curia

COVID Spike Protein Antibody Discovery with Curia Biologics

Carterra Symposium at Seattle

Xiaomei Ge, Ph.D.

Senior Scientist III

Curia Biologics (San Carlos, California)

May 31st, 2024

Site overview

Site

Welcome to Curia's San Carlos facility, located in California, US. Here, we support you across all of your early drug discovery needs.

Capacity

40,000 ft²
300+ DNA Molecular Construction
Gram scale DNA plasmid production
200+ L transient mammalian protein production
40 – 60+ annual Discovery & Engineering programs

Contact

201 Industrial Rd., Suite 300
San Carlos, CA 94070
650.288.4891 ext. 201
www.curiaglobal.com

Core technologies

- Pentamice™ platform
- Beacon® optofluidic system for single B cell discovery
- Tuna293™ and TunaCHO℠ expression systems
- XOMA® fully human scFv and FAB libraries
- High-throughput (HTP) protein production
- Carterra® LSA® platform and Octet® BLI system
- Sartorius® iQue screeners for HTP FACS

Quality & regulatory

- AAALAC vivarium accreditation
- San Carlos is a non-GMP site

PROUDLY, AN aaalac INTERNATIONAL ACCREDITED PROGRAM

Products & services

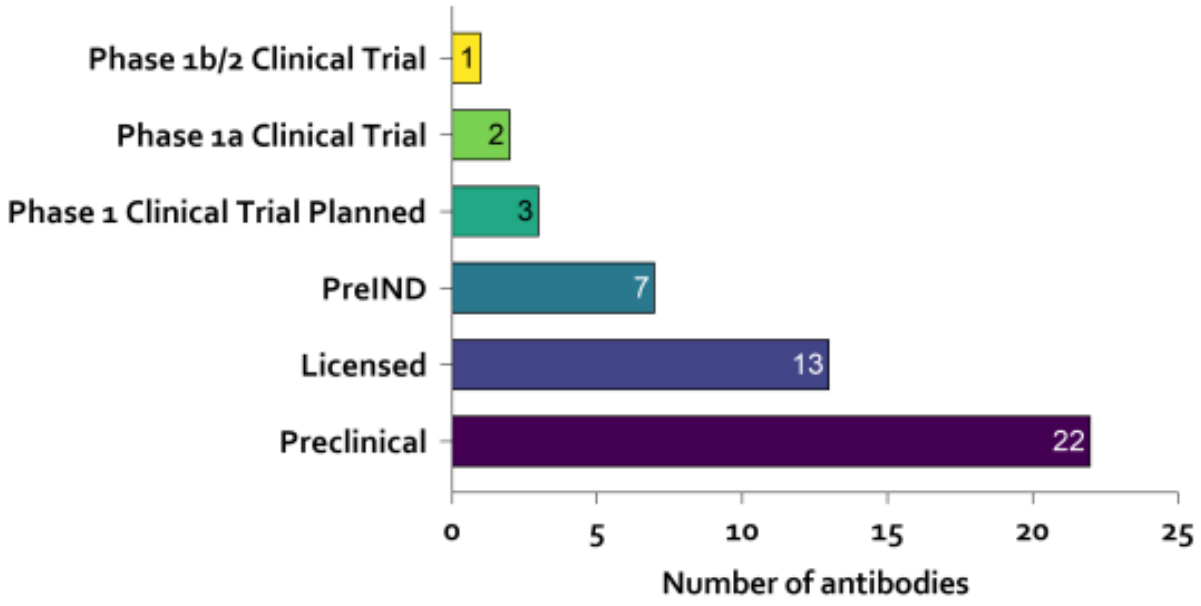
- Rodent, llama, and rabbit immunizations
- Hybridoma and B cell Ab discovery
- Phage and Yeast-Display Ab discovery
- Affinity maturation
- Recombinant DNA production
- Antibody Humanization
- Mammalian protein expression
- Developability analysis

Curia's Antibody Discovery, Engineering & Characterization (ADEC) Team & Services

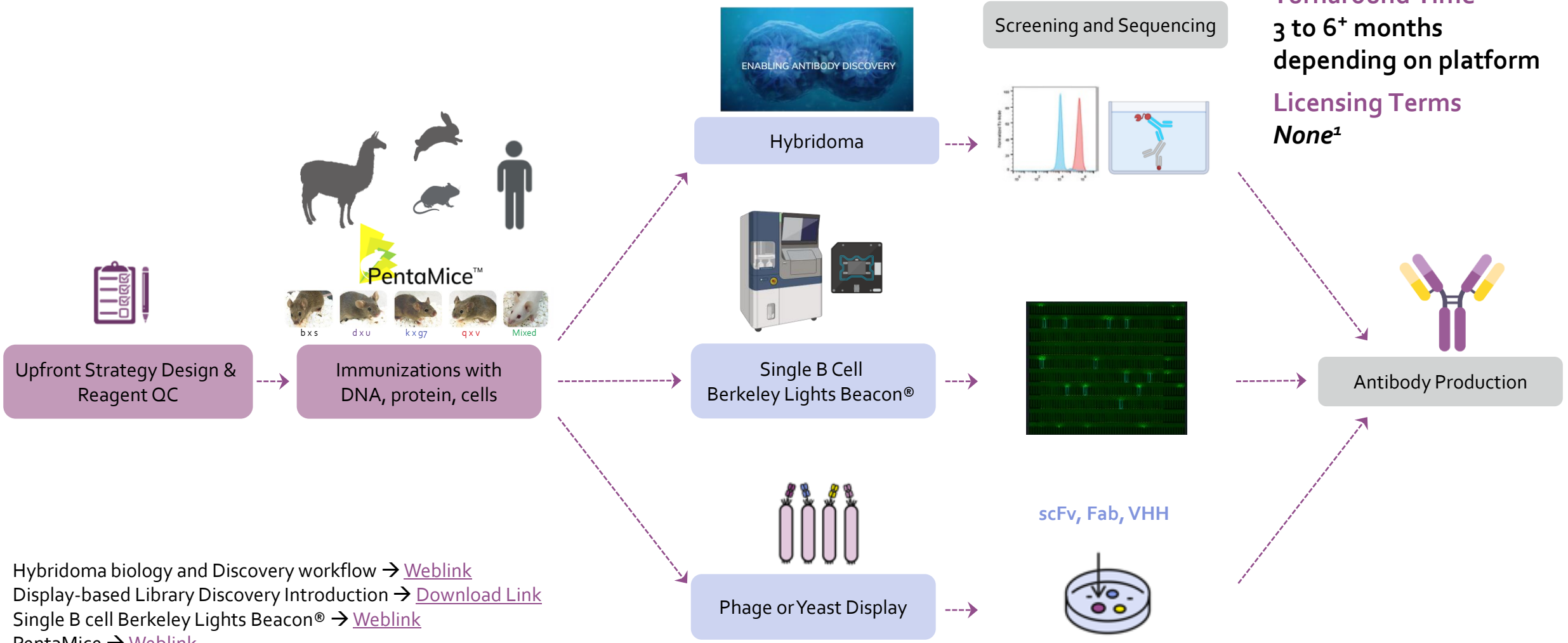
Discovering high quality antibodies!
200+ successful discovery campaigns

Biologics Discovered and/or Engineered with Curia in the Pipeline

✓ Tracking: Number of Lead Candidate Biologics Delivered to Clients in Preclinical Stages and Beyond



Antibody Discovery Workflows Utilize State-of-the-Art Hybridoma, Single B Cell, and Display Technologies to Deliver Quality Leads



Turnaround Time
3 to 6⁺ months depending on platform

Licensing Terms
***None*[1]**

Hybridoma biology and Discovery workflow → Weblink
Display-based Library Discovery Introduction → Download Link
Single B cell Berkeley Lights Beacon® → Weblink
PentaMice → Weblink

[1]Assumes use of in-house PentaMice®

Hybridoma and Single B cell Workflows for COVID Spike Protein Antibody Discovery

Highlights of standard workflow

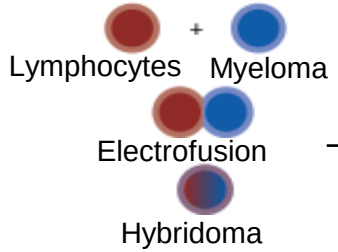
Hybridoma: screen ~10K cells, final 24 antibodies, 5.5 mo

Single B cell: screen ~50K cells, final 48-96 antibodies, 3.5 mo

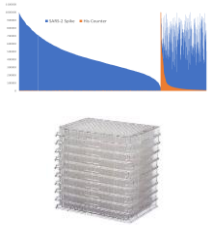
4 weeks

9-11 weeks

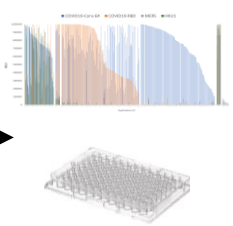
Hybridoma



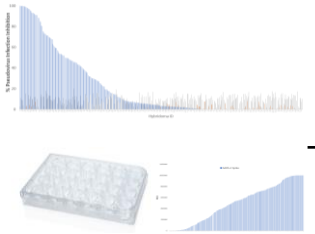
B cell + Proprietary Myeloma Electrofusion→Hybridoma



4,000 – 12,000 hybridomas screened by ELISA or FACS

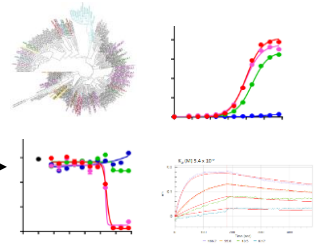


Up to 384 hits expanded → confirmation screen



Up to 384 confirmed hits expanded for cryopreservation and for saturated supernatants for binding, affinity and neutralization assays

Single cell cloning of 24 parental hybridomas to generate monoclonal hybridomas



V_H and V_L sequencing
EC_{50} binding
IC_{50} neutralization
K_D binding affinity
Epitope binning
Developability assessment

PentaMice™

Immunizations

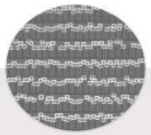
Single B Cell
Brucker Beacon®

1-3 days on-chip

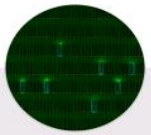
2 weeks

5-7 weeks

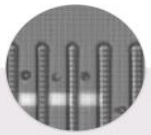
Import B cells into Beacon® system



Pen single B cells into 80K NanoPens™

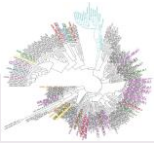


Screen ~50K B cells for binding and neutralization function

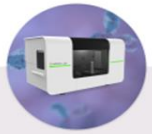


Automated cDNA synthesis

BCR sequencing by NGS for 1,152 mAbs



BCR analysis for mAb diversity and developability



High-throughput recombinant production and characterization of top 48-96 mAbs

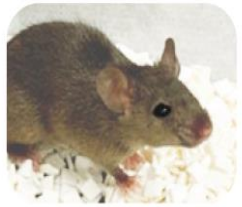
Curia's PentaMice Elevates and Expedites High Plasma Titers, a Key Success Indicator

