WHITE PAPER

Rapid discovery and characterization of monoclonal antibodies against the SARS-CoV-2 Delta spike protein

By combining our PentaMice® wild-type mice for optimal immunizations, single B cell selection with Opto® Plasma B Discovery 4.0 workflows on the Berkeley Lights® Beacon® Optofluidic System and speedy sequencing and developability analysis, Curia's First-to-Human antibody discovery service can progress from hits to leads in as little as 120–240 days.

Margaret Wong Ho, General Manager and Site Head, Curia Global, Inc.
Christine L. Hsieh, Senior Scientist II, Cellular Immunology Assay Development, Curia Global, Inc.
Xiaomei Ge, Senior Scientist II, Curia Global, Inc.
Dan Luo, Senior Scientist II, Curia Global, Inc.
Brian A. Zabel, Senior Director, Curia Global, Inc.

In the fall of 2021, the Delta variant of SARS-CoV-2 was the dominant strain in the US, being both more contagious than previous variants and more likely to lead to "long COVID" than subsequent Omicron variants. Here we describe the discovery and characterization of a large number of Delta spike-binding monoclonal antibodies (mAbs). By combining detailed DNA sequence analysis and binding assays, we identified 96 candidates for further analysis and development. Many of these hits exhibited neutralizing activity and also cross-reacted with one or more of the wild-type virus, Omicron 1.1.529, BA.2 and BA.5 variants.

The COVID-19 pandemic has led to a rapid and wide-ranging search for therapeutics to protect against and treat the illness. Besides the vaccines and antivirals that have been developed against SARS-CoV-2, a number of mAbs have been given emergency use authorization (EUA) by the FDA. Unfortunately, the neutralizing effect of mAb therapeutics can be reduced or negated by the evolution of viral variants. For this reason, the expedited discovery and development of new mAbs with neutralizing activity selective for novel variants is essential.

These techniques can also be used to rapidly discover antibodies for the treatment of cancers, neurodegenerative diseases (e.g., Alzheimer's) and emerging infectious diseases.

This white paper details the workflow used to discover and characterize Delta spike-binding mAbs. For more detailed information on each of the steps of this workflow, see our white paper, Antibody-based drug discovery at the speed of light.

1. **Immunization of PentaMice® wild-type mice**
2. **Single B cell screening and selection using the Beacon® Optofluidic System**
3. **cDNA synthesis and next generation sequencing (NGS)**
4. **Bioinformatics to assess diversity and developability**
5. **Rapid re-expression for validation and characterization**
6. **Binding affinity (K_D) analysis**
7. **Delta spike binding potency and cross-reactivity with other spike variants**
8. **Neutralization activity**
9. **Epitope binning by Carterra® LSA® platform**
10. **Additional on-chip assays**

"LakePharma (now part of Curia) is a great partner to work with. Their expertise enables them to generate large panels of antibodies that can be used as the foundation for our therapeutic candidates."

— Ronald Herbst, Ph.D., *CSO, Pyxis Oncology*

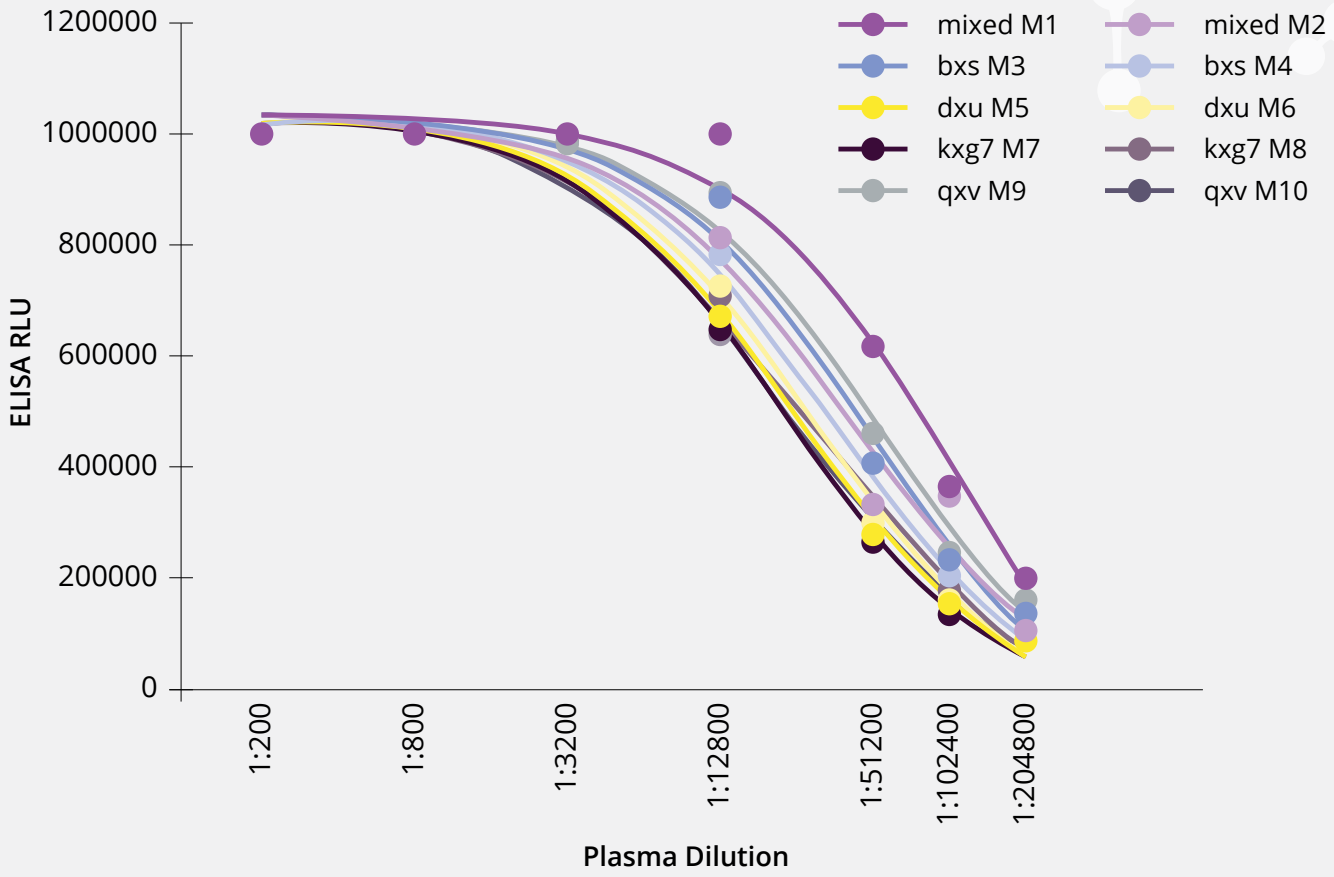
Immunization of PentaMice® wild-type mice with Delta spike protein

Curia's proprietary PentaMice® platform consists of five unique strains of genetically and immunologically diverse mice capable of presenting peptide antigen on 42 distinct MHC class II heterodimers derived from nine different MHC class II haplotypes. PentaMice genetics cast a wide net to load and present antigens to T helper cells that in turn promote B cell development into high-affinity plasma B cells. This strategy has proven to provide maximum titers across various antigens in a short amount of time.

