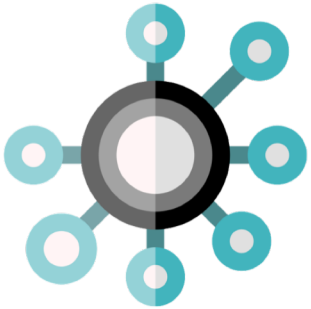
Coronavirus Immunotherapeutics Consortium



CoVIC

SUPPORTED BY:

COVID-19 Therapeutics Accelerator

A partnership of
The Bill & Melinda Gates Foundation
Mastercard, The Wellcome Trust and
others

A supplement from the
National Institute of Immunology and Infectious Diseases
to
The Consortium for Immunotherapeutics
Against Emerging Viral Threats (VIC)
CETR U19 AI142790

GHR FOUNDATION®

Goals

- **Primary - Therapeutic**
  - Evaluate promising therapeutic candidates in independent, standardized platforms (interest of NIAID)
  - Identify highly potent combination of 2 fully human neutralizing monoclonal antibodies against SARS-CoV-2 S protein for prevention of severe COVID-19 in low and middle income countries (LMIC; interest of BMGF)
- **Secondary - Academic**
  - Survey landscape of antibody activities against SARS-CoV-2 S protein using anonymized samples
  - Evaluate current assays for future use (i.e. do in vitro assays and animal models adequately correlate with success in humans? If not, why not?)

Use Cases: saving Lives is the highest priority

Treatment

Mild to moderate COVID-19

Treatment for individuals with mild to moderate COVID-19 who are at high risk for severe disease