PentaMice® immunizations

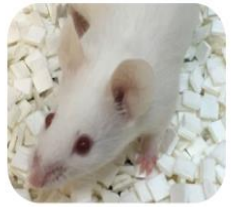
- Proprietary, royalty-free
- "Own your own molecules" PentaMice + Humanization
- **Maximum immunologically diversity based on MHC class II genetics**


b x s


d x u


k x g7


q x v


Mixed

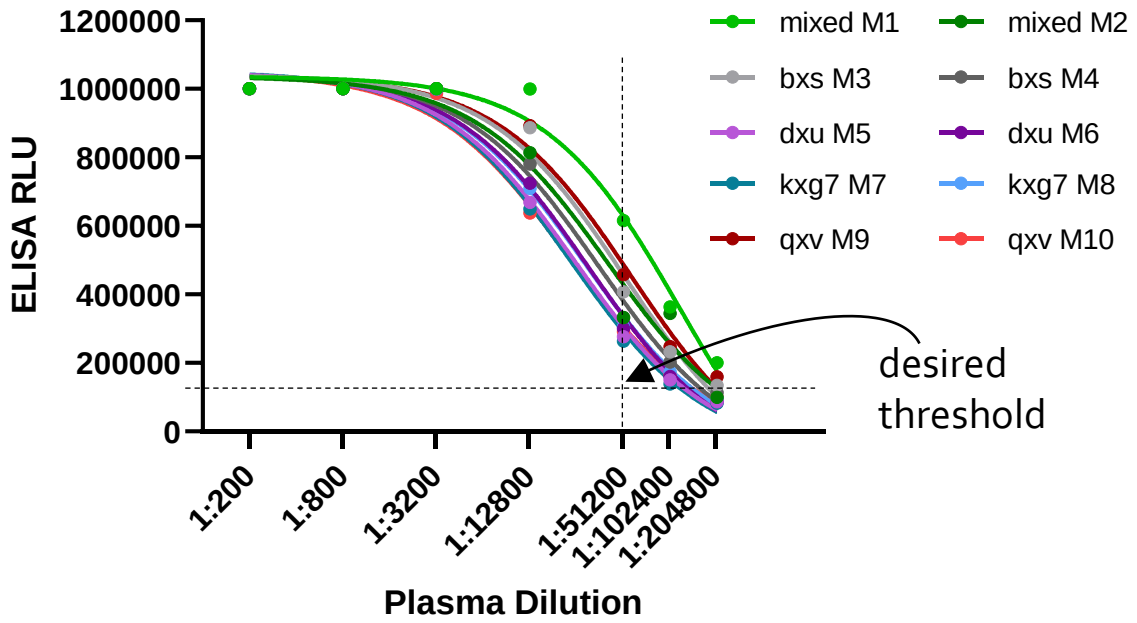
WHITE PAPER

White paper on Curia Insights

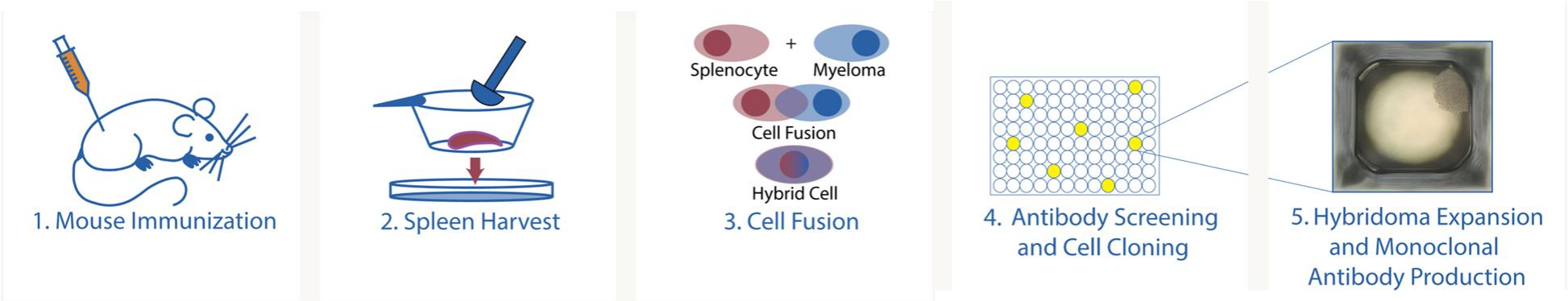
Leveraging the immunological diversity of the PentaMice® platform for COVID-19 antibody discovery

Margaret Wong Ho, General Manager and Site Head, Curia
Brian Zabel, Senior Director, Curia

Delta Spike ELISA Plasma Titer on day 17 of immunizations

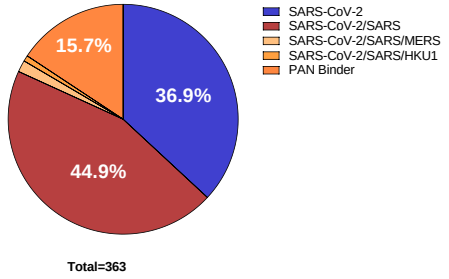


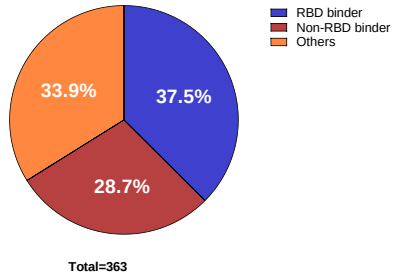
Hybridoma Workflow with High Hit Rate Delivers Highly Diversified Antibodies

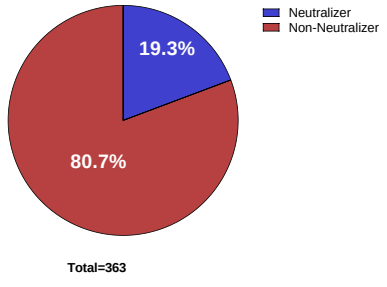


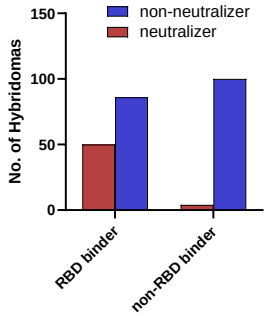
Highlights:

- **High hit rate (94.5%):** 363 in 384 saturated hybridoma supes confirmed binding of Spike protein
- **Diverse binding profile:** cross-reactivity with SARS1, MERS, and/or HKU1
- **Diverse binding epitopes:** RBD binders, S1 and/or S2 binders.
- **Early bio-functional characterization:** ~20% potent neutralizers, and identified druggable epitope on spike protein

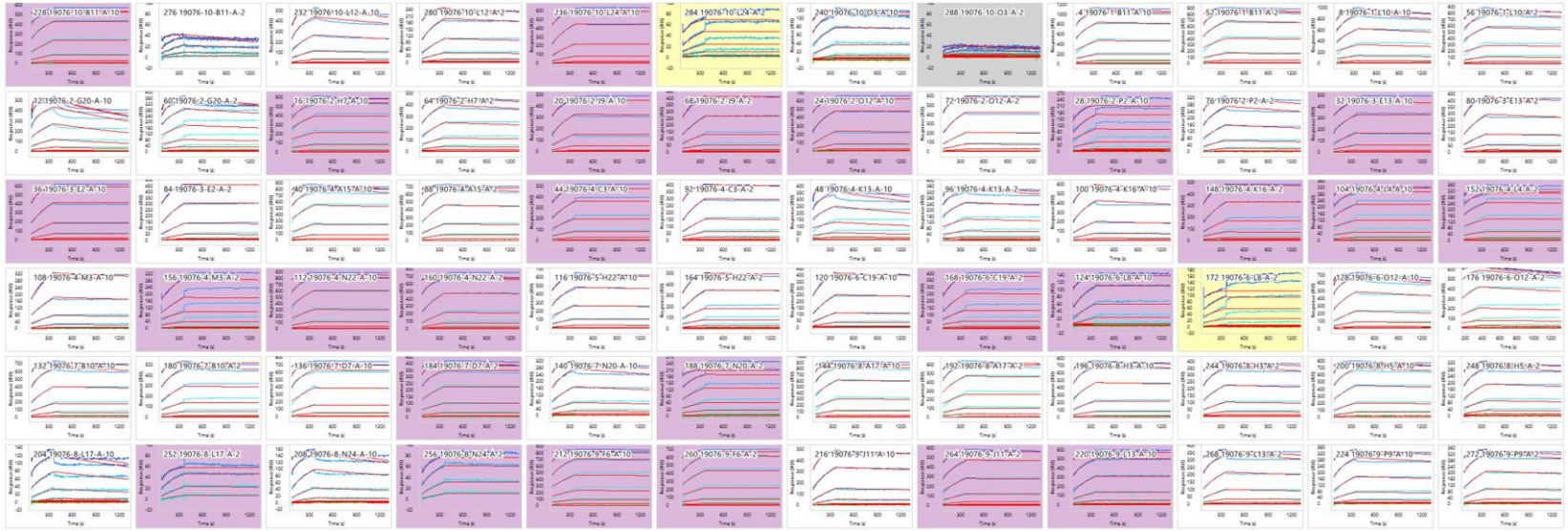


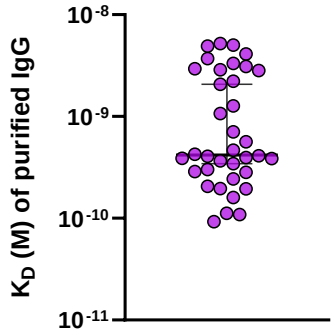






Rapid Kinetics Determination of mAbs by Arrayed SPR using Carterra® LSA®

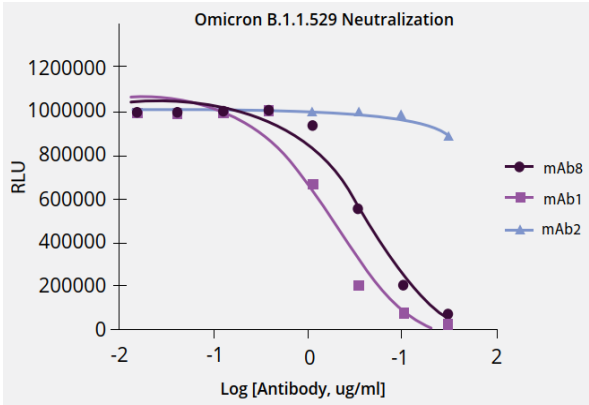


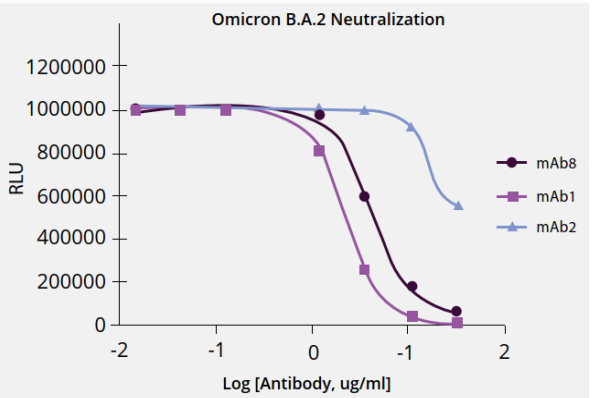


Delta Spike Binding affinity
median K_D= 0.42 pM

Highlights:

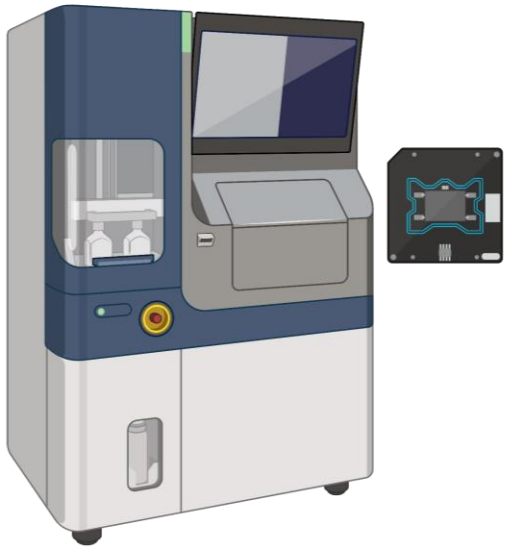
- High-throughput kinetic assay by Carterra® LSA® to determine binding affinity.
- Rapidly identified a group of picomolar binders with extremely slow off-rate.
- In vitro assays identified potent antibodies neutralizing spike protein variants.



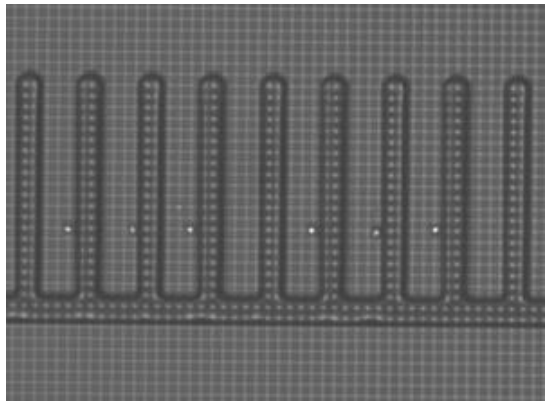


Case Study for Single B Cell Antibody Discovery: B Cells Are Penned by Using Nanofluidics and Screened by Using Fluorescent Microscopy

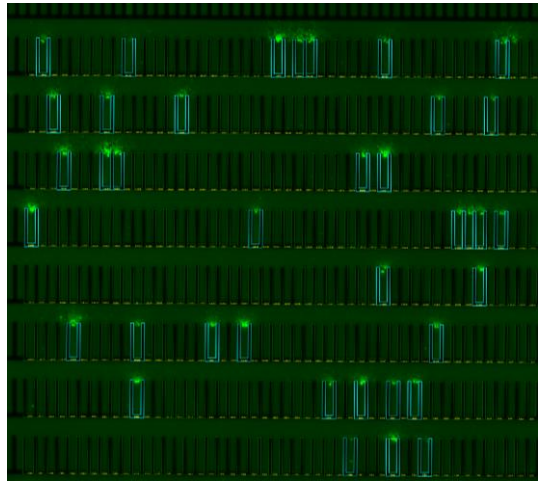
Bruker's Beacon 20K NanoPens/chip



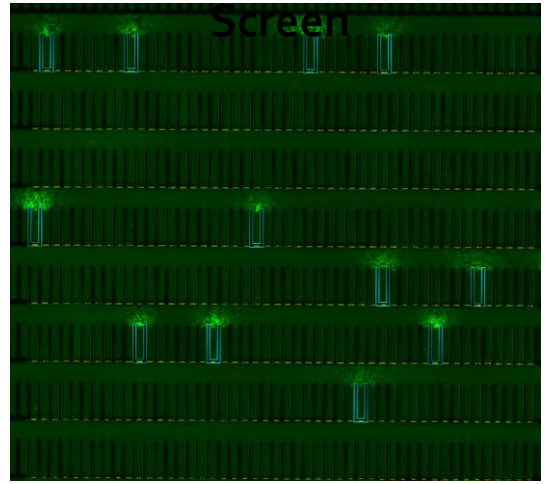
Magnified image of penned cells



Mouse IgG screen

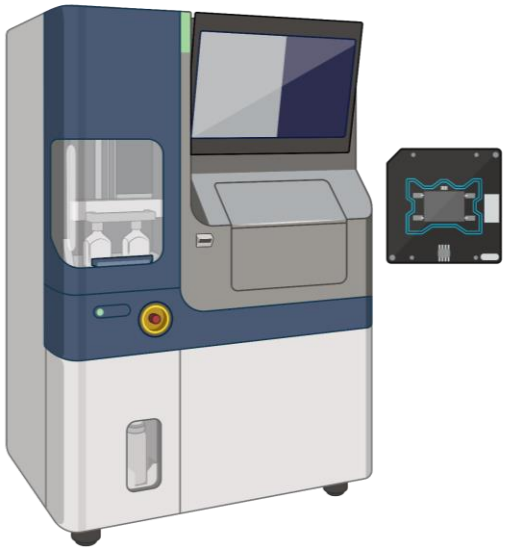


Delta Spike binding screen

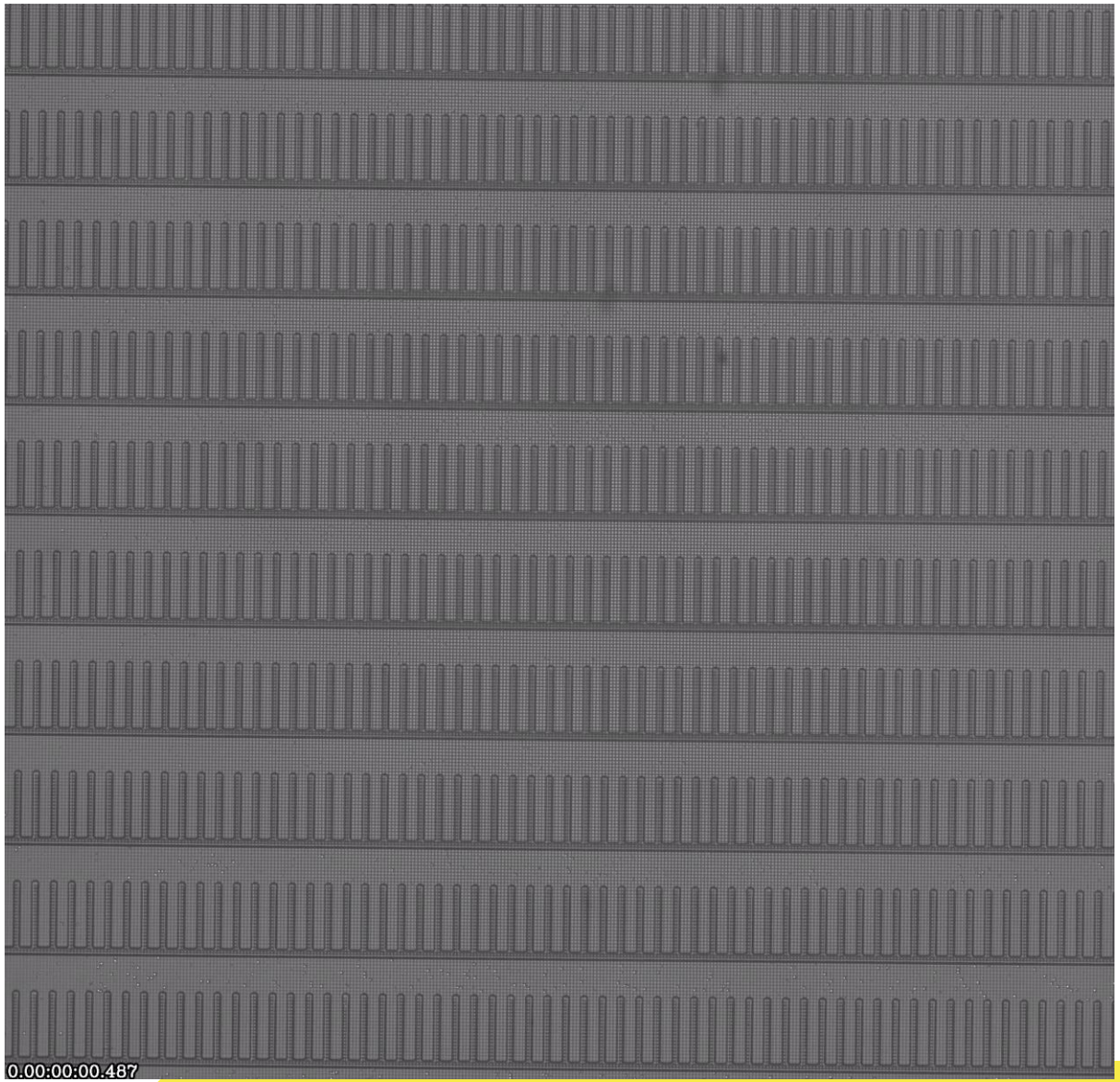


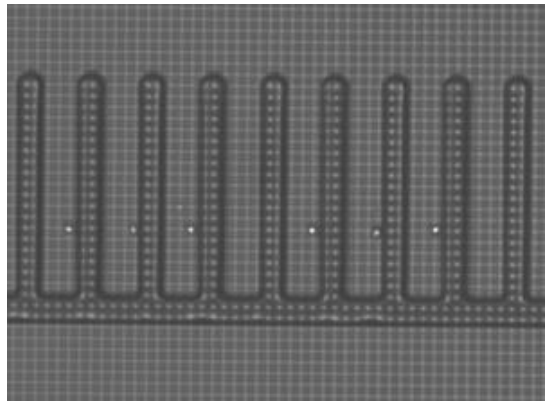
- ~600 NanoPens in a field of view
- 3 chip screen (60,000 NanoPens) **1-3 days**
- Delta spike+ pens: **1,894 (~3% hit rate)**