We immunized two mice per strain with the Delta spike protein for a total of ten mice per immunization cohort (Figure 1).

Rapid immune response and maximum titers were achieved in just 17 days. All five PentaMice strains performed well, indicating a high likelihood of discovering a diverse set of Delta spike-binding mAbs.

Figure 1. Delta spike ELISA titers



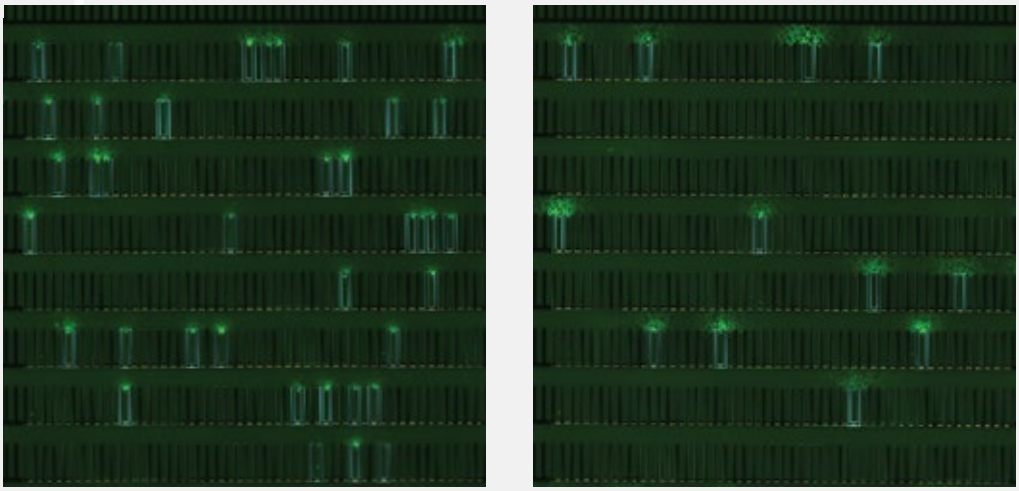
PentaMice wild-type mice were immunized with the Delta spike protein. Blood plasma was collected on day 17, diluted as indicated and tested for Delta spike binding by ELISA. RLU: relative light unit.

Single B cell screening and selection using the Beacon® Optofluidic System

We used magnetic beads to enrich CD138-positive B cells from splenocytes harvested from the immunized PentaMice wild-type mice. The enriched B cells were loaded onto three OptoSelect® 20k chips on the Beacon® Optofluidic System. Each chip contains approximately 20,000 NanoPen® chambers, each designed to isolate a single cell. To visualize the frequency of IgG-secreting B cells, anti-mouse IgG beads that capture the mouse antibodies secreted by each B cell were loaded onto the chips along with anti-mouse IgG conjugated to Alexa Fluor® 488 dye (green fluorophore). Beads bound to mouse IgG fluoresced green adjacent to the NanoPen chamber (Figure 2a). We then flushed the channels of the chip and, in a sequential antigen-binding screen, imported beads that were conjugated and tested in-house to Delta spike protein. Delta spike-binding mAbs were similarly identified by adding fluorescently labeled anti-mouse IgG, with green fluorescing blooms adjacent to the NanoPen chambers indicating a Delta spike-binding hit (Figure 2b). Overall we identified 1,894 Delta spike-binding hits (~3%) from the 60,000 NanoPen chambers in the experiment (Table 1).

Figure 2. On-chip mAb screening assays for mouse IgG and antigen binding

a b



(a) CD138+ plasma B cells from Delta spike immunized PentaMice wild-type mice were isolated and imported into 60,000 NanoPen chambers and screened on-chip by fluorescent microscopy for mouse IgG secretion and (b) for antigen-binding to Delta spike. Pictured is one field-of-view (FOV) out of 49 on an OptoSelect® chip containing 20,000-NanoPen chambers. In an automated manner, the Beacon® system identified hits by analyzing Alexa Fluor 488-positive blooms adjacent to NanoPen chambers and highlighted the pens in blue.

Automated, barcoded cDNA synthesis and sequence recovery

ON-CHIP BARCODED cDNA SYNTHESIS, HIGH-THROUGHPUT NGS AND AI-DRIVEN DEMULTIPLEXING FACILITATE ABUNDANT SEQUENCE RECOVERY

Automated sequence recovery begins with importing RNA capture beads into individual NanoPen chambers, each of which has a unique dual optical and genetic barcode to link sequences with each NanoPen chamber. The B cells were lysed on-chip, releasing mRNA that was captured by the beads and used as a template for cDNA generation. cDNA was multiplexed and then amplified. Then, amplicons from targeted amplification of variable heavy-chain (V_H) and variable light-chain (V_L) sequences were used to generate NGS libraries. For Delta spike mAb discovery, 1,082 beads were selected for export, and the export efficiency of this process was 84% (Table 1). NGS of the variable region genes of the cDNA revealed 248 unique, high-confidence paired heavy- and light-chain sequences.