Prophylaxis

Health Care Workers and First Responders

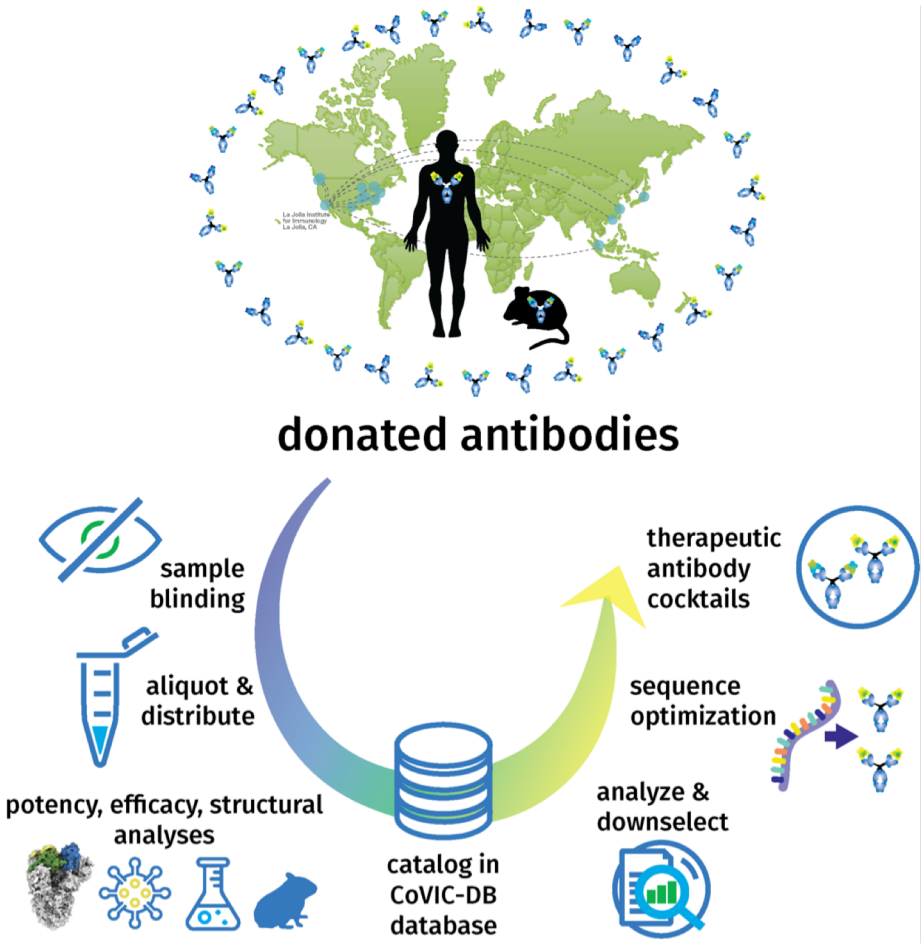
High risk Groups

Disease Outbreaks

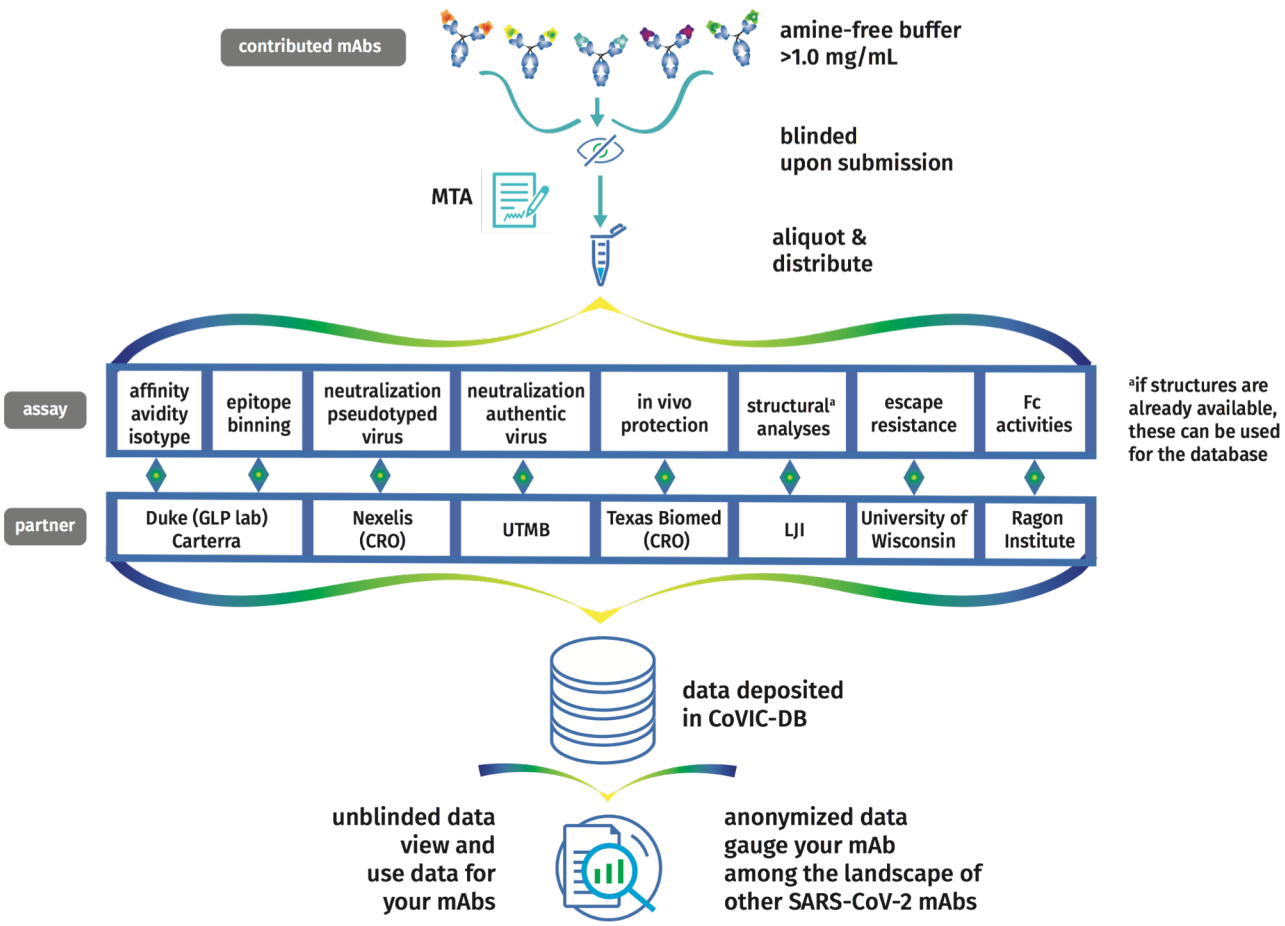
Immediate protection for 3-6 months for:

- Health care workers and first responders
- High risk groups (e.g., pregnant women)
- Ring vaccination-type response to disease outbreak

CoVIC workflow



CoVIC assays

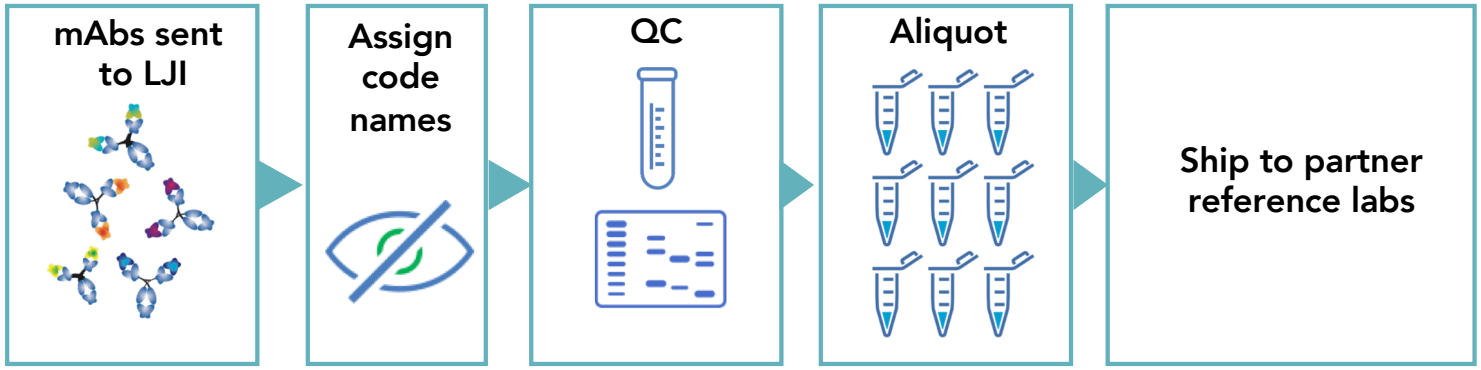


Entry criteria for mAbs

- Minimum:
  - Binding to SARS-CoV-2 S protein < 100 nM
  - Evidence of functional activity (e.g., block binding to ACE2, neutralization of pseudotyped or live virus)
  - Proper consent for mAbs isolated from human subjects
  - Simple MTA with LJI. Antibody owners retain all IP.
  - For BMGF, Global Access and Data Sharing Principles
- Desired:
  - Neutralization activity
  - Variety of epitopes
- Number of antibodies:
  - Groups of up to 20* mAbs

*more could be considered with sufficient rationale

Antibody contribution



- CoVIC PI and all reference labs are blinded to mAb name and source.
- OWS and BMGF Program Officers and CoVIC Program Manager are unblinded (but keep data confidential)
- Contributors know code names of their own mAbs, can view data as it is collected, can request re-analysis if data not as expected
- Contributors retain all IP and may publish and develop as they wish

Data sharing

- Blinding of antibody identity will be removed only upon consent of contributor
- Contributors are free to:
  - Publish data on their antibodies
  - Use data generated by CoVIC on their antibodies for publications or IND filings
  - Continue to develop their antibodies as they wish

