TWIST BIOSCIENCE

APPLICATION NOTE

Deep Repertoire Mining Uncovers Ultra-broad Coronavirus-Neutralizing Antibodies using Twist's High Throughput Antibody Production and SPR Screening

INTRODUCTION

As our bodies produce broadly neutralizing polyclonal antibodies and antigen-experienced immune cells to new, previously seen, or evolutionarily-related variants, pathogens co-develop immune evasion tactics to help conceal their neutralizing epitopes. A very deep scan of the pathogen-specific antibody repertoire can be employed to discover exceedingly rare and broadly neutralizing antibodies.

Most of the identified neutralizing binders against SARS-CoV-2 bind to either the receptor-binding domain (RBD), the S2 subunit, or the N-terminal domain (NTD), with the broadest binding observed for the S2 and RBD regions. The NTD is not particularly conserved, and the neutralizing antibodies typically bind to a single supersite and have limited genetic variability. NTD binders have been identified for neutralizing several variants of concern but are limited to binding only to SARS-CoV-2.

Study Goal: develop a universal or pan-coronavirus (CoV) vaccine to help protect against the ongoing emergence of increasingly diverse variants of concern or "evasion-strong" viruses.

WORKFLOW OVERVIEW

In general, patient samples were collected from early COVID-19 survivors who were subsequently vaccinated, subjected to sequence analysis and selection, expressed, purified, characterized biochemically, and tested functionally (**Figure 1**). More than 9,000 SARS-CoV-2-specific natively-paired mAbs were identified through multiplexed antigen screening using single-cell immune profiling technology (**Figure 2**). This screening method enabled the discovery of extremely rare but desirable Ab sequences and informing researchers of the broad pathogen-specific repertoire generated in infected and vaccinated patients. Lastly, substantial clonal coverage for SARS-CoV-2 specific mAbs was observed, whereby mAbs from various individuals coalesce around similar genetic solutions for pathogen recognition.

A high-throughput production and characterization workflow was employed to screen the functional properties of the 9,000 patient-generated mAb sequences identified (**Figure 3**). Briefly, a subset of 293 antibodies were recombinantly expressed using Twist Express Antibodies along with Twist Characterization Services of Affinity Ranking followed by Epitope Binning (**Figures 4** and **5**). For a detailed description of Materials and Methods, read Hurtado *et al*.

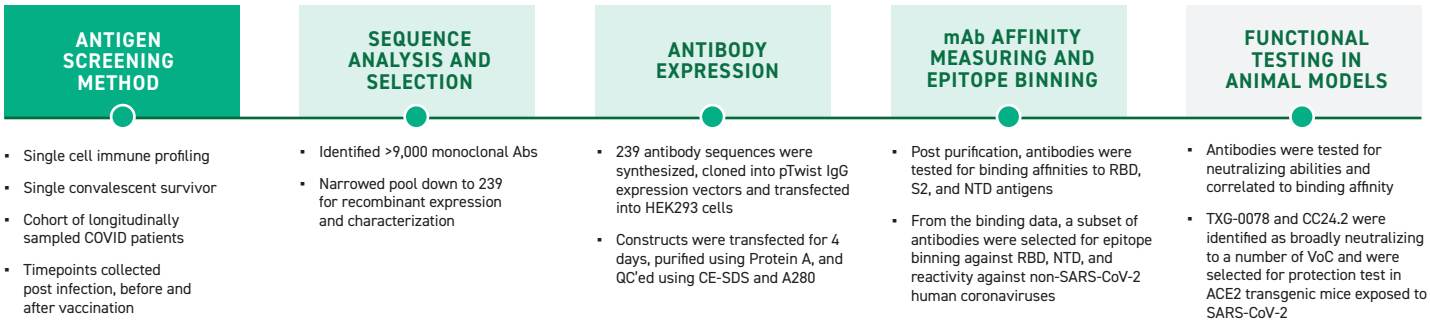

Figure 1. Experimental workflow for identifying broadly protective Abs.