> *"With LakePharma (now part of Curia), we really feel like we are interacting with scientists who are invested in our projects, and we trust their approaches and listen to their suggestions. We have a very high success rate for biologics discovery projects with LakePharma (now part of Curia) — and we did not give them easy targets, that's for sure."*
>
> – Ivo Lorenz, Ph.D., *Vice President of Biologics, Tri-Institutional Therapeutics Discovery Institute*

Table 1. SARS-CoV-2 Delta spike mAb recovery and analysis

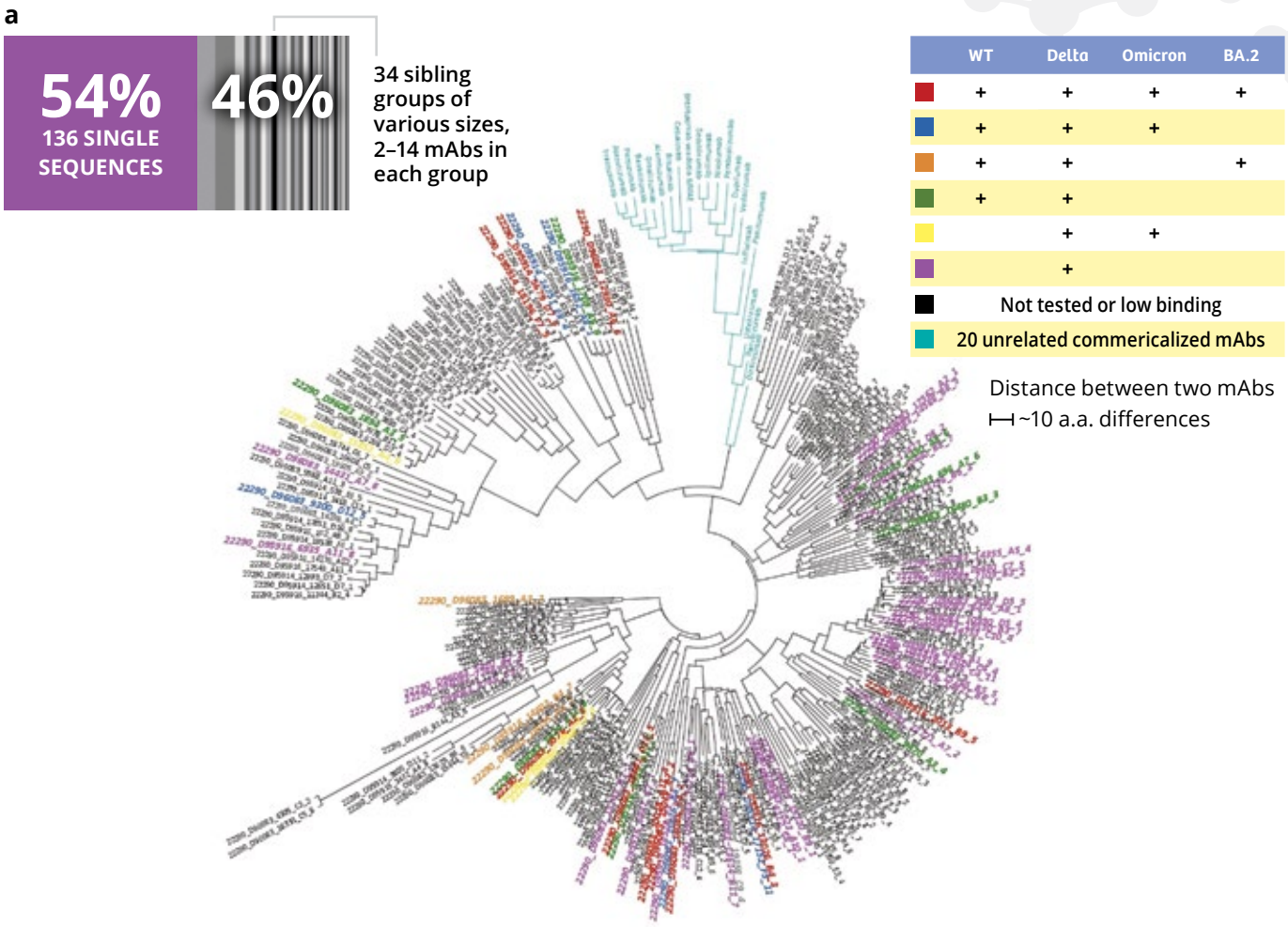
The results of screening 60,000 NanoPen® chambers with Berkeley Lights' Opto® Plasma B Discovery 4.0:

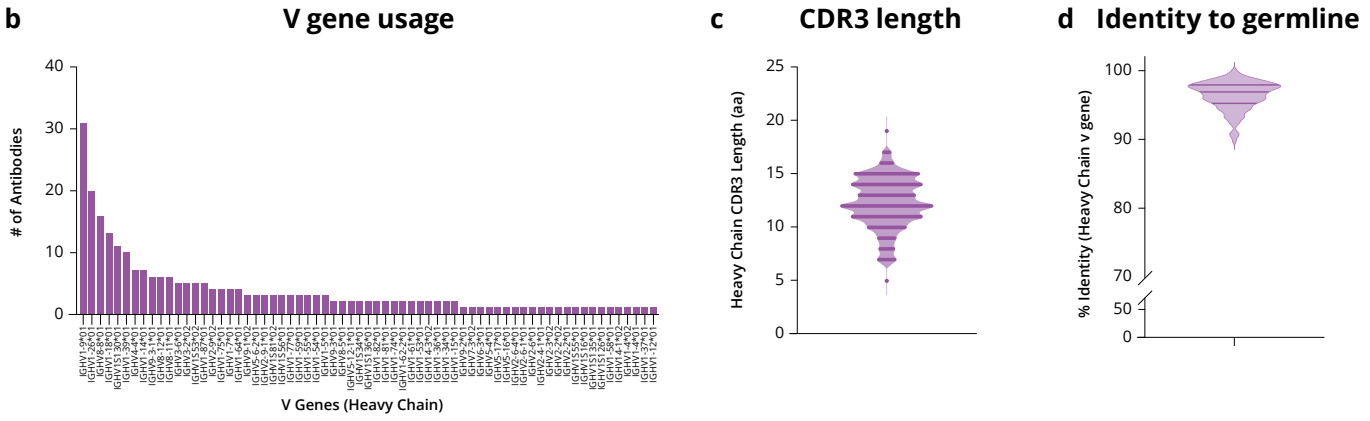
- **Delta spike+ pens: 1,894 (~3% hit rate)**
- **Export efficiency: 84%**
- **248 unique high-confidence paired heavy- and light-chain sequences using NGS**
- **170 clonal families, using a diverse set of 67 V_H genes and 54 V_L genes**
- **Rapid re-expression of Delta-selective mAbs validated with demonstrated cross-reactivity to WT, Omicron and/or BA.2 SARS-CoV-2 spike protein**
- **Good developability: More than half of the hits have fewer predicted liabilities than 20 commercialized blockbuster mAbs**