B Cells Are Penned Using Nanofluidics and Optoelectropositioning



Bruker® Beacon® and Optoselect Chip allow screening up to 80,000 NanoPens;
~600 NanoPens in a field of view





PentaMice were immunized with SARS-CoV-2 Delta Spike Trimer

B cells were purified and 'penned'

Time-lapse Imaging Video of Our Delta-Spike Antigen Binding Screen

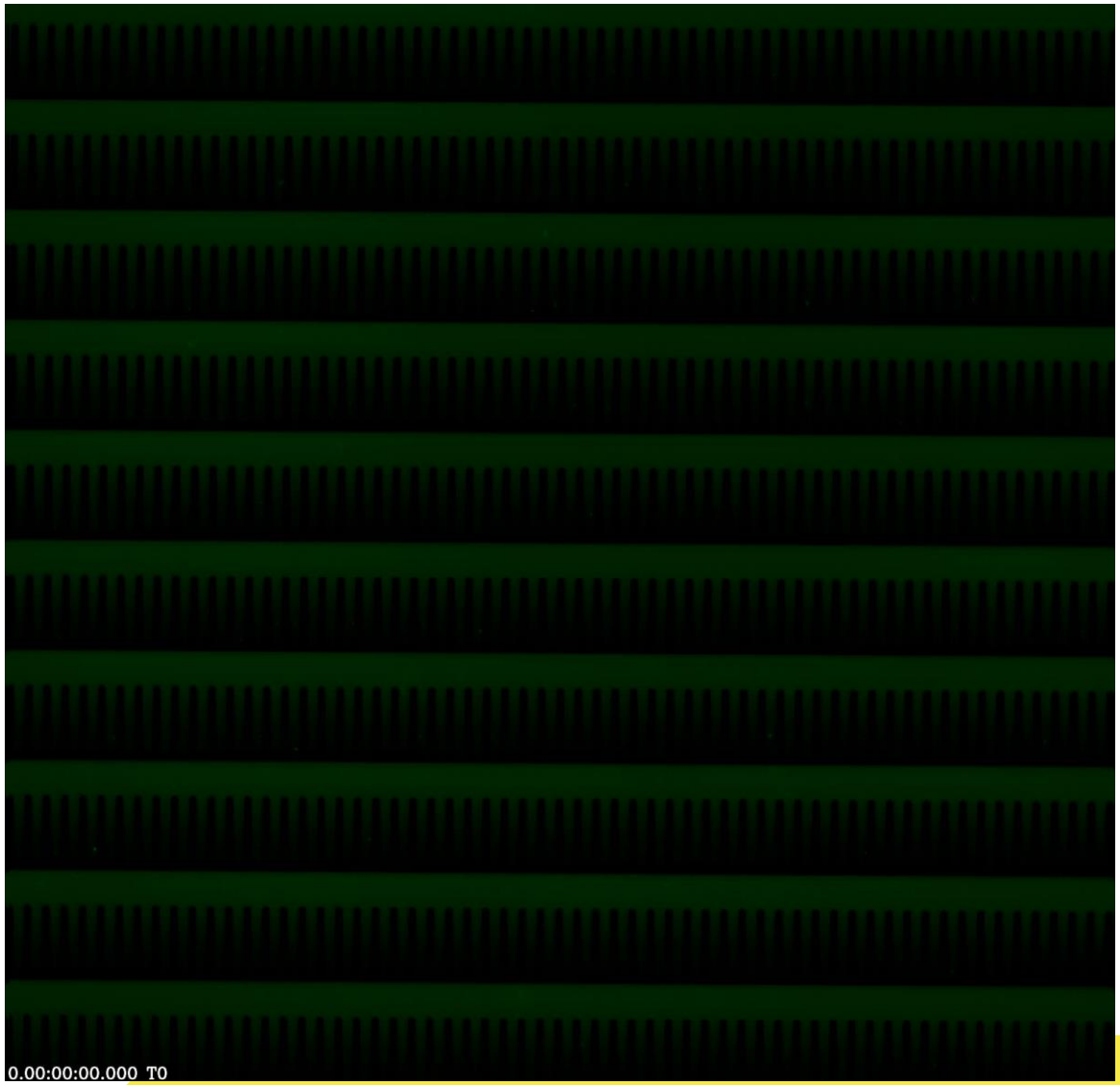
Beads bound to Delta Spike protein imported into channels above NanoPens

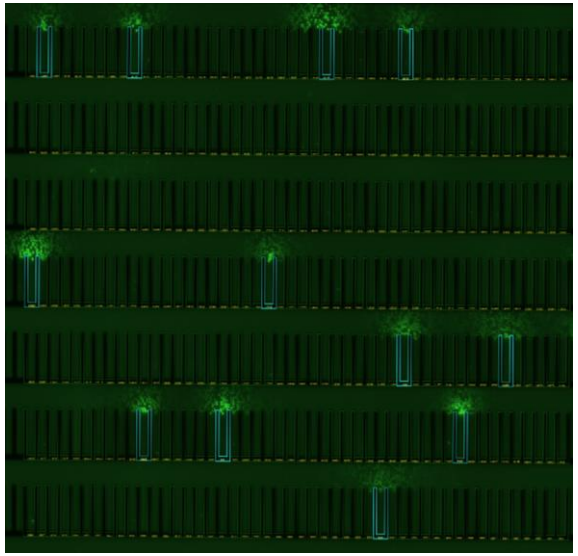
Light microscopy of chip

Mouse anti-Delta Abs bind to Delta beads above the NanoPens

Binding is detected by adding anti-mouse AF488

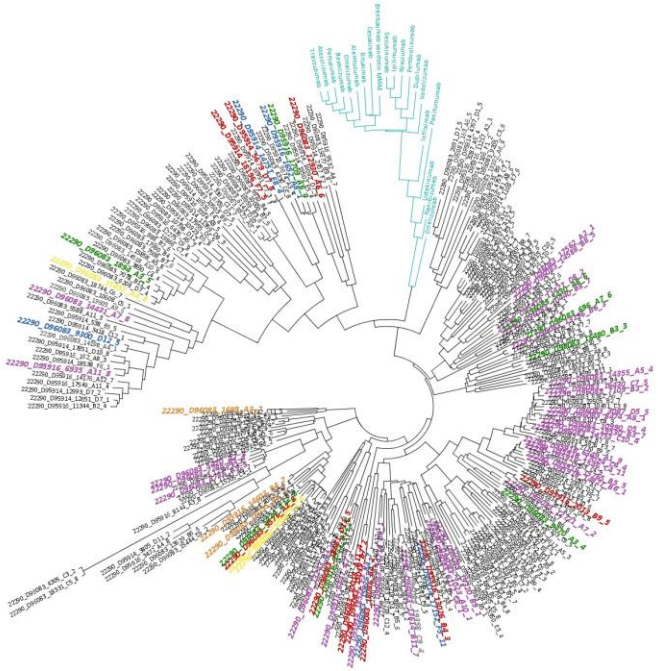
Fluorescent plumes of anti-Delta spike mAbs, ~2000 hits





- 3 chip screen with Opto Plasma B Discovery 4.0 Workflow (60,000 NanoPens)
- Delta spike^{+} pens: **1,894 (~3% hit rate)**

Abundant Unique Antibodies Discovered with Diverse Sequence and Functional Binding Profiles



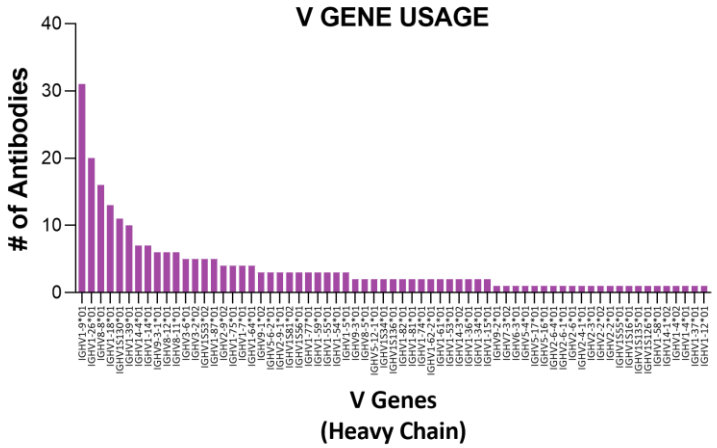
	WT	Delta	Omicron	BA.2
	+	+	+	+
	+	+	+	
	+	+		+
	+	+		
		+	+	
		+		
	Not tested or low binding			
	20 Commercialized mAbs			

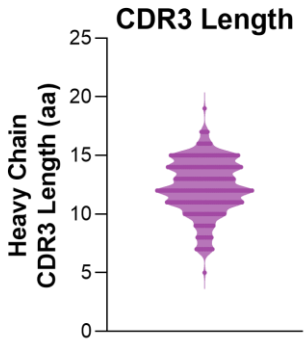
Sum of radial branch distances between two mAbs

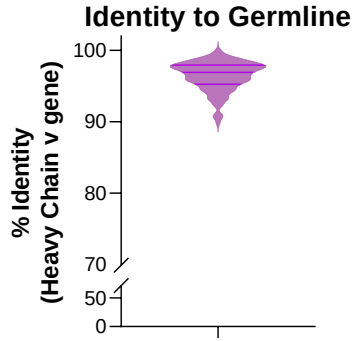
~10 a.a. differences

Highlight: High diversity of 248 antibodies discovered

- **248 unique** high-confidence paired of V_H - V_L sequences were identified by Next Generation Sequencing
- **170** clonal antibody families were discovered using a total of **67** V_H genes, **54** V_L genes , and a broad range of CDR3 lengths.
- **Diverse binding profile**: cross-reactivity with Delta, WT, Omicron, BA.2 and/or BA.5 spike proteins