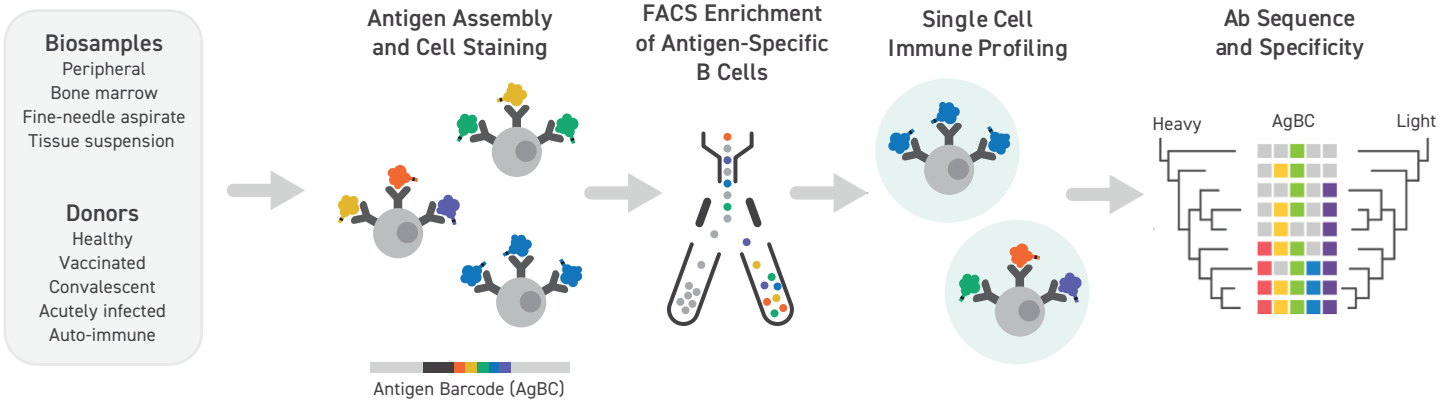

Figure 2. Overview of the multiplexed antigen screening method. Figure is adapted from Hurtado *et al.*