Bioinformatics analysis reveals broad mAb diversity and developability

The V_H and V_L sequences of all 248 Delta-binding antibodies were compared to each other, as well as to 20 unrelated commercialized mAbs, in a lineage tree, or polar dendrogram (Figure 3a). The shorter the radial distance between two hits, as represented on the dendrogram, the more closely their sequences are related. The dendrogram shows the broad heterogeneity of antibodies discovered. All 20 of the commercialized mAbs (e.g., Rituxan, Herceptin) are distantly related to the spike antibodies as expected (Figure 3a, teal). There are 170 families of clonal Delta spike-binding antibodies represented, comprising 136 single sequences and 34 sibling groups of various sizes, each having 2–14 members per group (Figure 3a), using a total of 67 different V_H genes (Figure 3b) and 54 different V_L genes. The variability in the heavy-chain CDR3 length shows that this panel of hits displays great diversity (Figure 3c), and the divergence from germline sequences provides a measure of the somatic hypermutation that occurred as part of the *in vivo* affinity maturation process (Figure 3d).

Figure 3. mAbs discovered from the Beacon® system exhibit extensive diversity





(a) A custom bioinformatics pipeline following NGS was used to identify 248 unique high-confidence paired heavy- and light-chain sequences, depicted as a rooted dendrogram. The antibody sequences were grouped into 170 clonal families (defined as individual sequences sharing <94.5% identity with any other antibody (n=136), or sibling groups of antibodies sharing >94.5% identity within a family (n=34 sibling groups) (inset, left). Rapid re-expression of selected mAbs identified diverse cross-reactive binding profiles to Delta, WT, Omicron and/or BA.2 SARS-CoV-2 spike proteins, denoted by the indicated colors (inset, right). (b) Analysis of the variable heavy-chain gene use identified 67 different heavy-chain V genes in the 248 mAbs with a (c) broad range of CDR3 lengths and (d) considerable divergence from germline sequences, reflecting the extent of *in vivo* somatic hypermutation.

RAPID RE-EXPRESSION OF GENE SEQUENCES IN HEK293T

Additional assays after NGS were used to winnow the number of promising hits. A subset of the Delta-selective mAbs was chosen for re-expression to maximize diversity and to select representative family members from sibling groups with the fewest predicted manufacturing liabilities. DNA sequences from heavy- and light-chain variable regions of 96 hits were amplified by PCR and used to transiently transfect HEK293T cells. Secreted IgG was collected within five days and assessed by ELISA.

Among the Delta-selective mAbs selected, many showed cross-reactivity to the wild-type, Omicron and/or BA.2 SARS-CoV-2 spike protein (Figure 3a).

THE MAJORITY OF DELTA SPIKE-BINDING mAbs HAVE FAVORABLE DEVELOPABILITY PROFILES

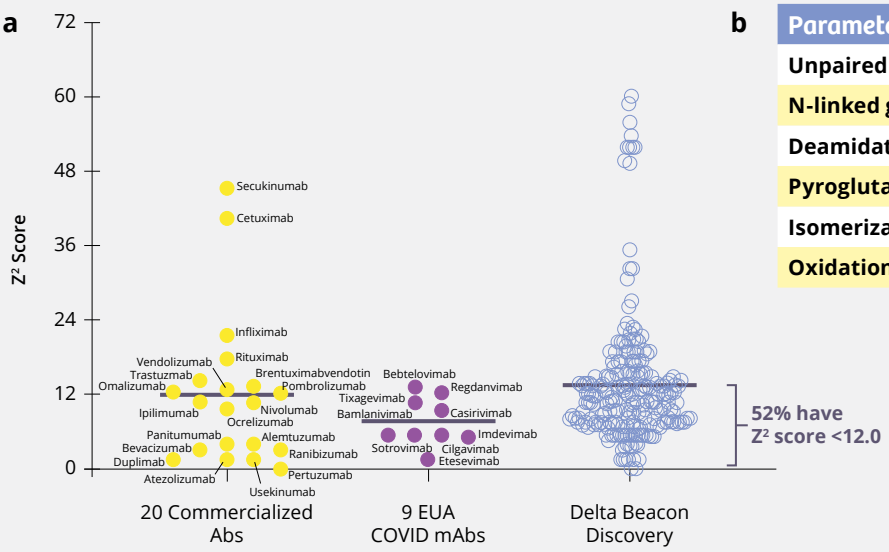
Curia's proprietary Z^2 liability score is an assessment of certain theoretical developability issues via sequence-based identification of six common potential liability parameters (Figure 4b), each of which is weighted based on its frequency in a set of 20 FDA-approved, manufactured and marketed monoclonal antibodies (yellow). The worst sequence-based liability is having an unpaired cysteine, as this increases the chances of protein misfolding and aggregation during manufacturing.

While this is a good way to gauge the developability of a hit, a high Z^2 score does not necessarily eliminate a candidate. There are examples of successfully manufactured, FDA-approved mAbs with high Z^2 scores, like Secukinumab, which has an unpaired cysteine, an isomerization potential and an oxidation potential, giving it a Z^2 of 45.2; however, these mAbs often require specialized production workflows.

As shown in Figure 4a, more than half of the Delta-selective mAbs tested have a developability score below 12, well below many successfully commercialized mAbs.

Figure 4. *In silico* developability analyses can be used to help select candidate mAbs with relatively low potential developability risks

a



b

Parameter	Score
Unpaired cystsine(s)	40.0
N-linked glycosylation	13.3
Deamidation	6.7
Pyroglutamate formation	4.0
Isomerization	3.6
Oxidation in CDRs	1.5

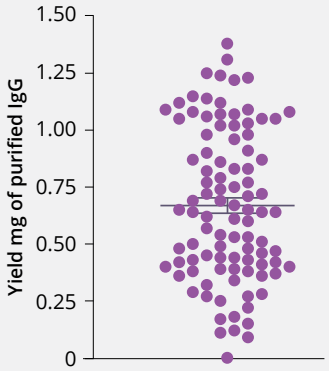
(a) Z^2 scores of 20 commercialized mAbs, 9 COVID-19 mAbs granted emergency use authorization and the Delta-selective antibodies discovered at Curia using the Beacon® system. (b) Liabilities that can adversely affect developability used to determine the Z^2 score.