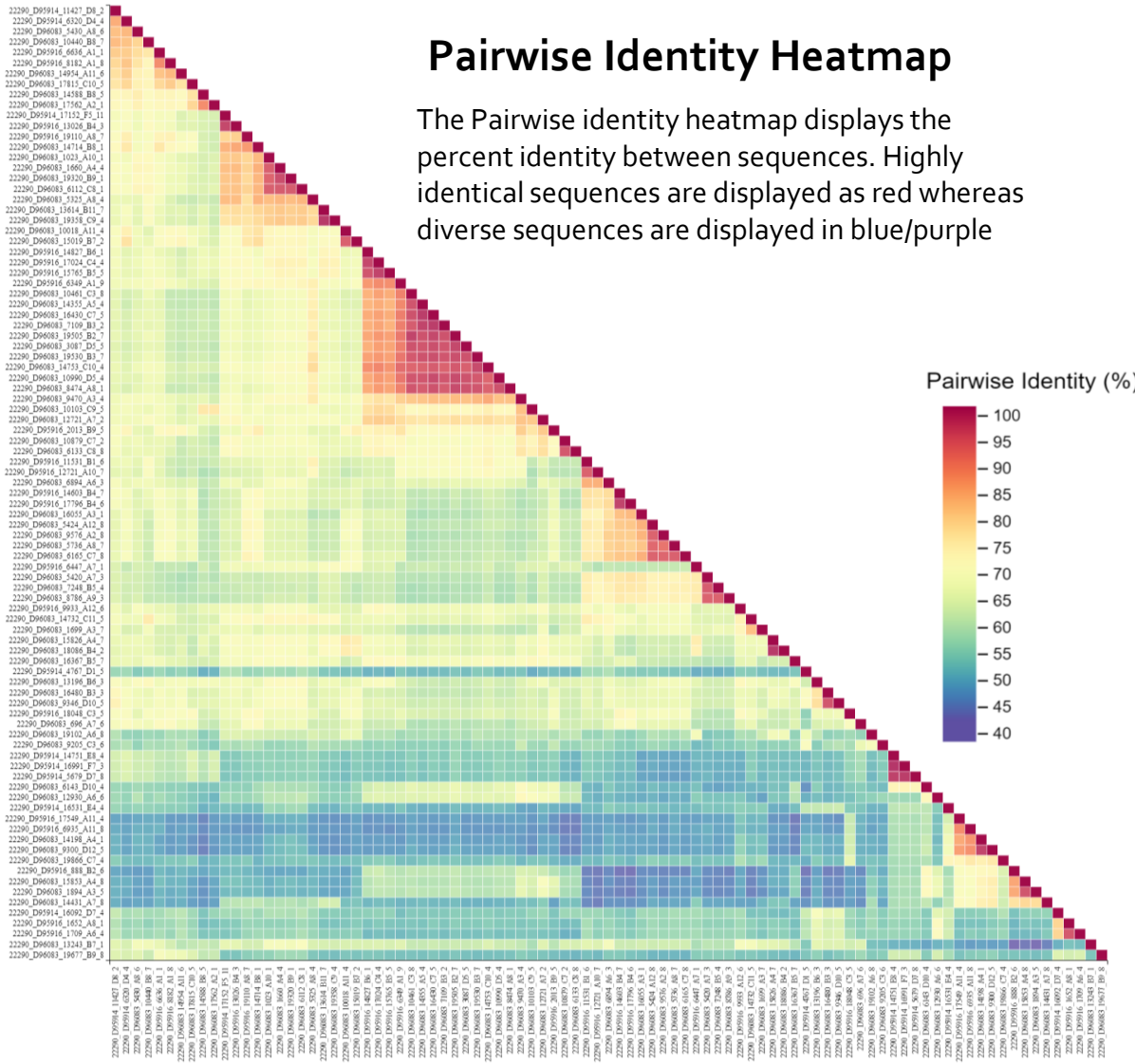




Custom Bioinformatics for Visualization of Antibody Diversity

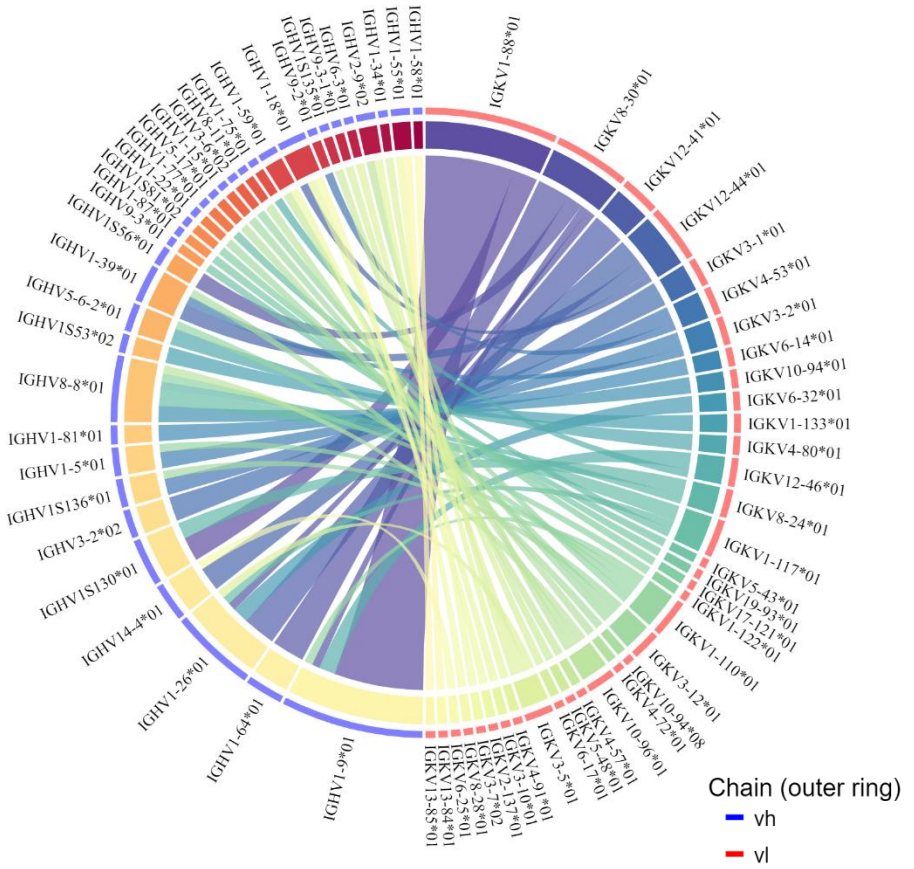
Pairwise Identity Heatmap

The Pairwise identity heatmap displays the percent identity between sequences. Highly identical sequences are displayed as red whereas diverse sequences are displayed in blue/purple

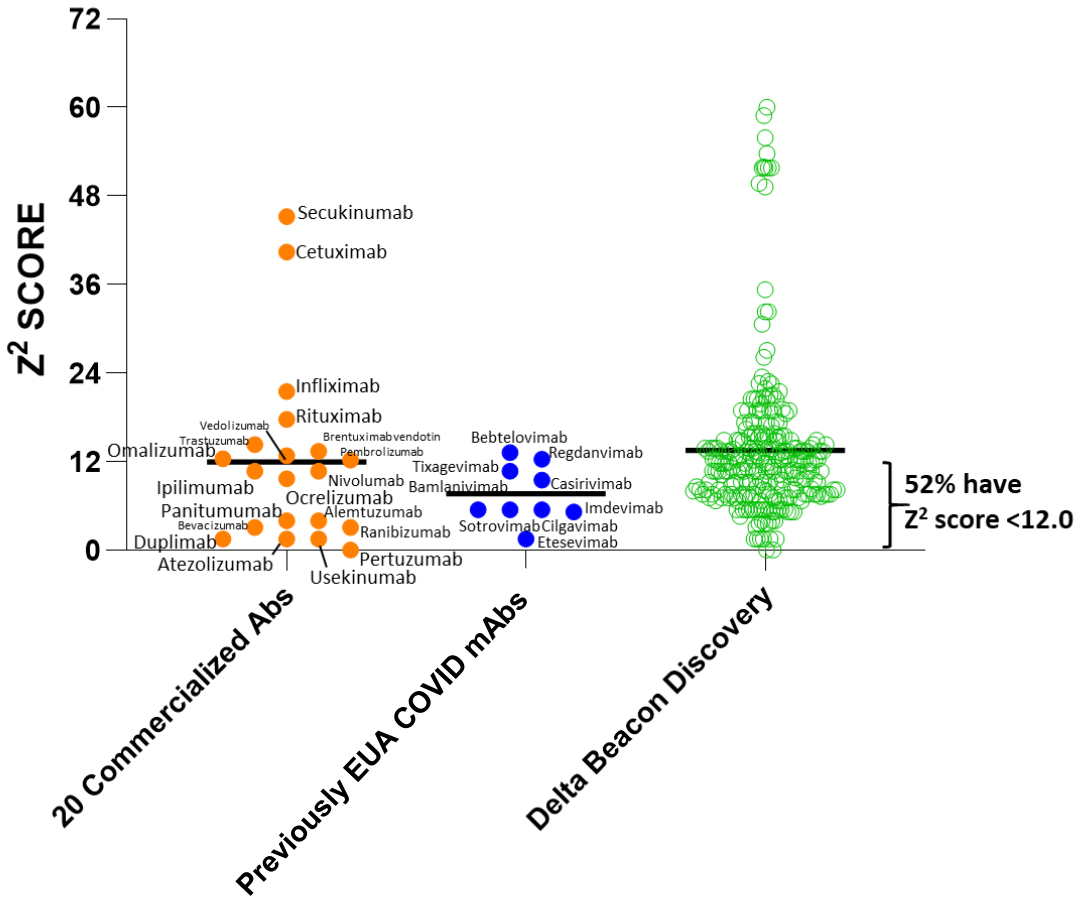


Circos plot

The circos plot represents data in a circular layout and is useful for exploring relationships between different variables.
This plot shows how Heavy Chain V genes pair with Light Chain V genes. We can also see how many V genes are represented in our data set. This is another look into sequence diversity.