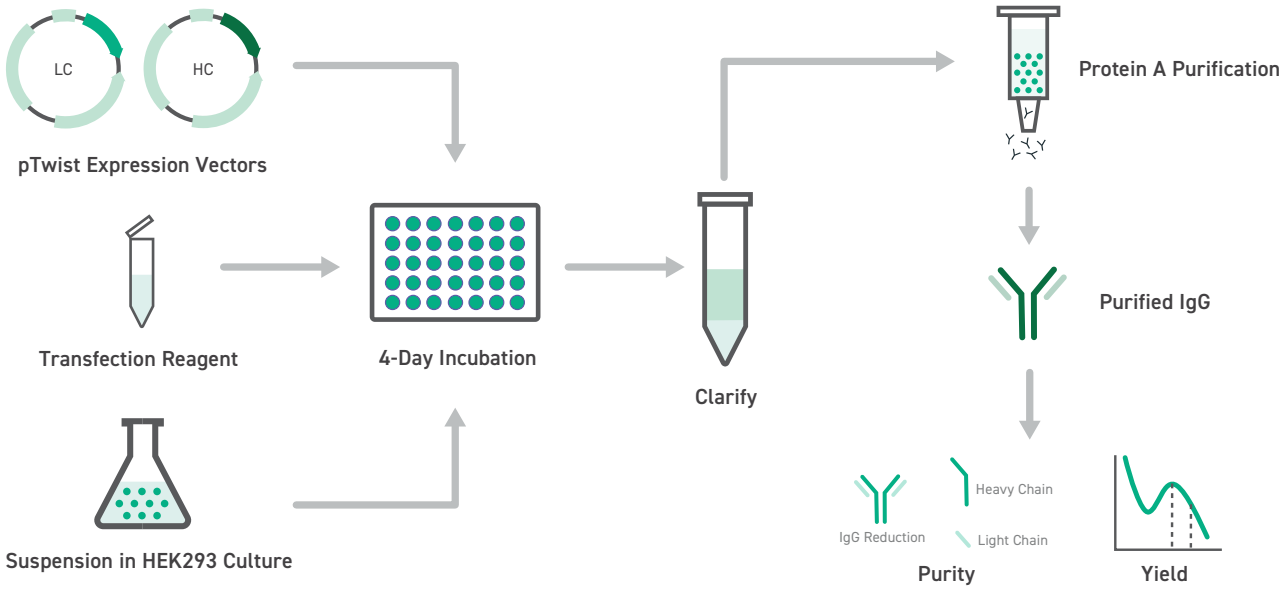

Figure 3. Ab expression, purification, and characterization workflow. mAb variable regions were expressed in an IgG1 backbone vector, pTwist, and transfected into HEK293 cells grown in suspension. Following a 4-day incubation period, IgG was purified. Yield was measured using ABS280, and purity was assessed using capillary electrophoresis sodium dodecyl sulfate (CE-SDS).

RESULTS

After identifying paired sequences and screening through binders to S2, RBD, and NTD, a subset of 239 antibodies were selected for recombinant expression and detailed characterization. Epitope binning revealed five specificity groups (**Figure 4**). Three groups are bound to RBD epitopes targeted by currently approved therapeutics, while the two other groups have distinct specificity for epitopes in the NTD. One group (**red group; Figure 4**) could not bind to the Beta and Omicron S-protein, while the other (**yellow group; Figure 4**) could not bind to the Omicron S-protein (**Figure 5**).

The specific antibody response to SARS-CoV-2 was also quantified using an expanded array of antigens designed to measure binding across various viral variants. In a longitudinal study involving individuals who had recovered from SARS-CoV-2 infection and received subsequent vaccination, the authors identified 6,302 paired mAbs. The utilization of heavy chain variable genes among SARS-CoV-2-specific mAbs exhibited significant diversity, and the authors did not detect notable enrichment of particular heavy chain variable genes among cross-reactive mAbs (**Figure 6**).

Ultimately, two antibodies of interest were identified as potential future candidates for pan-CoV vaccine development. A variant, CC24.2, was identified as targeting a novel RBD epitope while exhibiting pan-variant binding to BA, BQ, and XBB variants. Additionally, TXG-0078 was identified as an NTD-specific binder of a diverse range of alpha- and beta-CoVs.

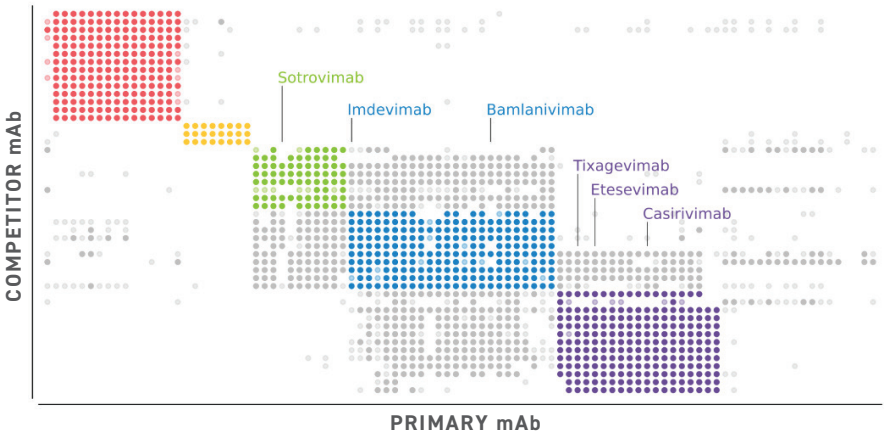

Figure 4. A subset of 239 antibodies was chosen for recombinant expression using Twist Express Antibodies, irrespective of their original isotype. Epitope binning was performed using a high-throughput SPR competitive binding assay on the Carterra LSA platform, revealing five main specificity groups. Among these, three groups target RBD epitopes, which are also the focus of therapeutic antibodies, while the remaining two groups correspond to NTD epitopes, not targeted by any marketed biologics. Figure is adapted from Hurtado *et al.*