RAPID RECOMBINANT PRODUCTION OF LEAD mAbs IN THE TunaCHO℠ PLATFORM

Using a combination of sequence analysis and rapid re-expression data, the top 96 antibody sequences were selected for reformatting and rapid recombinant expression using Curia's TunaCHO seven-day transient antibody production process. The antibodies were expressed as mouse IgG2a kappa, and the average yield from 10 mL cultures was 0.67 mg (Figure 5).

Figure 5. Recombinant antibody yields following small-scale high throughput production in Curia's TunaCHO℠ platform

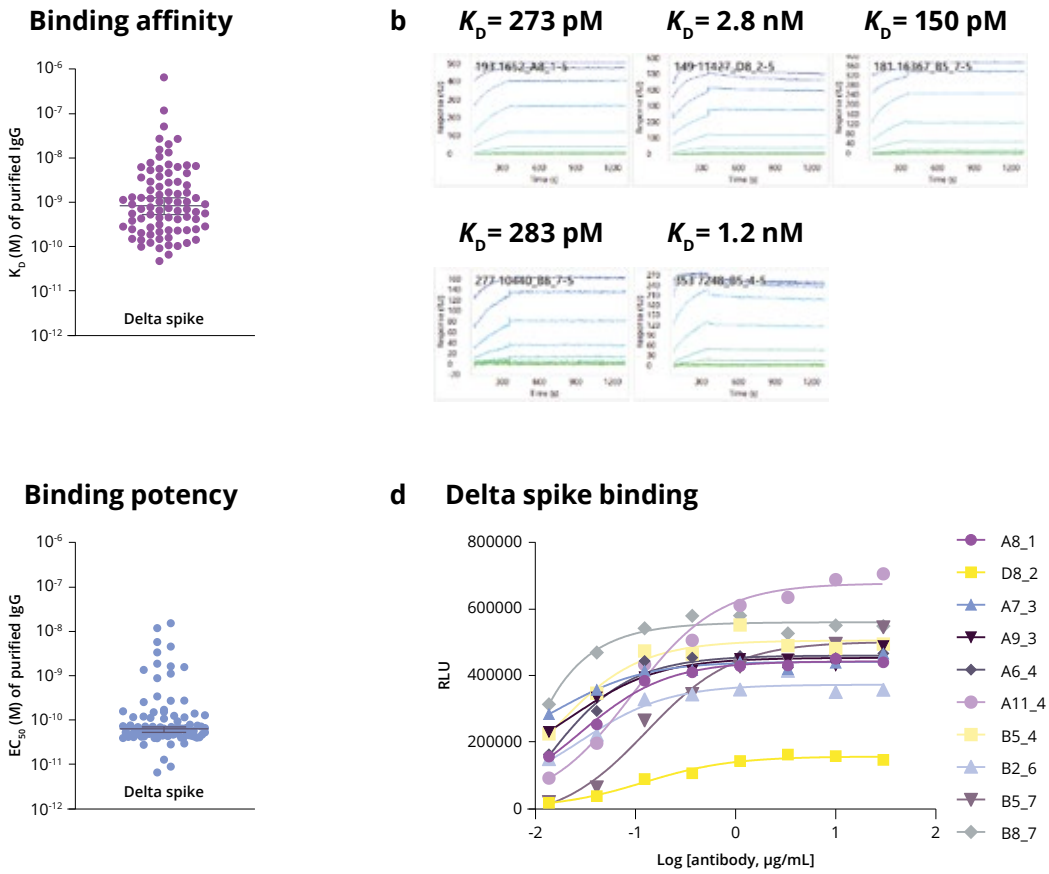
Antibodies were all produced as mIgG2a kappa and purified using Protein A purification methods (n=96, mean ± SEM, 0.67 ± 0.03 mg).



QUANTITATIVE ASSESSMENT OF DELTA SPIKE BINDING ACTIVITY

Using purified antibodies generated from TunaCHO productions, we determined the Delta spike binding affinities (K_D) by surface plasmon resonance using the Carterra® LSA® platform (Figure 6a,b) and the binding potency (EC_{50}) by ELISA (Figure 6c,d). Many high-affinity, highly potent antibodies were discovered having subnanomolar K_D (median=837 pM) and EC_{50} (median=64 pM) against Delta spike.

Figure 6. Quantitative analysis of Delta spike binding affinity and potency



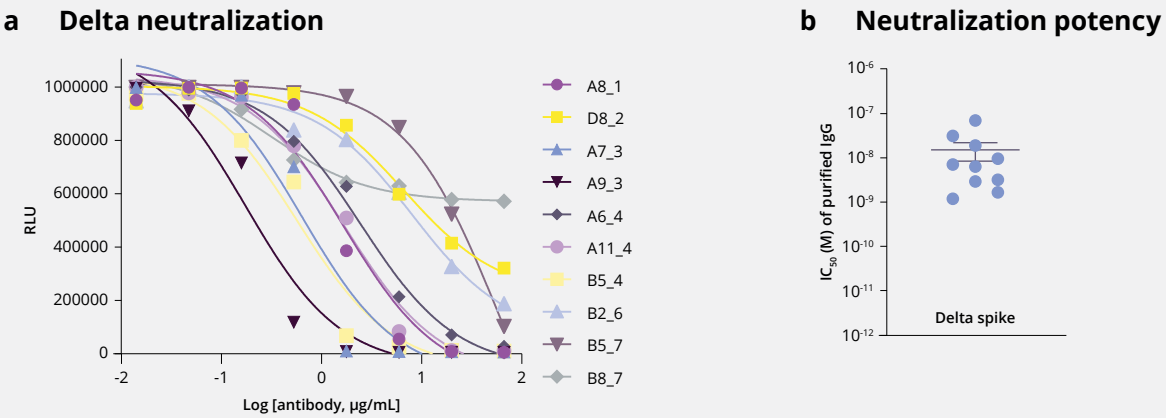
Binding affinities (K_D) were determined by surface plasmon resonance using the Carterra® LSA® platform for recombinant mAbs discovered with the Beacon® system. (a) Median K_D= 837 pM ± 95% CI shown. (b) Carterra® LSA® platform sensorgrams illustrate the binding kinetics of 5 Delta spike mAbs that neutralize huACE2 receptor binding. (c) Delta spike binding potencies were determined by ELISA. Median EC_{50}=64 pM ± 95% CI shown. (d) ELISA binding curves for the top 10 Delta neutralizing mAbs.