In silico Developability Analysis Identifies Antibodies with Fewer Sequence-based Liabilities

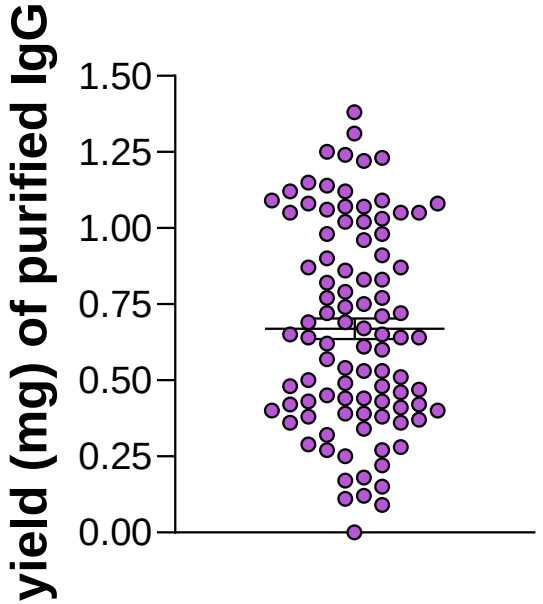


Z² scores are calculated on sequence-based parameters

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

High-throughput Small Scale Transient Expression of Top 96 mAbs in TunaCHO Cells

Highlight: TunaCHO cell platform offers high yield, high throughput, consistent scalability, and streamlined production into stable cell development

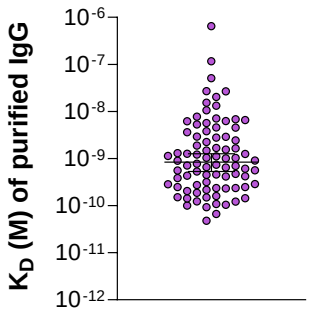


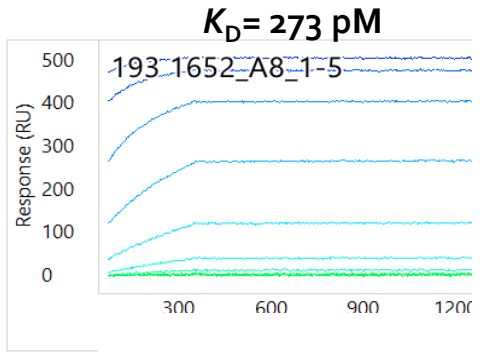
Recombinant antibody yields from 7 day expression in TunaCHO system (10 mL)
mean ± SEM, 0.67 ± 0.03 mg

Antibody Characterization Using Carterra® LSA® and ELISA Assays

Delta Spike Binding affinity

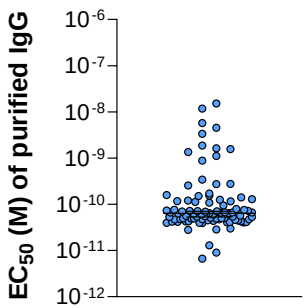
median K_D= 837 pM

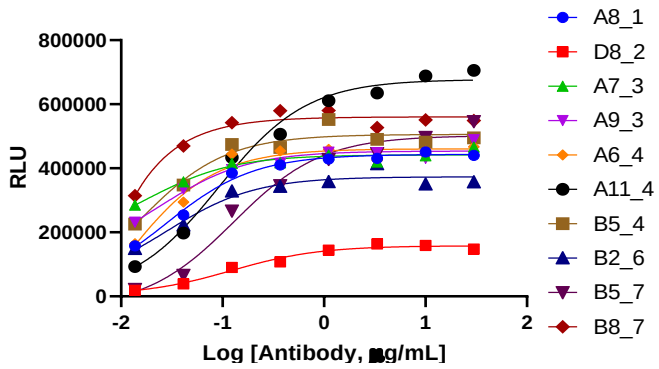




Delta Spike Binding potency

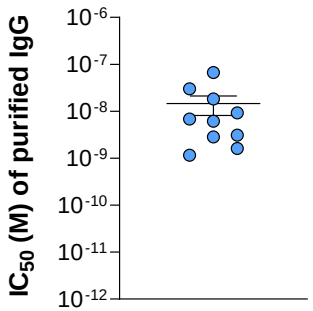
median EC_{50}=64 pM

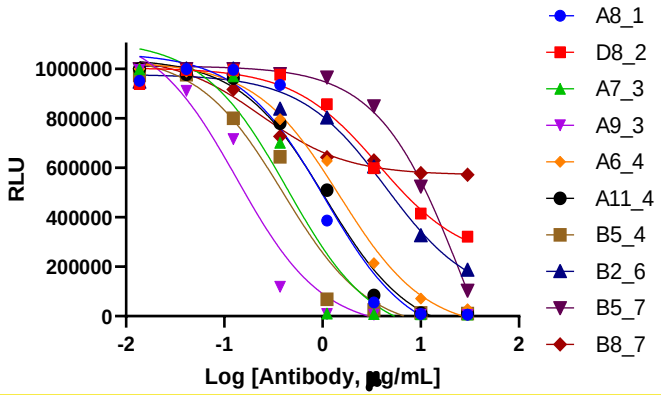




Top 10 Delta Spike Neutralization

median IC_{50}=14.6 nM





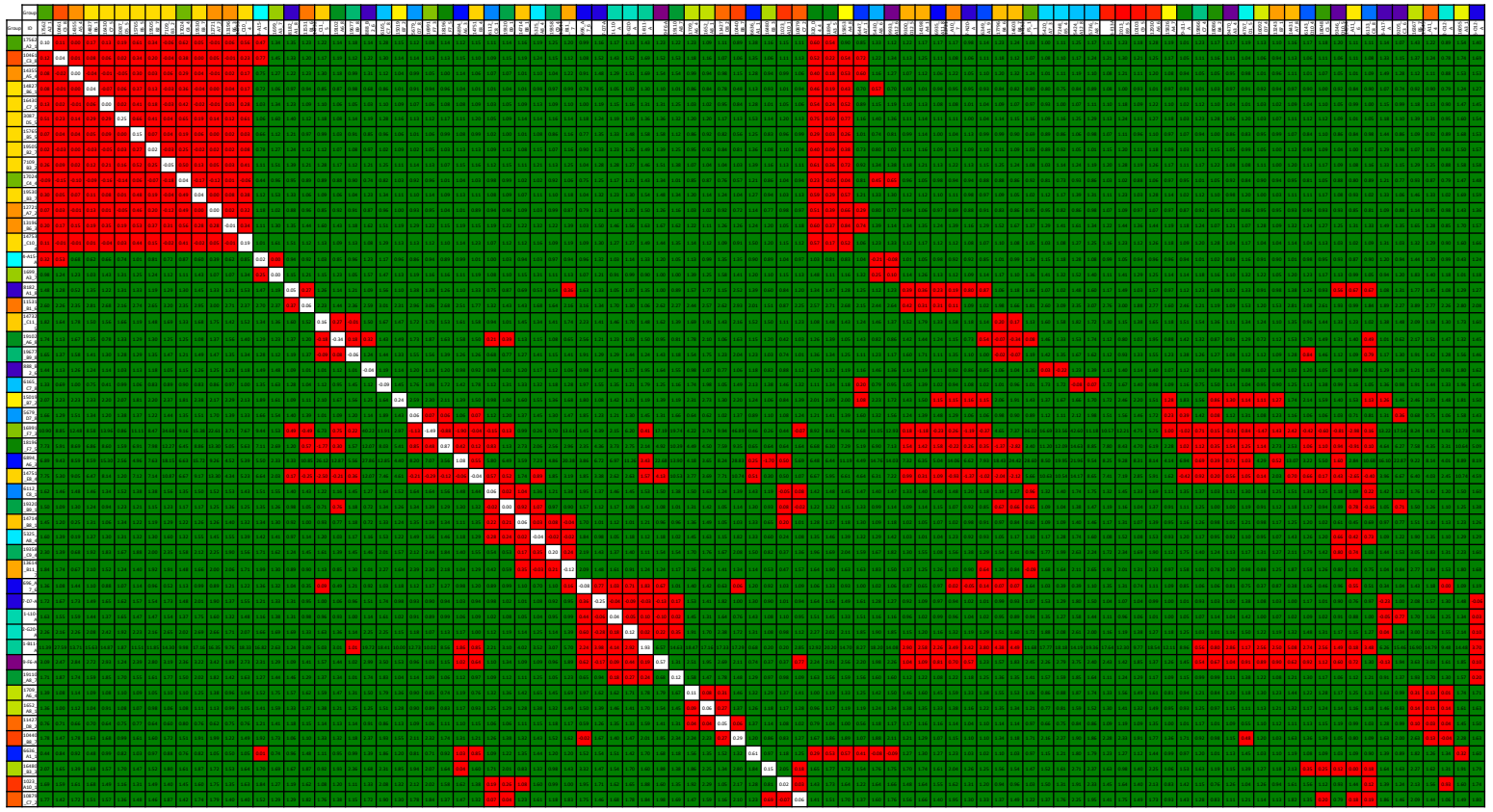
Highlights

- High-throughput kinetic assay for binding affinity by SPR (median K_D: sub-nanomolar high affinity mAb)
- Potent binders by ELISA
- In vitro assays identified functional antibodies (potent neutralizers)

Epitope Binning Heatmap Using Carterra® LSA® of Delta Spike mAbs (94 x 94)