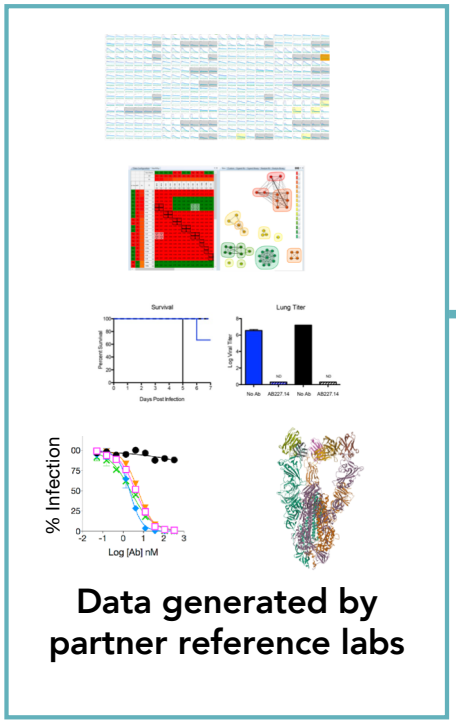
Benefits of contributing

- Opportunity to contribute to finding a solution to a global health crisis and protect the most vulnerable populations
- Access to a broad range of assays and complete analysis at no cost
- Gauge relative performance of mAb as part of parallel analyses using standardized antigens, reagents, and assay platforms
- Satisfy Operation Warp Speed requirement for independent verification on independent, standardized platforms
- Possible identification of mAbs that pair with contributed mAb to achieve optimal efficacy and escape resistance
- Sequence evaluation for developability and identification of risk residues
- Possibility to generate variants including modification of risk residues and Fc engineering
- Data will be analyzed by experts in the field

Complementing, not competing

- CoVIC will not compete with your antibody discovery effort
- We understand the tremendous investment you have made in therapeutic molecules- the world needs your life-saving therapies *now* and CoVIC will not impede this progress. We intend to accelerate your progress.
- CoVIC provides additional and complementary data that you can use for your own purposes
- CoVIC offers the opportunity for your molecules to be evaluated by the Operation Warp Speed (OWS) and the Therapeutics Accelerator. The Therapeutic Accelerator can further assist mobilization of therapeutic molecules to save lives in LMIC.

CoVIC database: a profile of therapeutic antibodies against SARS-CoV-2 Spike protein



Data generated by partner reference labs

CoVIC Database
CoVIC-DB

Data analysis
Landscape of activities of anonymized mAbs

Contributors can view

- Unblinded data for their mAbs
- How their mAbs compare with other blinded mAbs in the panel

Contributors will receive

- Email confirmation of mAb submission
- Email alerts when new data are available
- Data that can be used for IND filings

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Bjoern Peters

Amounts needed

Opt-in[a]	Assay	Amount (mg)
	Biochemical assays (affinity for full-length, HexaPro S D614 and D614G, S RBD and NTD; ACE-2 binding inhibition; epitope binning)	2
	Pseudovirus neutralization (VSV backbone displaying D614 or D614G)	2
	Live virus neutralization (high-throughput GFP readout)	2
	In vivo testing (hamster and TgACE-2 mice- amt. dependent on selected dose)	3-40[b]
✓	High resolution structural analyses (cryo-EM and crystallography)	5-8
	Fc systems serology	1
	Cell-based assays of Fc activity	1
	Glycan analyses	1
	Escape analyses	1
✓	Half-life and ADE resistance in ACE2, TRPMSS2 hFcRN knockin mice	2

[a]Request to include your antibodies in these analyses
[b]Amount dependent on dose selected from pilot studies with reference mAbs

Shipping and antibody storage

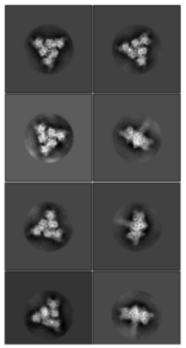
Shipping Address

- La Jolla Institute for Immunology
  Attn: Sharon Schendel, CoVIC
  9420 Athena Circle
  La Jolla, CA 92037 USA
  Phone: 858.752.6555
- For FedEx, specify "Priority Alert". World Courier and DHL are reliable for international shipments. Consult with Sharon about language for Commercial Invoice needed to clear customs.
- International shipments: To avoid delays clearing customs, include a Commercial Invoice, stateing that materials are for research purposes only and are highly purified proteins that are completely non-hazardous. Consult with Sharon for sample invoice

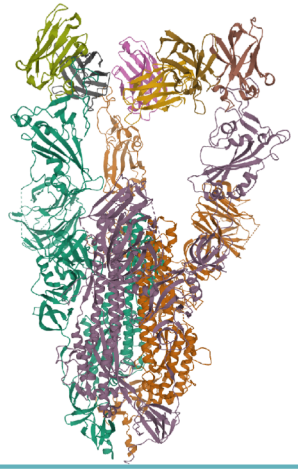
Antibody storage

- No buffers with free amines (e.g., Tris, His, azide); PBS is preferred buffer
- At least 1 mg/mL concentration
- Ship on ice, frozen, or lyophilized
- Complete antibody submission template (to be supplied)

High-resolution structural analyses



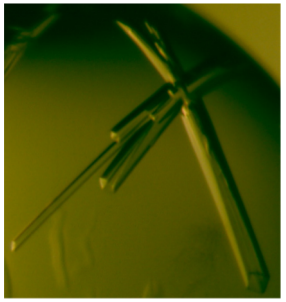
Cryo-electron Microscopy

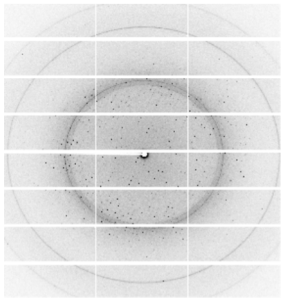


mAb Fab domains in complex with:

- Full-length S
- RBD or NTD
- transmembrane surface spike

Intact mAbs in complex with FcR and Spike





X-ray Crystallography

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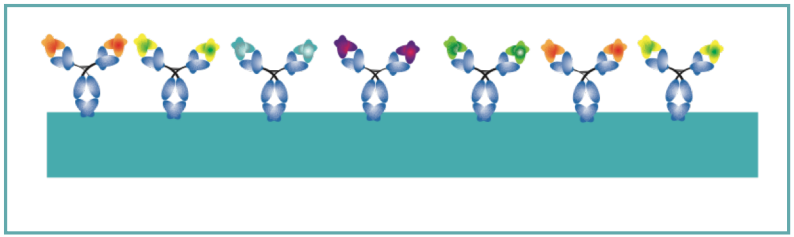
Erica Ollmann Saphire