DELTA NEUTRALIZATION

SARS-CoV-2 enters cells by binding to the ACE2 receptor on host cells. An effective neutralizing antibody will bind to the Delta spike protein and prevent the virus from binding to ACE2. We tested this *in vitro* with an ELISA neutralization assay. This gives us an IC_{50} value, the measurement of the concentration of the mAb needed to block 50% of the binding of the spike protein to ACE2. The lower the IC_{50}, the more potent the neutralization.

Ten percent of the Delta spike binding antibodies we tested were potent Delta neutralizers, with an average IC_{50} of ~15 nM (Figure 7).

Figure 7. Quantitative analysis of Delta spike neutralization activity

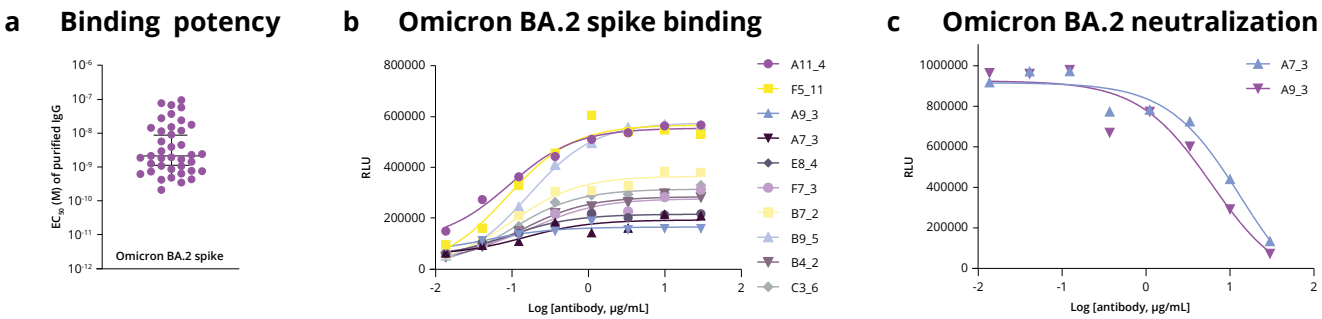


(a) Delta neutralization potencies for 10 of 96 mAbs were determined using an ELISA-based biofunction assay. Plates were coated with huACE2 and mAb-dependent inhibition of Delta spike binding was determined. (b) IC_{50}=14.6 ± 6.5 nM, mean ± SEM shown.

OMICRON CROSS-REACTIVITY AND NEUTRALIZATION

More than 40% of the Delta-binding antibodies also bound to Omicron BA.2 with single-digit nanomolar binding potencies (median EC_{50}=2.1 nM) (Figure 8a,b). Interestingly, two antibodies also effectively neutralized both Delta and Omicron BA.2 binding to huACE2 (Figure 8c).

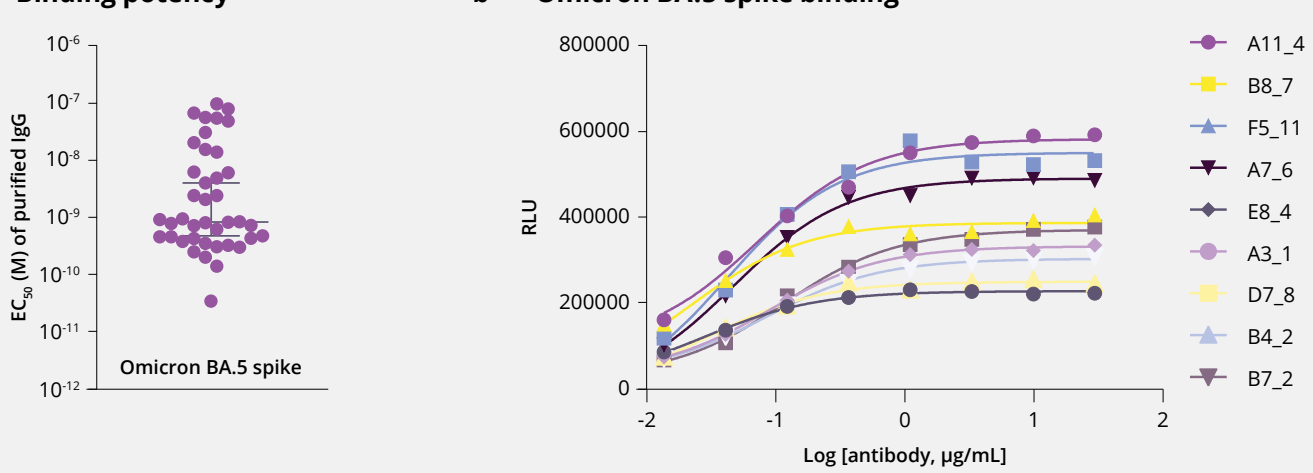
Figure 8. Quantitative analysis of Omicron BA.2 spike binding potency and neutralization activity



(a) Omicron BA.2 spike binding potencies for 39 of 96 mAbs were determined by ELISA. EC_{50}=2.1 nM, median ± 95% CI shown. (b) ELISA binding curves demonstrate binding activity of the top 10 Omicron BA.2 binding mAbs. (c) Omicron BA.2 neutralization potencies were determined using an ELISA-based biofunction assay. Plates were coated with huACE2 and mAb-dependent inhibition of Omicron BA.2 spike binding was determined. IC_{50}=30.3 (A9_3) and 57.6 (A7_3).

More than 40% of the delta-binding antibodies also bound to Omicron BA.5, currently the most prevalent variant of SARS-CoV-2 in the US (Figure 9).

Figure 9. Quantitative analysis of Omicron BA.5 spike binding.