Analyte IgG

Ligand IgG



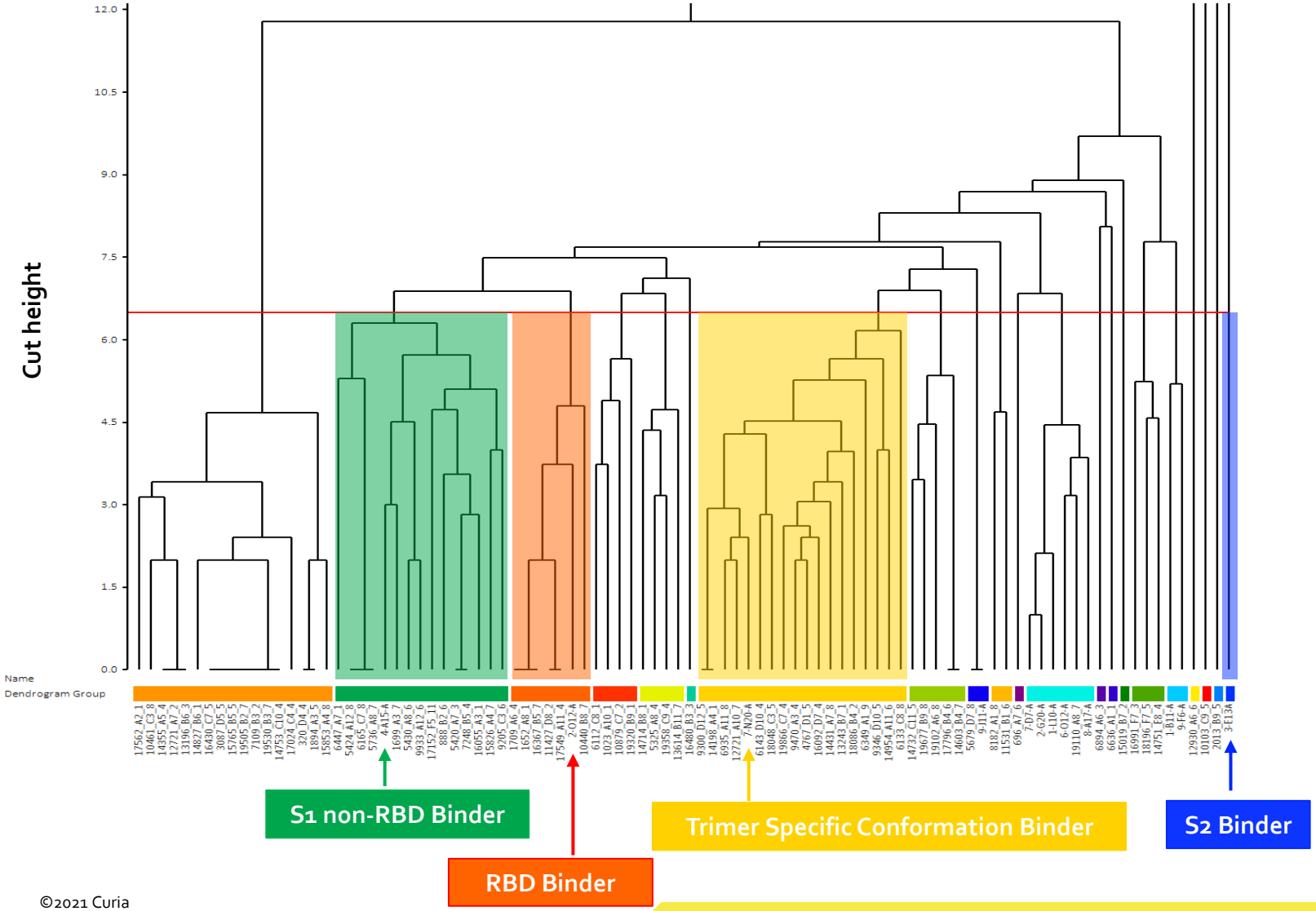
Heat Map Legend

- Non-binding analyte IgG
- Binding analyte IgG
- Self-self competition

Notes

- 44 of the 94 antibodies showed weak antigen binding after immobilization. However, binding as analyte was observed. These IgG were excluded as ligands, but they were still evaluated as analytes.
- Optimization of coupling condition could improve ligand IgGs binding profile.
- Colors above correlate to Groups shown on the subsequent slides and are determined based on the selected 6.5 cut height on following slide.

Epitope Analysis Bins and Dendrogram of Delta Spike mAbs (94 x 94)



Combined Dendrogram

Hierarchical clustering is applied to the sorted heat map to generate dendrograms, which progressively group mAbs.

The grouping stringency for communities is adjusted by the red horizontal cut height bar on the dendrogram. The cut height bar can be adjusted to allow for larger groupings or more stringent groups.

.

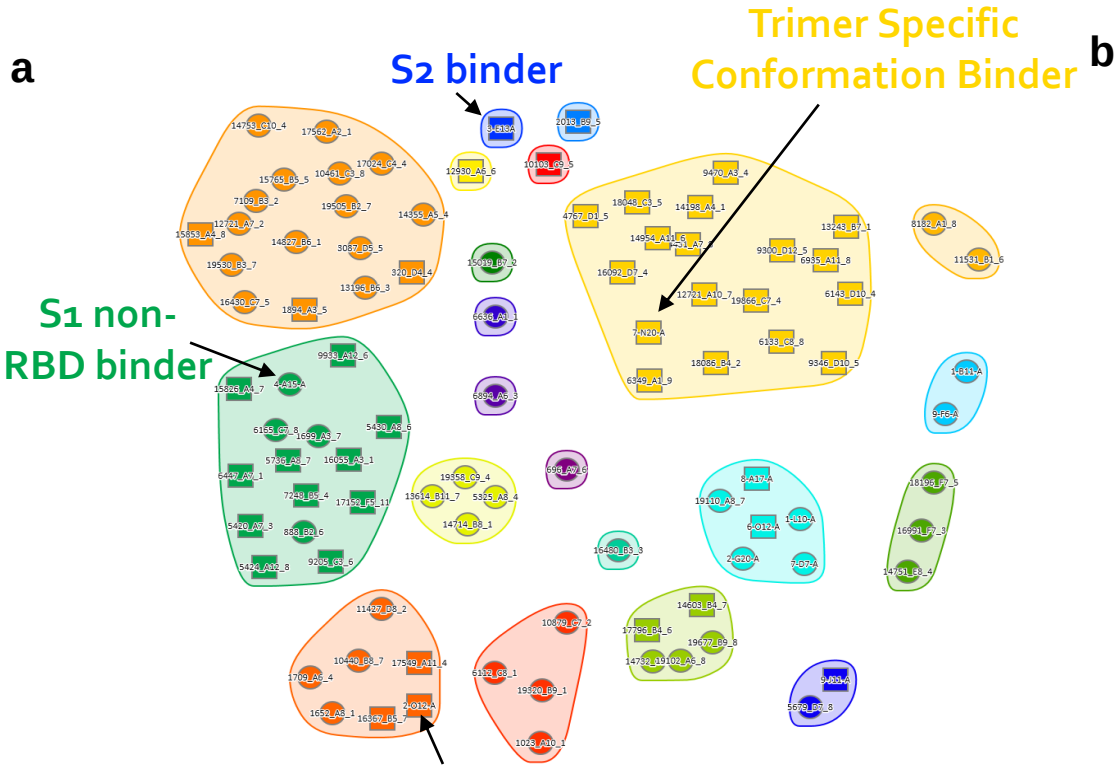
Highlights

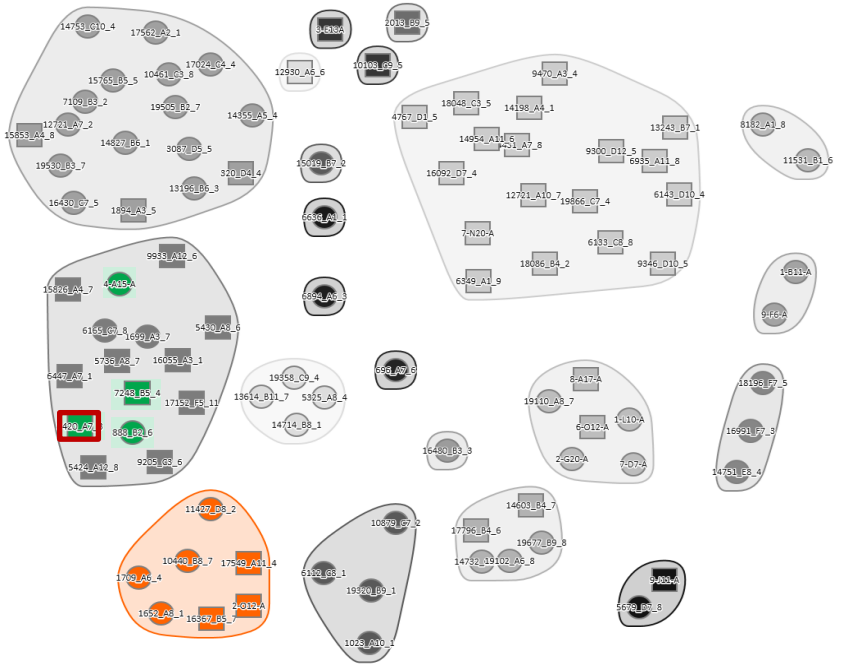
- 21 groups were identified after clustering based on a cut height of 6.5.
- The highlighted mAbs with known binding epitopes in different branches to help assign the cut height for assigning bins.

Epitope Binning Community Plot Using Carterra® LSA® of Delta Spike mAbs (94 x 94)

Highlight

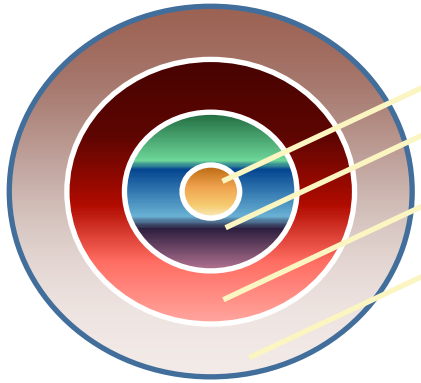
Antibody Characterization: Epitope binning identifies antibody communities to help select antibodies for further analysis/development





neutralizes Delta spike, RBD binding bin
neutralizes Delta spike, S1 binding bin
neutralizes Delta and Omicron BA.2 spike

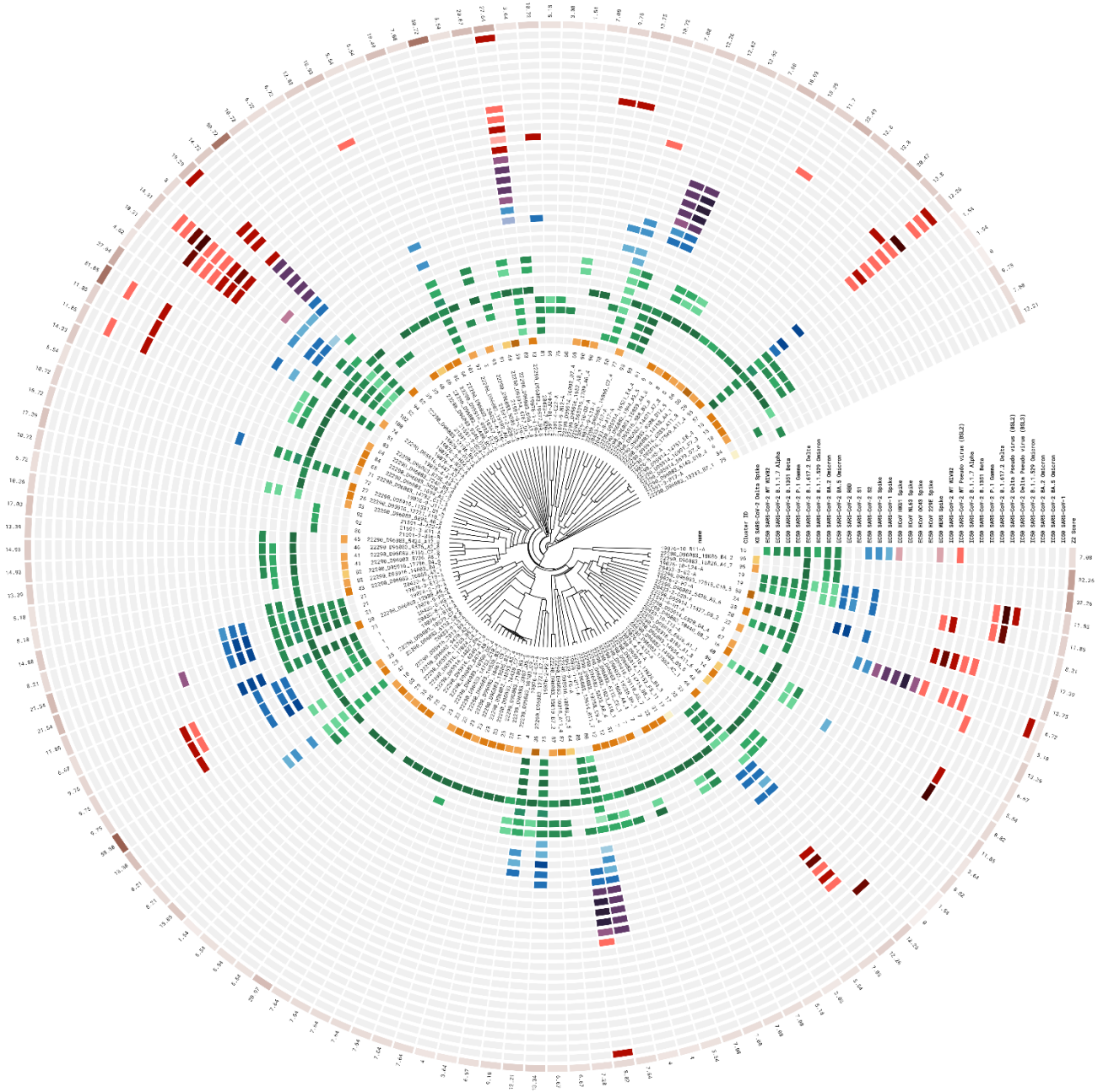
Custom Multiparameter Data Visualization of Curia's SARS-CoV-2 mAbs



