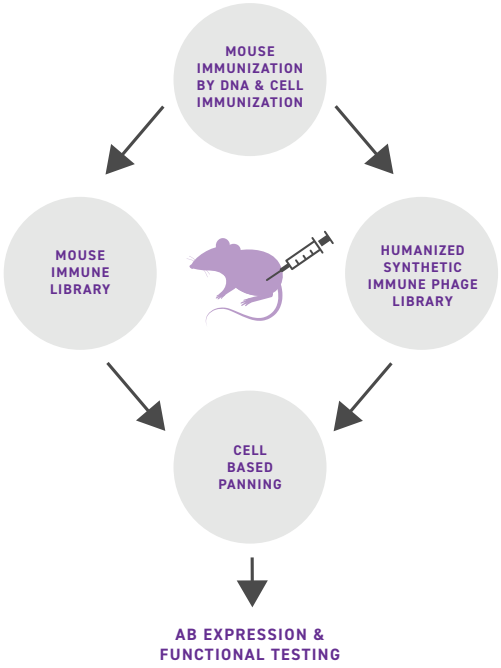
An emerging approach allows for the combination of both library types, giving researchers the best of both worlds.

A DUAL APPROACH TO ANTIBODY DISCOVERY

In a recent study, Twist researchers used a dual antibody discovery approach **(Fig. 1)** combining both immune and synthetic immune phage libraries libraries to rapidly identify high-affinity, humanized antibodies targeting the human complement component 5a receptor (C5aR1). This difficult-to-target G-protein coupled receptor (GPCR) participates in immune-driven inflammatory signaling and is a potential therapeutic target in numerous conditions including Alzheimer's disease, COVID-19, and asthma.

To develop therapeutic anti-C5aR1 antibodies, Twist researchers began by immunizing proprietary, hyperimmune mice, DiversimAb™, with human wildtype C5aR1. Next, RNA was collected and sequenced to create the immune library. Simultaneously, RNA was collected and sequenced to identify common antibody motifs. This data informed the construction of a synthetic humanized antibody library in which mouse complementarity determining regions (CDRs) were placed within human antibody frameworks.

This combined approach enabled the team to proceed into antibody screening with a large, diverse, yet antigen-specific cohort of prospective antibodies.



SYNTHESIZING SUCCESS

Biopanning of both the immune and synthetic immune phage libraries produced several promising binders, of which the top 96 were selected to be reformatted, expressed as full-length IgG1 antibodies, and tested for their ability to specifically bind to human C5aR1.

Binding assays were performed using a cell line stably expressing human C5aR1 protein **(Fig. 2)**. Several promising candidates were identified, with the top 10 most specific binders representing a near-even mixture of antibodies derived from either synthetic immune phage or immune libraries.

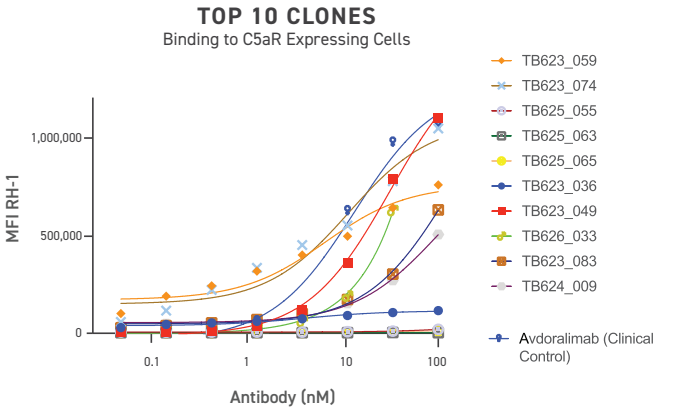


Figure 2. Cell binding assay demonstrates specific and comparable binding affinity against C5aR1 relative to the clinical control Avdoralimab. A higher MFI (median fluorescence intensity) ratio indicates higher binding specificity. Candidates with odd TB numbers (i.e. TB623_059) are derived from the immune library, whereas even numbered candidates come from the synthetic library.