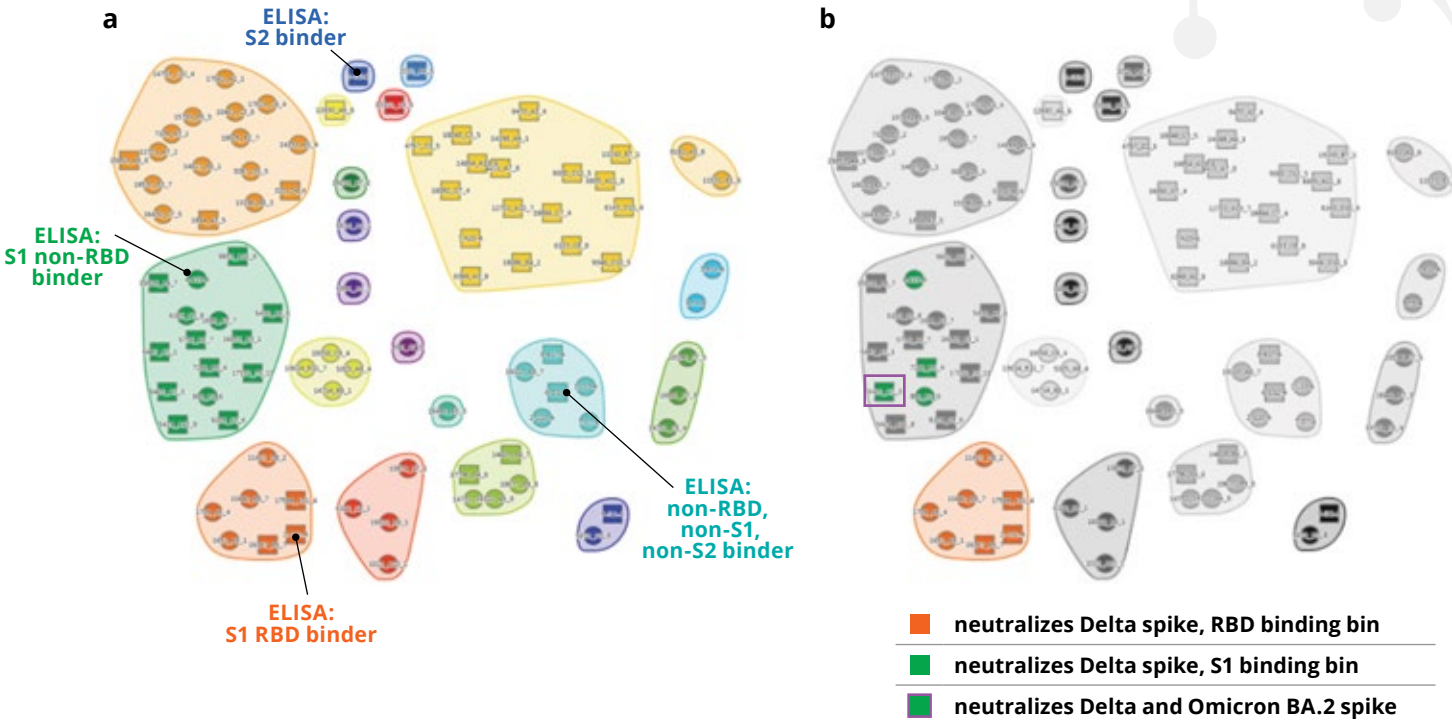


(a) Omicron BA.5 spike binding potencies for 40 of 96 mAbs were determined by ELISA. Median EC_{50}=807 pM ± 95% CI shown. (b) ELISA binding curves for the top 10 Omicron BA.5 binding mAbs are shown.

EPITOPE BINNING

As another approach to assess the binding diversity of the panel of newly discovered Delta antibodies, we determined the breadth of Delta spike epitopic coverage. We performed epitope binning using surface plasmon resonance technology with the Carterra® LSA® platform by coating a chip with 82 Delta spike-binding mAbs and 12 anti-spike mAbs discovered in a previous Curia hybridoma-based mAb discovery campaign, some of which have known binding domains. We then added Delta spike protein and assessed pairwise sandwich binding of each of the 94 spike mAbs. In an AI-assisted process known as binning, an analysis pipeline catalogs the extent and quality of sandwich binding and clusters antibodies that share similar binding profiles into bins. We discovered six antibodies that binned with a known receptor-binding domain (RBD) binder; 14 antibodies that binned with a known S1 non-RBD binder; and five antibodies that binned with an antibody that binds to a conformational surface not represented by recombinant RBD, S1 and S2 in isolation (Figure 10a). Notably, all of the Delta mAbs that binned with the known RBD-binder neutralized Delta spike binding to its receptor huACE2 (Figure 10b). This provides important validation for the assignment of bins, as it is known that most RBD-binding mAbs have neutralizing activity. Three mAbs that binned with S1 non-RBD binders also had Delta neutralizing activity, including A7_3, which also neutralized Omicron BA.2 spike (Figure 10b). In total, a diverse set of 19 different Delta spike epitope-binding bins were identified for the 82 Delta mAbs, with functional neutralizing mAbs largely clustered together with two types of S1 binders (either RBD or non-RBD binders).