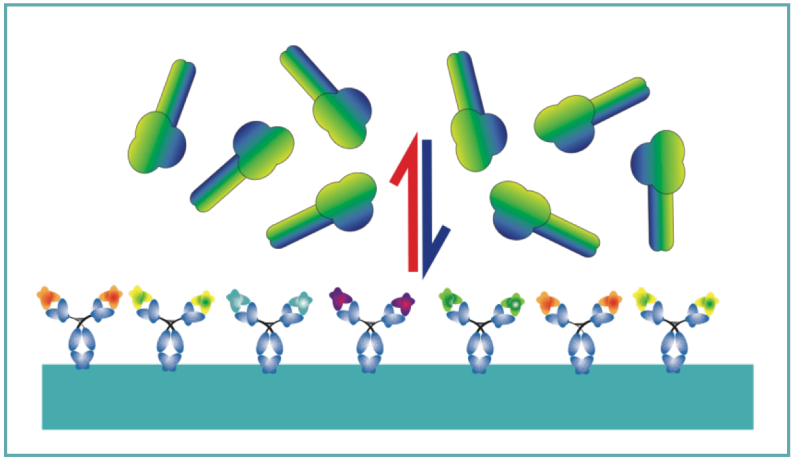
Structural-biology grade antigens

Antigen	Residues	Tags	Stabilizing Mutations	Other modifications	GenBank accession	Ref.	Provider
Full-length ectodomain Spike (SARS2_dfurin_Hexa Pro fib_HRV_8XHISdStrep) D614	aa 1-1208	C-terminal TwinStrep Tag, 8XHisTag	F817P, A892P, A899P, A942P K9896P, V987P Furin site mutated: RRAR>GSAS (aa682-685)	C-terminal T4 fibritin trimerization motif; HRV3C protease cleavage site	Base sequence (GenBank): MN908947 From Wuhan Hu-1 2019-nCoV S isolate gene sequence	Hsieh et al. Science 32703906 and Wrapp et al. Science 32075877	Saphire
Full-length ectodomain Spike D614G	*Ibid* except for Gly substitution for Asp at aa 614					Korber et al., Cell 32697968	Saphire
RBD	aa318-591	w/ and w/o C-terminal EK-dStrep-Avi	na	na	GenBank: MN908947	Wrapp et al. Science 32075877	Saphire
NTD	aa14-305	w/ and w/o C-terminal EK-dStrep-Avi	na	na	GenBank: MN908947	Wrapp et al. Science 32075877	Saphire
ACE-2	Na	w/ and w/o C-terminal EK-dStrep-Avi2xStrep	na	Fc-fusion protein	UniProt: Q9BYF1	Tipnis et al. JBC 10924499	GH-VAP University of Washington

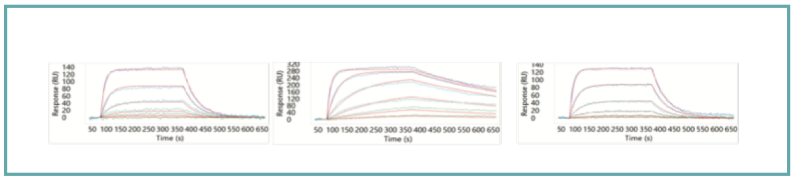
SPR Binding analyses



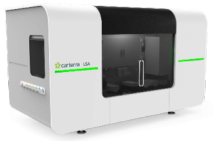
Immobilization of antibodies (*5µg/mL*) on substrate (*HC30 chip*) via Fc- or direct coupling



Collect sensorgrams with LSA platform for ascending titration of antigen (*FL: 0.06-250 nM; RBD: 0.17-714 nM; RBD: 0.14-1,112 nM*)



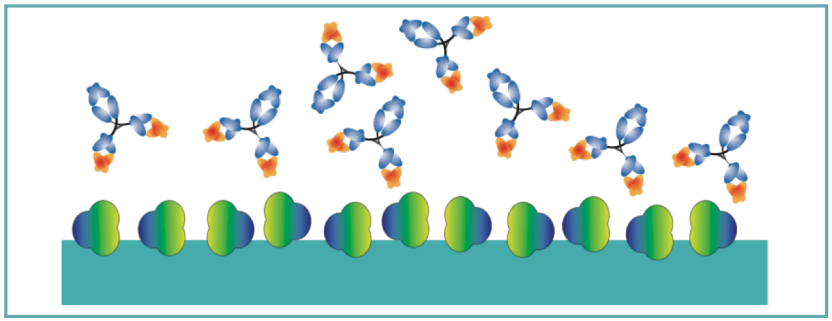
Calculate K_a, K_d, K_D (Langmuir binding model with average of triplicate measurements)



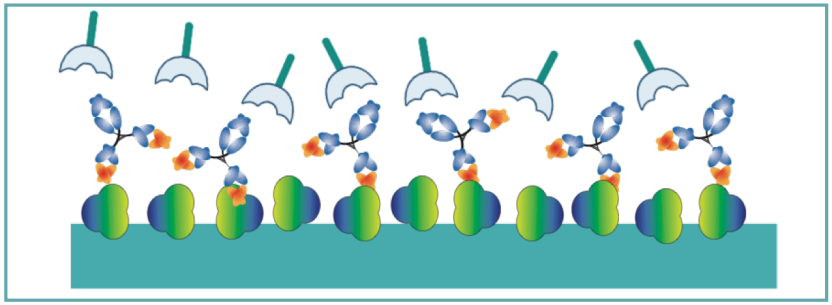
Georgia Tomaras
Duke Human Vaccine Institute
Duke University School of Medicine

Dan Bedinger
carterra

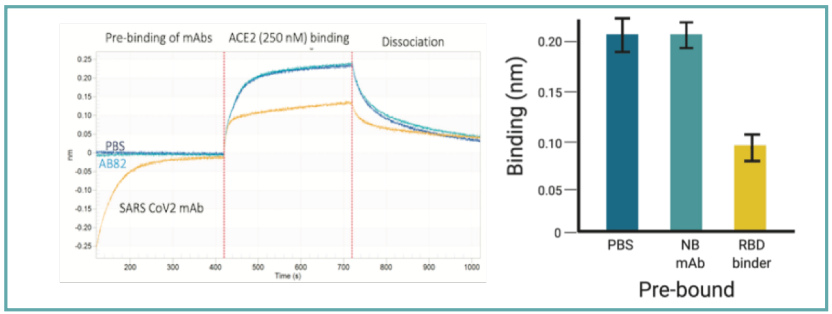
Antibody inhibition of Spike-ACE-2 interaction