Affinity (K_D)
Potency (EC_{50})
Neutralization (IC_{50})
Sequence Liability (Z^2)

137 mAbs
32 distinct assays

K_D	EC_{50}			IC_{50}	Z^2 Sequence Liability Score	
	SARS-CoV-2 Variants	SARS-CoV-2 Domains	Related CoV Virus			
					60 55 50 45 40 35 30 25 20 15 10 5 0	< 0.1 nM
						< 1 nM
						< 10nM
						< 100 nM
						< 655 nM
						No binding or Not Tested



COVID Spike Protein Antibody Discovery with Curia Biologics

Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

HOME

New Results

Follow this preprint

Mouse Antibodies with Activity Against the SARS-CoV-2 D614G and B.1.351 Variants

Larisa Troitskaya, Nelson Lap Shun Chan, Brendon Frank, Daniel J. Capon, Brian A. Zabel, Xiaomei Ge, Dan Luo, Rachel Martinelli, Jing Jin, Graham Simmons

doi: https://doi.org/10.1101/2021.07.05.451203

Abstract | Full Text

Abstract

With the rapid sprea
authorized for emerg
protect patients aga
antibody repertoires
a different or broade
characterized so far.
neutralizing potency
antibodies may have
exposed to new SAI

December 12, 2022

TONIX PHARMACEUTICALS

Tonix Pharmaceuticals Announces Exclusive License of Potential Therapeutic or Preventative Humanized anti-SARS-CoV-2 Monoclonal Antibodies from Curia Global, Inc.

Immunocompromised Individuals, Including Organ Transplant Recipients, are at Increased Risk of Severe COVID-19 and Poor Clinical Outcomes

SARS-CoV-2 has Mutated to Evade the Existing EUA-Approved Therapeutic Monoclonal Antibody Therapies

CHATHAM, N.J., Dec. 12, 2022 (GLOBE NEWSWIRE) -- Tonix Pharmaceuticals Holding Corp. (Nasdaq: TNXP), a clinical-stage biopharmaceutical company, today announced that it has obtained an exclusive license from Curia Global, Inc., a leading contract research, development and manufacturing organization, for the development of three humanized murine monoclonal antibodies (mAbs) for the treatment or prophylaxis of SARS-CoV-2 infection. SARS-CoV-2 is the cause of COVID-19.

"We believe that the licensing of these mAbs strengthens our pipeline of next-generation therapeutics to treat COVID-19," said Seth Lederman, M.D., Chief Executive Officer of Tonix Pharmaceuticals. "Immunocompromised individuals, including organ transplant recipients, are at increased risk of severe COVID-19 and poor clinical outcomes[1]. Although five monoclonal antibody products, containing seven distinct monoclonal antibodies, have received Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA) for either treatment or prophylaxis of COVID-19, only a single product, Evusheld®, is still recommended for use as a prophylaxis by the National Institutes of Health COVID-19 Treatment Guidelines Panel or FDA[2,3]. Moreover, concerns have been raised about the ongoing ability of Evusheld® to prophylax in the face of new variants[4]. We believe there is a need for second generation mAb treatments and prophylactics for COVID-19[5]. To date, the EUA-approved products have been derived from the blood of COVID-convalescent patients or a humanized mouse[6,7]. The Company believes that humanized murine monoclonal antibodies discovered by Curia and licensed by Tonix represent a potential new approach to treating SARS-CoV-2 infection. The Company believes that murine monoclonal antibodies have the potential for neutralizing a broader spectrum of SARS-CoV-2 variants and may be harder for SARS-CoV-2 to evade as we face a 'variant soup' from both convergent and divergent evolution."[8]

curia

WHITE PAPER

Antibody-based drug discovery at the speed of light

The combination of the PentaMice® platform and single B cell screening with the Berkeley Lights Beacon® Optofluidic system increases speed to market for monoclonal antibody therapeutics

Grant J. Carr, Vice President, Global R&D Discovery, Curia
Margaret Wong Ho, General Manager and Site Head, Curia
Brian A. Zabel, Senior Director, Curia
Christine L. Hsieh, Senior Scientist II, Cellular Immunology Assay Development, Curia

Capabilities and technology combine to provide First-to-Human antibody discovery, development and clinical manufacturing. Speed, scientific expertise and efficiency can surmount the high attrition rates of early antibody discovery and achieve first-to-market delivery of new therapeutics.

Slow processes that generate a limited number of recombinant monoclonal antibodies hinder success. For a blockbuster $1B per annum biologic in an increasingly competitive market, every month of delay in getting to market can result in the loss of up to $83 million in revenue per month. Worse, any delay increases the chance of a competitor filing a patent claim for the sequence and utility of the antibody you have discovered before you do.

curia

WHITE PAPER

Leveraging the immunological diversity of the PentaMice® platform for COVID-19 antibody discovery

Margaret Wong Ho, General Manager and Site Head, Curia
Brian Zabel, Senior Director, Curia

Hybridoma technology is a popular method for antibody discovery, but th
approach of using a single inbred mouse strain for immunization fails to g
and antibody titers needed to maximize the discovery of high-quality lead
introduces an alternative immunization approach — the PentaMice platfo
wildtype mouse strains bred in-house for increased MHC class II diversity
Curia is leveraging it for COVID-19 antibody discovery.

Most approved therapeutic antibodies on the market today were derived from hybridomas,[1] a technology that has remained largely unchanged since its invention by Köhler and Milstein 47 years ago. To create a hybridoma, animals are first immunized with a target antigen, after which their B cells are isolated and fused to immortal myelomas. Hybridoma clones are then screened and selected for target reactivity. After a target-specific clone has been identified, the originating hybridoma serves as an endless source for further production of the clonal antibody.

With hybridoma technology, antibody diversity and plasma titers, which are predictive of antibody discovery success, are generated by the B cells

of immunized animals. Th
histocompatibility comple
to present the target antig
activating them and causi
stimulatory molecules an
1a). These signals converg
amplification and high affi
Maximizing this response
which is driven by T cell re
peptides presented by MH

MHC II molecules are high
means there is substantial
among MHC class II genes,
which means that each ali

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.

US 20230115257A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2023/0115257 A1**

ZABEL et al. (43) **Pub. Date: Apr. 13, 2023**

(54) **SARS-COV-2 SPIKE PROTEIN ANTIBODIES**

(71) Applicant: **CURIA IP HOLDINGS, LLC**, Albany, NY (US)

(72) Inventors: **Brian A. ZABEL**, Redwood City, CA (US); **Dan LUO**, Newark, CA (US); **Ling ZHANG**, Menlo Park, CA (US); **Vydehi KANNEGANTI**, Hayward, CA (US); **Joyce YU**, Menlo Park, CA (US); **Sophie YANG**, Mountain View, CA (US); **Lequn ZHAO**, c/o Curia IP Holdings, LLC, CA (US); **Hua TU**, Flower Mound, TX (US); **Xiaomei GE**, Foster City, CA (US)

(21) Appl. No.: **17/746,859**

(22) Filed: **May 17, 2022**

Related U.S. Application Data

(60) Provisional application No. 63/216,406, filed on Jun. 29, 2021, provisional application No. 63/317,441, filed on Mar. 7, 2022, provisional application No. 63/189,635, filed on May 17, 2021.

Publication Classification

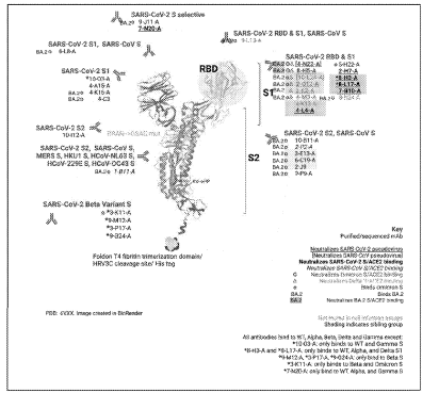
(51) **Int. Cl.**
C07K 16/10 (2006.01)
G01N 33/569 (2006.01)

(52) **U.S. Cl.**
CPC ***C07K 16/10*** (2013.01); ***G01N 33/56983*** (2013.01); *C07K 2317/24* (2013.01); *C07K 2317/76* (2013.01); *C07K 2317/92* (2013.01); *G01N 2333/163* (2013.01); *G01N 2800/26* (2013.01)

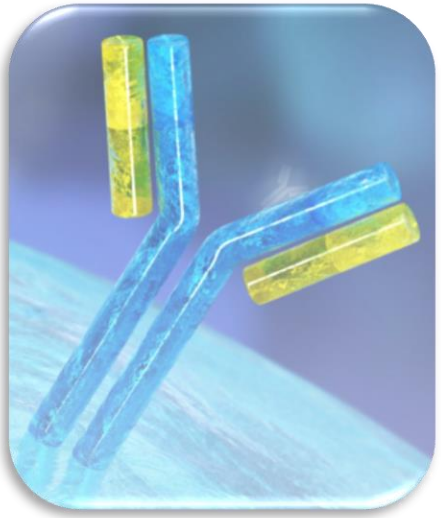
(57) **ABSTRACT**

Embodiments include monoclonal antibodies (mAbs) that recognize SARS-CoV-2 spike protein. The mAbs are capable of distinguishing among variants of the virus. The present disclosure also provides a composition and methods of making and using such a composition for treating, preventing, and/or detecting SARS-CoV-2 infection.

Specification includes a Sequence Listing.



Thanks and Let's Connect To Discover Great Antibodies!



Curia Global, Inc.

Web: Connect with an Expert

Email: antibody@curiaglobal.com

Tel: 877-275-2674