Figure 10. Epitope binning of Delta spike mAbs discovered on the Beacon® system



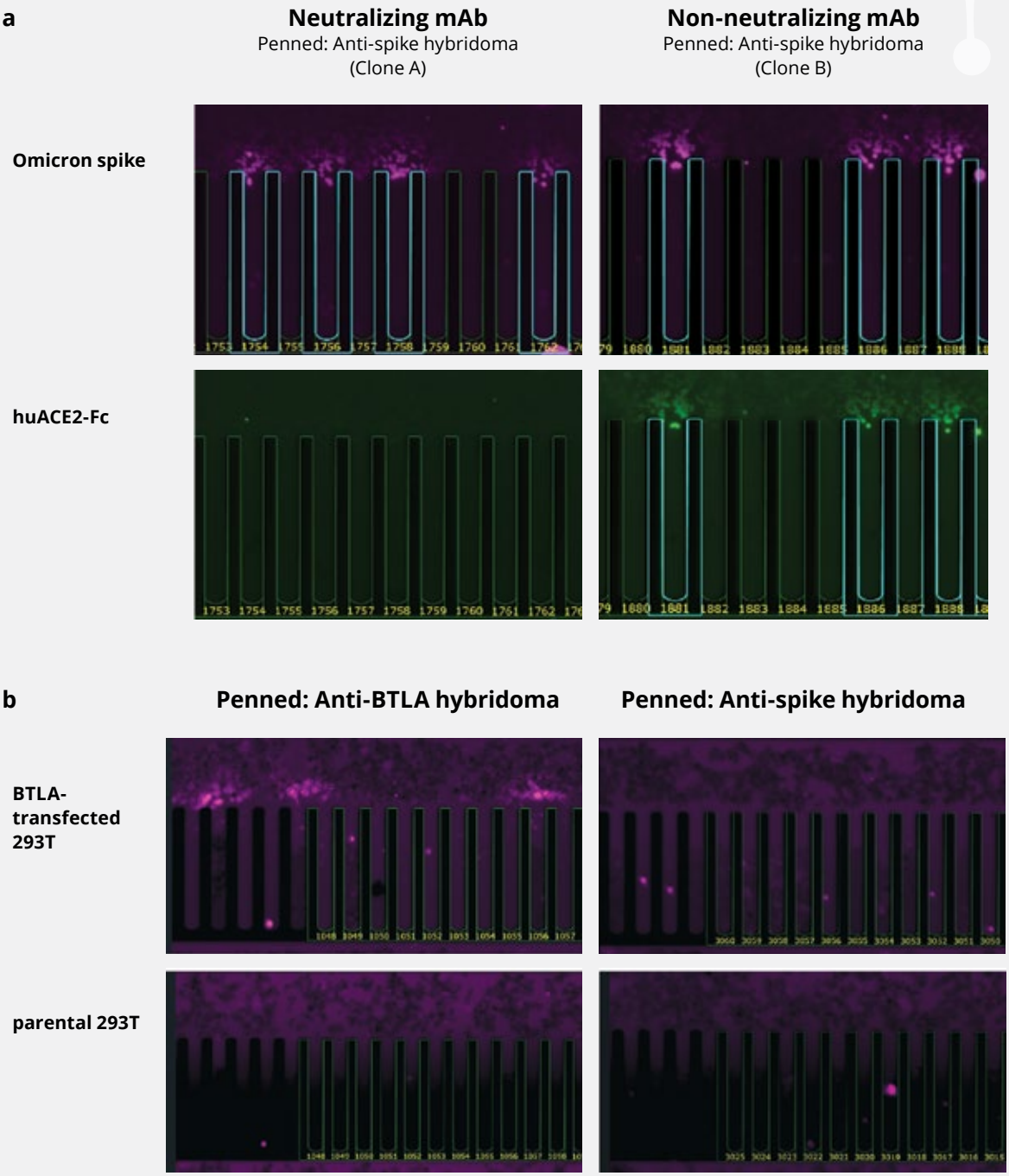
Communities of anti-Delta spike mAbs were defined based on epitope binning experiments. Surface plasmon resonance for competitive binding to full-length Delta spike protein was performed on a Carterra® LSA® instrument. (a) Twenty-one communities were classified with guidance from reference antibodies that were hybridoma-derived anti-spike mAbs with known binding to various spike domains (e.g., S2 binder in ELISA assays). (b) A community plot highlights mAbs that effectively blocked Delta and/or Omicron BA.2 spike-binding to huACE2 *in vitro*.

ADDITIONAL ON-CHIP ASSAYS

In addition to binding protein antigens, it is also possible to perform neutralization assays directly on-chip to enrich for the discovery of functional antibodies. To develop this assay, we imported two different hybridomas secreting anti-Omicron spike mAbs into NanoPen® chambers. In ELISA assays, clone A neutralized Omicron spike binding to ACE2, while clone B did not. After penning the hybridomas into specific fields of view (FOVs) on the same chip, we flooded the channels with assay reagents to assess mAb secretion and neutralization. In NanoPen chambers with confirmed anti-Omicron spike mAb (purple signal in the channels adjacent to the hit NanoPen chambers indicated in blue), no huACE2 binding was detected (green signal) for neutralizing mAb-secreting hybridoma clone A (Figure 11a, left panels). In contrast, the non-neutralizing mAb secreted by clone B failed to block huACE2 binding (green signal) to Omicron spike (purple signal, Figure 11a, right panels).

Finally, intact cells can also be used for screening in on-chip binding assays. Here we assessed the specificity of anti-spike antibodies against unrelated cell surface antigens. We imported two different hybridomas secreting either anti-spike or anti-BTLA (B and T lymphocyte attenuator) mAbs into NanoPen chambers. After penning the hybridomas into specific FOVs on the same chip, we loaded the channels sequentially with BTLA-transfected HEK293T cells or HEK293T parentals. Cell surface BTLA binding was detected only in the channels adjacent to chambers containing anti-BTLA secreting hybridomas and not spike-secreting hybridomas (Figure 11b). Together, these studies demonstrate that both functional and cell-based assays can be deployed on-chip to enhance screens and allow discovery of antibodies with specialized activities.

Figure 11. On-chip mAb screening assays, including a functional blocking assay, that can be performed on the Beacon® system



Antibodies were screened in a competitive binding assay to identify receptor/ligand blocking functions. (a) An antibody discovered at Curia outcompetes huACE2 for binding to Omicron spike (left panels), while another antibody does not block receptor binding (right panels). (b) Validation of specificity of the hybridoma anti-spike antibodies. Hybridomas were screened for binding to cell surface antigens, such as BTLA (B and T lymphocyte attenuator), expressed on stably transfected 293T cells (upper panel), and counter-screened against the parental cell line (lower panel).

Fast-tracking discovery of monoclonal antibodies

This study demonstrates the rapid and successful discovery and analysis of a highly diverse set of high-affinity and potent mAbs that bind and neutralize SARS-CoV-2 Delta and Omicron spike proteins. This approach should prove helpful in the ongoing need for diagnostics and treatments specific for novel virus variants that continue to arise, as well as other emerging infectious diseases and indications.

Learn more about rapid antibody discovery and development at curiaglobal.com/beacon.

ABOUT CURIA

Curia is a Contract Development and Manufacturing Organization with over 30 years of experience, an integrated network of 29 global sites and over 3,500 employees partnering with customers to make treatments broadly accessible to patients. Our biologics and small molecule offering spans discovery through commercialization, with integrated regulatory and analytical capabilities. Our scientific and process experts and state-of-the-art facilities deliver best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we deliver every step to accelerate and sustain life-changing therapeutics. ***Learn more at curiaglobal.com***

CONTACT US

www.curiaglobal.com

curia

© 2023 Curia Global, Inc. All rights reserved. The trade/service marks used herein are the property of Curia Global, Inc. or their respective owners. Use of the ® symbol indicates that the mark has been registered in at least the United States. Use of the ™ or ℠ symbol indicates that the mark is in use in at least the United States.