In Forte Octet 384 or HTX instrument, RBD immobilized on BLI biosensors is dipped into wells (triplicate) containing saturating amount of antibody (20µg/mL)



Antibody-bound RBD sensors are dipped into soluble ACE2 (250nM) for 300 s and dissociation is followed for 300 s



The percentage inhibition is determined relative to buffer only and non-RBD-binding control mAb

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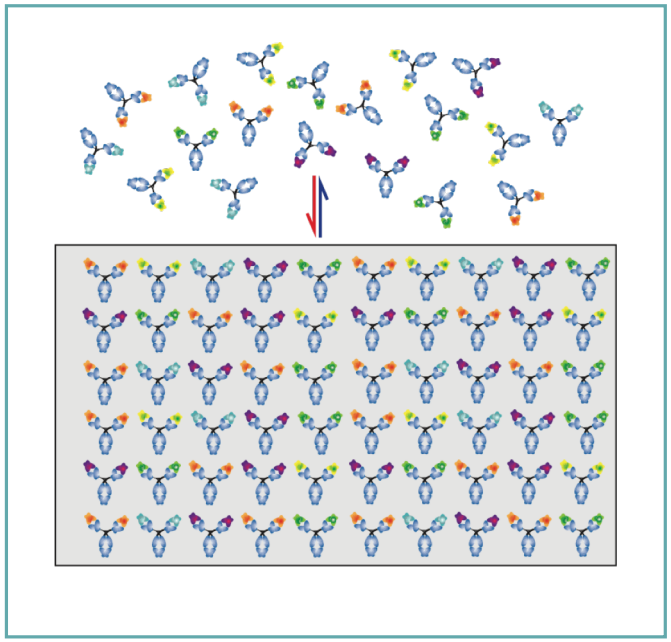
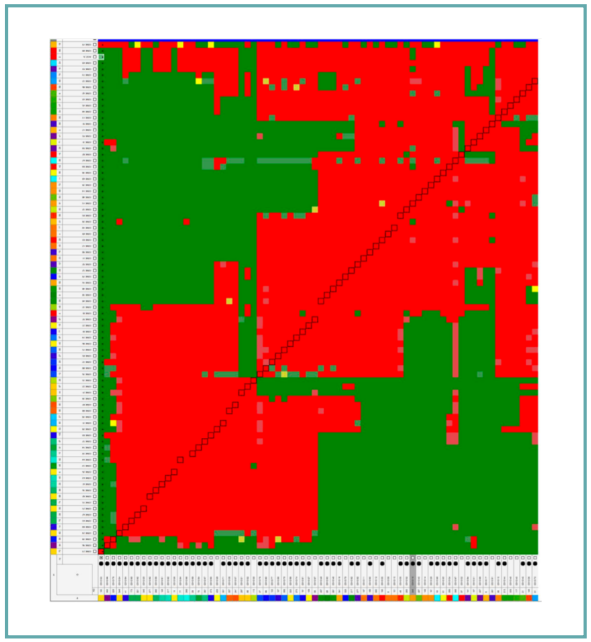
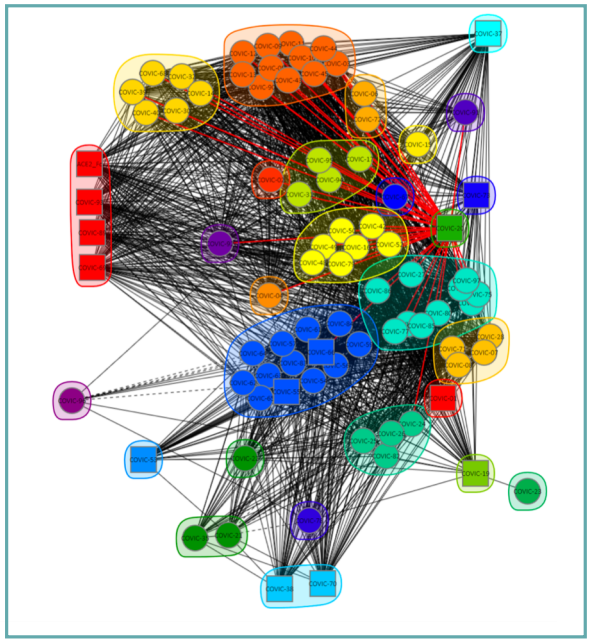
Epitope binning



Antibodies arrayed on chip and a second set of antibodies is flowed across the chip



Heat map for 72 unique CoVIC mAbs, binned in a ligand (rows) x analyze interaction matrix
Red: blocking pairs; Green: sandwiching pairs



Network plot; epitope bins are colored and correspond to heat map plot

Dan Bedinger

carterra

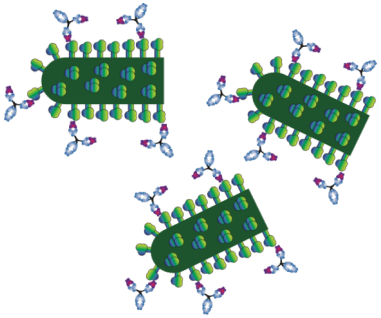
Pseudovirus neutralization

SARS-CoV-2 Spike source: Wuhan Hu-1 strain; NC_045512

Base pseudovirus: VSVΔG (Kerafast)

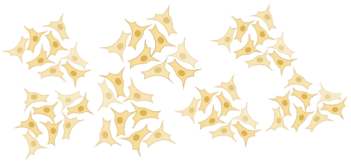
Pseudovirus transduction: HEK293K cells transfected with SARS-CoV-2ΔCT (C-term 19 aa deleted)

Pseudovirus: VSVΔG-SpikeΔCT



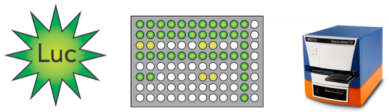
Pre-incubate pseudovirus with CoVIC mAbs at 0.004-3.6 µg/mL in 11 pt. concentration curve for 1 hr at 37 ˚C