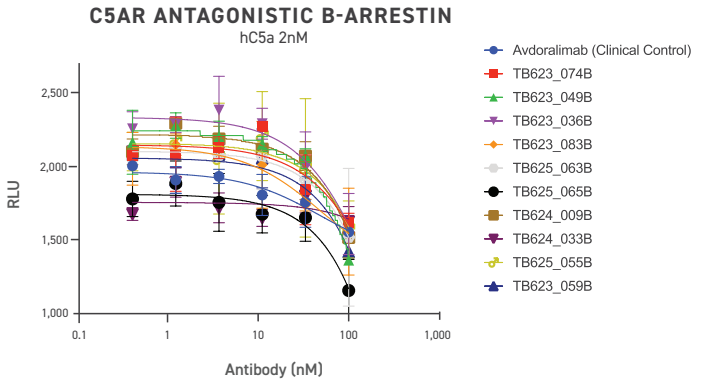


Figure 3. B-arrestin recruitment assay, demonstrating decreasing recruitment (measured in relative light units, RLU) with increasing antibody titers. Candidates whose name end in an odd number (i.e. TB623_059) are derived from the immune library, whereas even numbered candidates come from the synthetic library.

With the goal of inhibiting checkpoint signaling, the team next assessed the candidates' ability to disrupt signaling from C5aR1 in a β-arrestin recruitment assay **(Fig. 3)**. Results showed strong inhibition of β-arrestin recruitment on par with the clinical control, Avdoralimab.

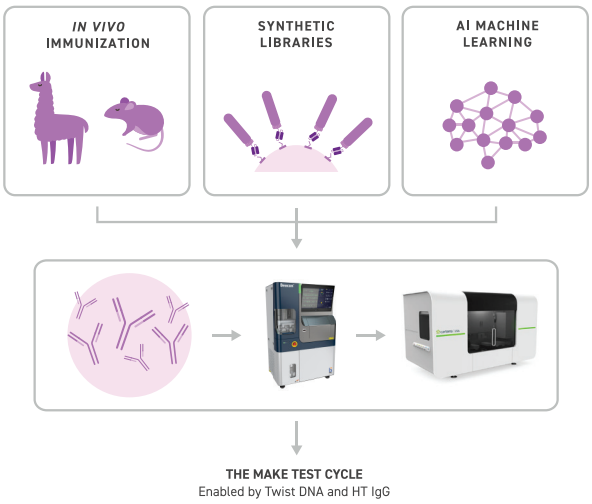
This data shows that without optimization, several antibody candidates have been identified with comparable performance to a clinical control, indicating their high potential for clinical development.

These results highlight the benefits of using a dual approach to antibody discovery. Generation of an immune library enabled the rational construction of a large synthetic library, which gave researchers a broader pool of potential candidates to test, ultimately resulting in a greater quantity of promising candidates.

TWIST ANTIBODY DISCOVERY AND OPTIMIZATION

Twist Biopharma Solutions has developed a suite of tools to help accelerate the antibody discovery and optimization process, including:

- **Immunization strategies:** Using mice, alpaca and rabbit coupled with rapid single B-cell screening or immune library construction.
- **Library-of-libraries:** A rich trove of synthetic antibody libraries designed against hard-to-drug targets (such as GPCRs) for rapid, large-scale screening.
- **Antibody Humanization and Optimization:** A platform that incorporates machine learning to rationally humanize and optimize promising candidates to improve developability.
- **Out Licensing:** Did you know Twist has already identified candidates ready for therapeutic development available for licensing? Email us to find out more.



TO LEARN MORE ABOUT TWIST BIOPHARMA SOLUTIONS' ANTIBODY DISCOVERY AND OPTIMIZATION SOLUTIONS, CONTACT THE TWIST BIOSCENCE TEAM.

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