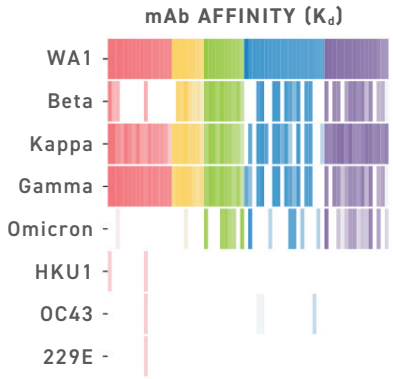

Figure 5. SPR binding screening against a wide panel of SARS-CoV-2 variants of concern and seasonal coronaviruses demonstrates a range of cross-reactivity. The heatmap depicts antibody binding affinity (KD). Figure is adapted from Hurtado *et al.*

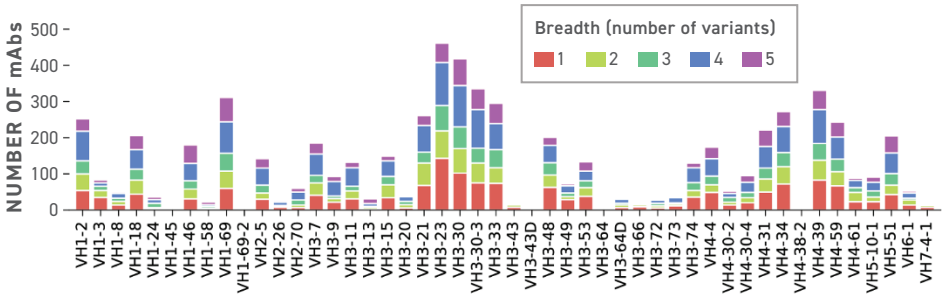

Figure 6. The diversity of heavy chain variable genes among SARS-CoV-2-specific mAbs, categorized by their ability to recognize multiple variants. There was no notable overrepresentation of particular variable genes among antibodies that cross-reacted with different variants. Leveraging Twist HT IgG and characterization services facilitates the comprehensive identification of genotype-phenotype relationships. Figure is adapted from Hurtado *et al.*

CONCLUSIONS

Identifying conserved, neutralizing epitopes continues to be challenging for identifying effective, escape-resistant antibody therapeutics. This manuscript demonstrates how multiplexed antigen screening with single-cell immune profiling technology enables deep insight into determining functional immune repertoires, identifying over 9,000 CoV-specific, fully human antibodies.

Following the identification of these sequences, the next challenge arises in synthesizing, expressing, and purifying the antibodies at scale and characterizing hundreds or thousands of leads for binding affinity, specificity, and epitope analysis. Twist Biopharma Solutions' High Throughput Antibody (HT IgG) production is a gene-to-protein workflow that relieves this bottleneck by producing tens to thousands of diverse antibodies for screening biophysical and pharmacokinetic properties. HT IgG production is enabled by Twist Biosciences' silicon-based DNA synthesis platform, which can precisely write thousands of genes per run, enabling the rapid and high-throughput production of antibodies for discovery and screening.

Delivery of up to 2 mg of each antibody at scale enabled the identification of rare, NTD-specific neutralizing antibodies that bind a broad range of alpha- and beta-coronaviruses and protect against in vivo challenge by SARS-CoV-2. The authors also report the discovery of CC24.2, a neutralizing antibody broadly neutralizing pan-sarbecoviruses targeting a novel RBD epitope.

Twist HT IgG enables the interrogation of many antibody leads identified from large-scale, functional analysis of circulating B cells. Using the single-cell multiplexed antigen screening approach, the authors could simultaneously couple genetics with the binding specificities of antibodies identified from thousands of B cells, enabling the analysis of pathogen-specific humoral response at single antibody resolution. This approach can accelerate our understanding of SARS-CoV-2 and infectious disease biology more broadly, allowing rapid identification of ultra-rare antibodies with ideal specificities and more durable immunity in the face of a rapidly evolving infectious disease landscape.