Target cells: Vero E6



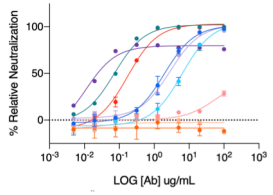
Incubate antibody-pseudovirus complexes with target cells for 20 ± 2 hr at MOI=0.009

Reporter: Luciferase
Lysis reagent: One-Glo (includes luciferase substrate)
Plate reader: SpectraMax i3



Lyse cells, read luminescence

Calculation method: 4-PL curves
Software: SoftMax pro GxP



Generate neutralization curves from average of triplicate measurements; calculate IC_{50}, IC_{80}, IC_{90} values

nexelis

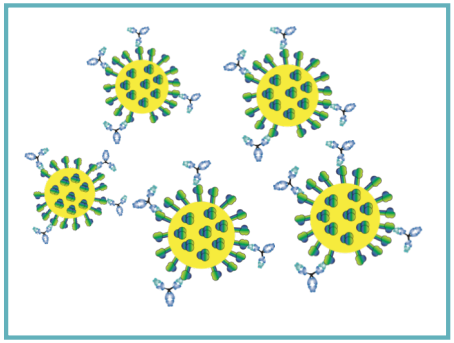
Luc Gagnon

Authentic virus neutralization

Virus: SARS-CoV 2019-nCoV/USA_WA1/2020 (MN985325)

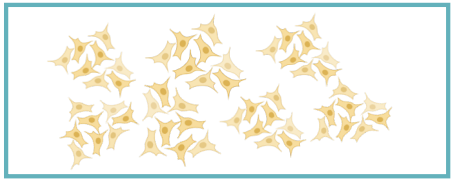
Modified virus: SARS-CoV-2-mNG with mNeonGreen replacing Orf7 (PMID: 32289263; passage ≤3)

Containment: BSL-3



Pre-incubate SARS-CoV-2-mNG with CoVIC mAbs at 0.048-200 µg/mL in 7 pt. concentration curve for 1 hr at 37 ˚C

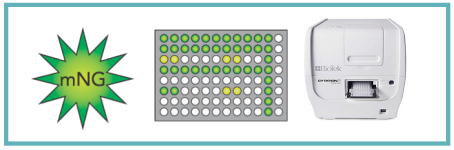
Target cells: Vero E6



Apply antibody-pseudovirus complexes to target cells for 48 hr at MOI=0.005

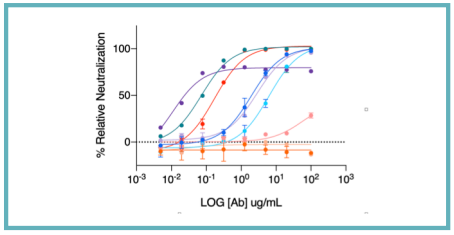
Reporter: mNeonGreen GFP

Plate reader: Bioteck Instruments Cytation 2



Quantitate mNeonGreen readout

Calculation method: 4-PL curves



Generate neutralization curves from average of triplicate measurements; calculate IC_{50}, IC_{80}, IC_{90} values

Alexander Bukreyev

utmb Health
Galveston National Laboratory

In vivo efficacy: hamsters

Animals: Golden Syrian hamsters (Charles River Laboratories (049); 4-6 weeks-old; ~80 g;

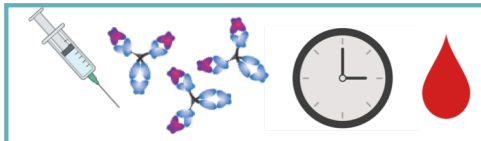
Containment: BSL-3

Acclimated for 10 days after delivery

n=13/mAb

mAb Dose: TBD from pilot study of 1, 3, 10, 30 mg/kg

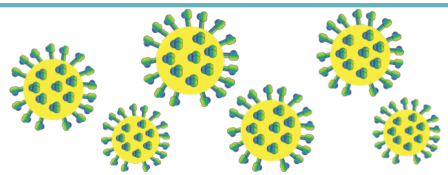
Delivery: I.P. 24 hr before challenge



Antibodies delivered IP 24 hr before virus challenge; blood samples collected for PK studies

Challenge virus: SARS-CoV-2/human/USA/WA-CDC-WA1/2020 Passage 6 (MN985325); Stocks confirmed by NGS to be 100% identical to BEI original P4 stock; Bristol deletion and other deletions/mutations confirmed to be absent from working stocks

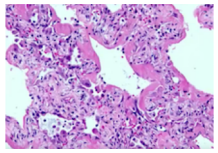
Virus inoculum: 2 x 10^5 PFU

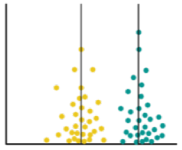


Virus delivered intranasally (25 µL/nostril)

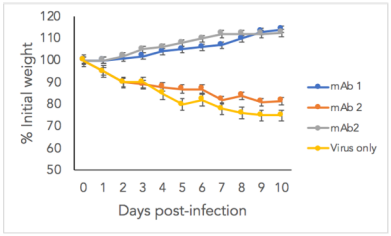
Divide animals into two groups

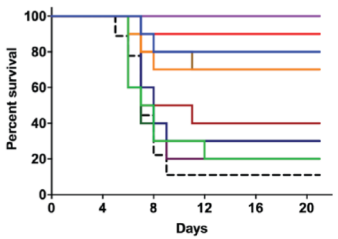
Group 1: (n=8) Analyze gross pathology; viral load in nasal turbinates and lung, histopathology on 2 (n=4) and 4 (n=4) dpi





Group 2: (n=5) Followed through 10 dpi for morbidity (weight loss) and mortality (survival)





TEXAS BIOMEDICAL RESEARCH INSTITUTE

Jordi Torelles

A post-exposure therapeutic dose-finding study for 24 h post-infection is planned for candidates that perform well in prophylaxis studies

In vivo efficacy: mice

Animals: K18 hACE2 transgenic (The Jackson Labs (034860)
4-6 weeks-old; ~20-30 g;

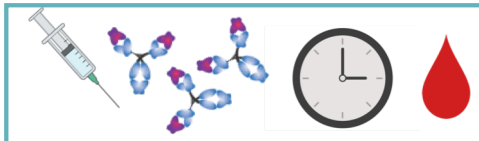
Containment: BSL-3

Acclimated for 10 days after delivery

n=13/mAb

mAb Dose: TBD from pilot study of 1, 3, 10, 30 mg/kg

Delivery: I.P. 24 hr before challenge



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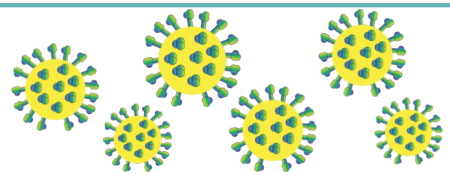
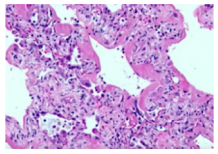
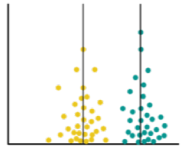


Virus delivered intranasally (25 µL/nostril)

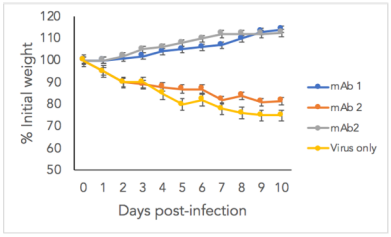
Divide animals into two groups

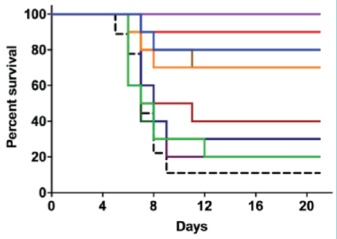
Group 1: (n=8) Analyze gross pathology; viral load in nasal turbinates and lung, histopathology on 2 (n=4) and 4 (n=4) dpi





Group 2: (n=5) Followed through 10 dpi for morbidity (weight loss) and mortality (survival)





TEXAS BIOMEDICAL RESEARCH INSTITUTE

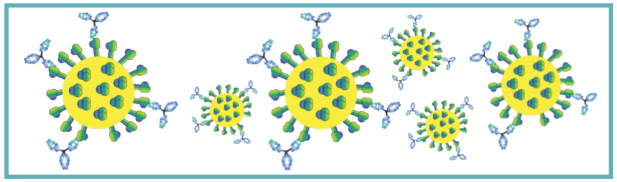
Jordi Torelles

A post-exposure therapeutic dose-finding study for 24 h post-infection is planned for candidates that perform well in prophylaxis studies

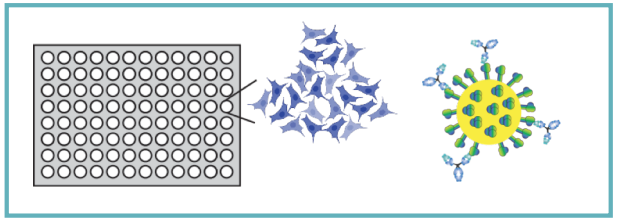
Mapping escape risk in vitro

SARS-CoV-2 virus:
SARS-Cov-2/USA357 WI1/2020
(GenBank: MT039887.1)
SARS-CoV-2 isolate 177-11368 (D614G)

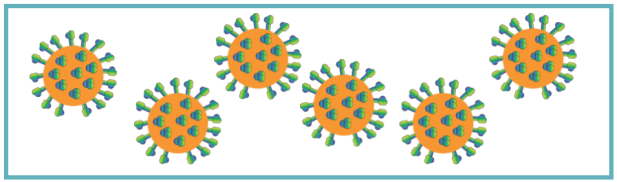
Target cells: Vero E6/TMPRSS2
(PMID: 32164551)



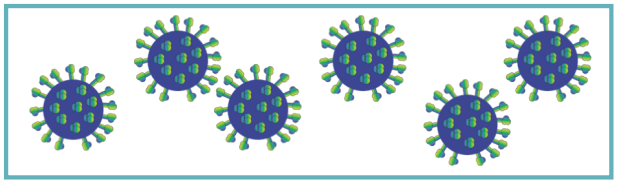
Incubate 50, 500, or 5,000 PFU virus with 0.02-10 µg/mL CoVIC mAb for 1hr at 37˚C



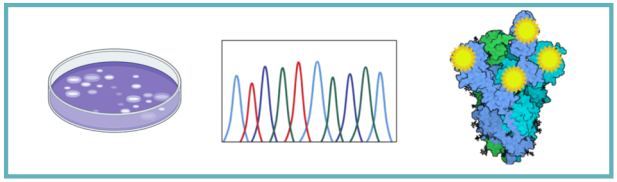
Add to target cells in 96 well-plates, culture until 80% cell death or 7 dpi



Harvest P1 virus; repeat first two steps



Harvest P2 virus



Plaque purify virus, amplify in Vero-E6T cells, sequence S gene to identify escape mutations

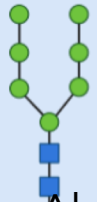
Yoshihiro Kawaoka

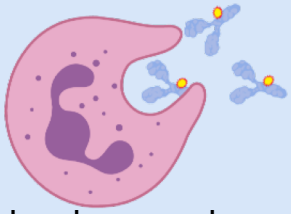
Peter Halfmann

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

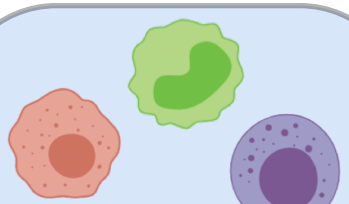
Immune profiling of mAbs against SARS-CoV-2 Spike

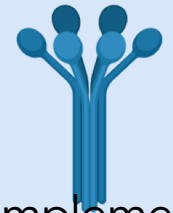

mAb-FcR binding profiles


mAb glycosylation


mAb-dependent phagocytosis


NK cell degranulation


Innate immune effector cell activation


complement deposition

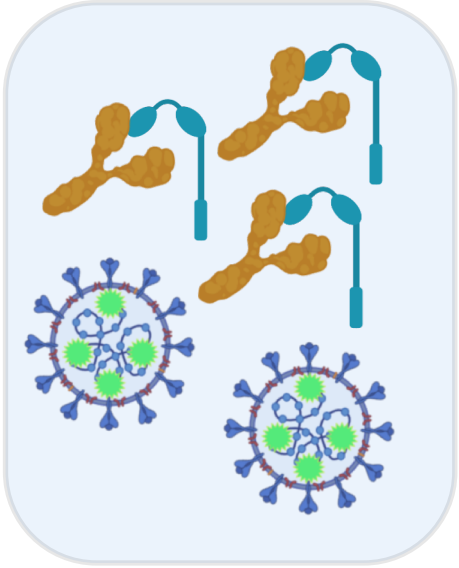

Galit Alter

Ragon Institute of MGH, MIT and Harvard

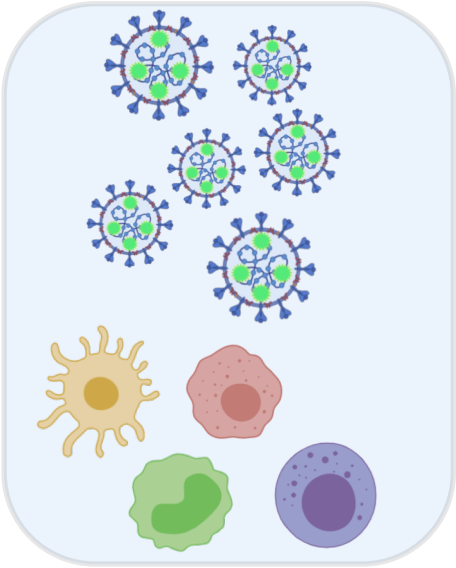
Approach: Use high-throughput systems serology to profile Fc-driven activities of anonymized mAbs. With database, determine which features (footprint, approach angle, avidity, Fc type and other factors) link to in vivo protection.

Cellular studies to examine resistance of therapeutic candidate mAbs to ADE

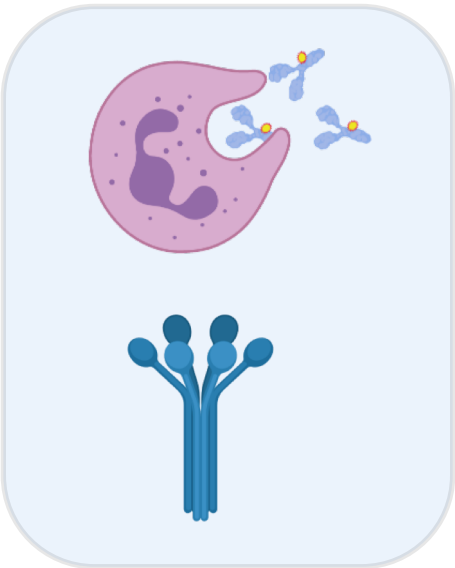
Bio-layer interferometry (BLI) to determine binding kinetics and affinity of mAbs for FcRs



Examine effect of blocking FcɣR on mAb inhibition of infection by SARS-CoV-2 with Neon-Green reporter



Assess ADE propensity using live SARS-CoV-2 with Neon-Green reporter* to infect various human myeloid cell types



Determine phagocytic score and complement deposition to characterize role of Fc effector function in ADE

Alexander Bukreyev

utmb Health
Galveston National Laboratory

*Xie et al. *Cell Host Microbe* 2020 PMID 32289263

In vivo analyses of antibody half-life and ADE resistance in human Fc-FcRn binding setting*

Triple knock-in mice expressing human ACE2, FcRN and TMPRSS2

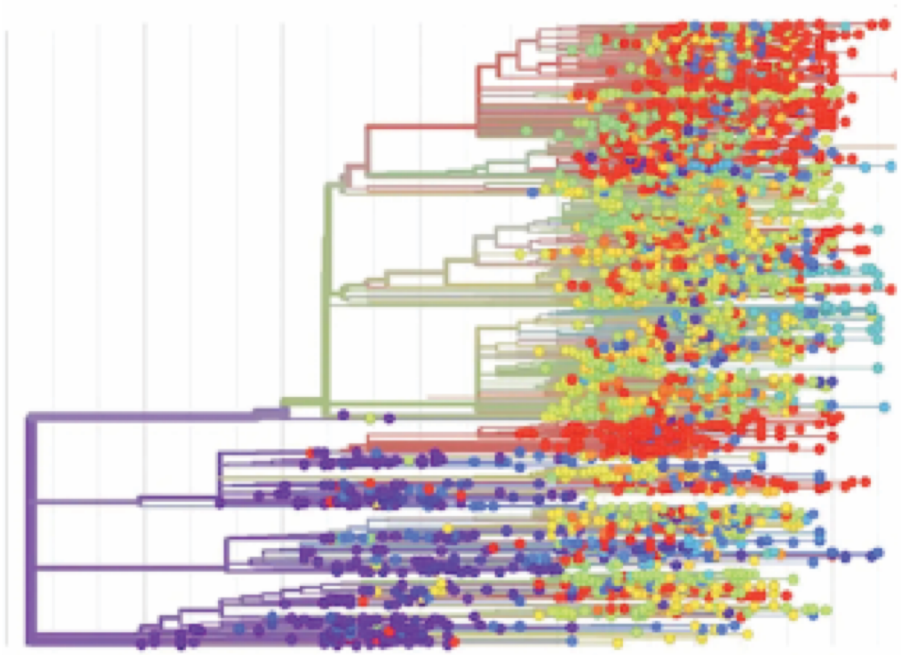
- Evaluate novel mouse model for predictive efficacy
- Analyze serum and tissues for viral loads, kinetics and histopathology after intranasal infection with SARS-CoV-2 and treatment with candidate mAbs
- Determine half-life for each mAb that has efficacy without ADE

Sujan Shresta

La Jolla Institute FOR IMMUNOLOGY | Life Without Disease.®

*Antibody owners may opt-in to this study

Tracking SARS-CoV-2 Spike mutations in real-world, human-to-human transmission



Los Alamos NATIONAL LABORATORY

Bette Korber

- Monitor GISAID to identify recurrent spatial and regional changes in mutation frequency
- Develop new tools for mutational tracking
- Keep researchers abreast of emerging mutations
- Suggest therapeutic candidates that remain responsive to emergent mutations

Questions? Feedback?
Join us!

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