Deep mining of early antibody response in COVID-19 patients yields potent neutralisers and reveals high level of convergence

John McCafferty

FairJourney Biologics

IONTas

Flow Eighteen30

Deep mining of early antibody response in COVID-19 patients

Bullen et al (2020)

https://www.biorxiv.org/content/10.1101/2020.12.29.424711v1

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- Introduction
- Donor-derived phage libraries
- Analysing donor responses
- Identifying and characterising neutralising antibodies
- Effect of viral variants

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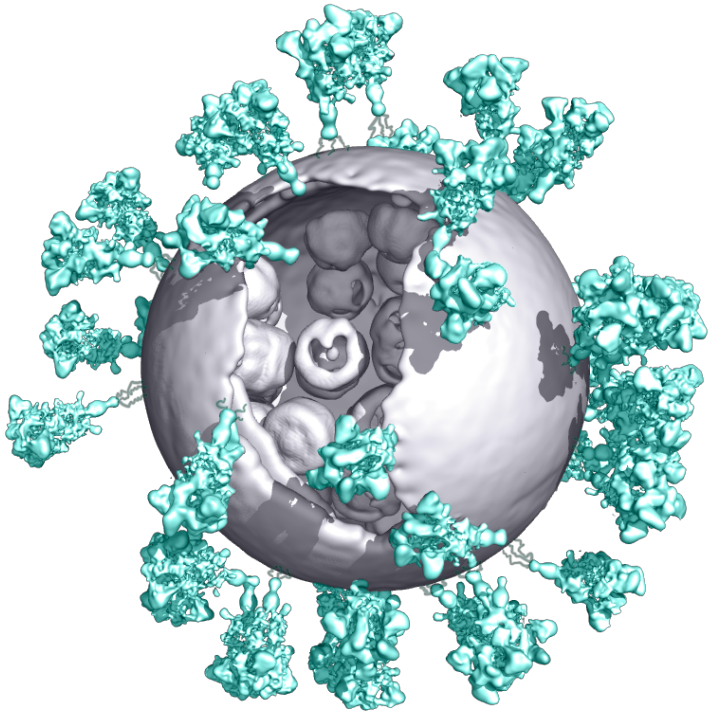
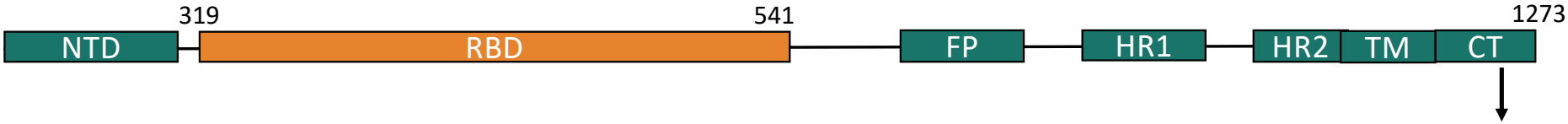
“Molecular Architecture of the SARS-CoV-2 Virus“



- 30kb RNA genome
- 29 proteins
- Spike protein enables viral entry through ACE-2
- Average of 26 spike proteins/virus
  - Yao et al (2020)

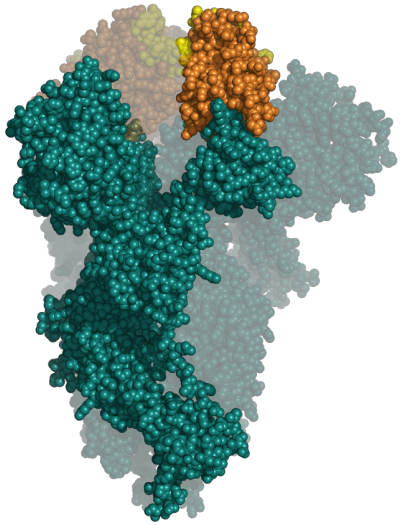
Yao et al (2020) Cell *183*, 730–738

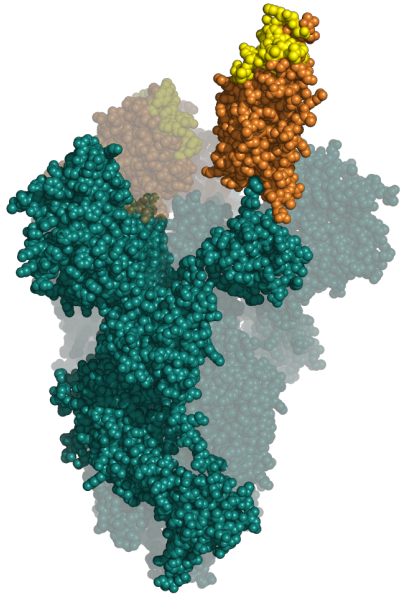
SARS-Cov-2: The spike protein



cytoplasmic tail
….8/37 are Cys!

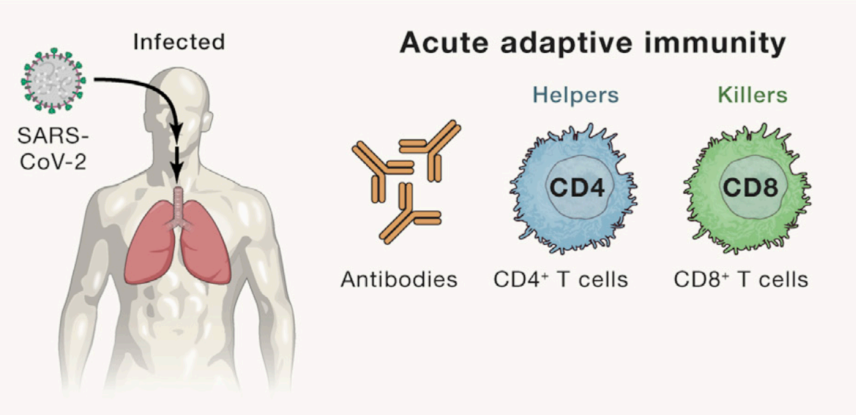
Closed



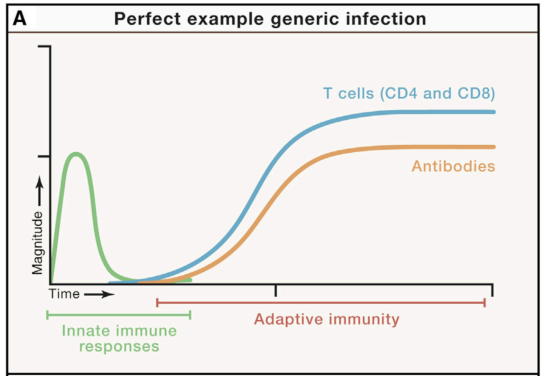


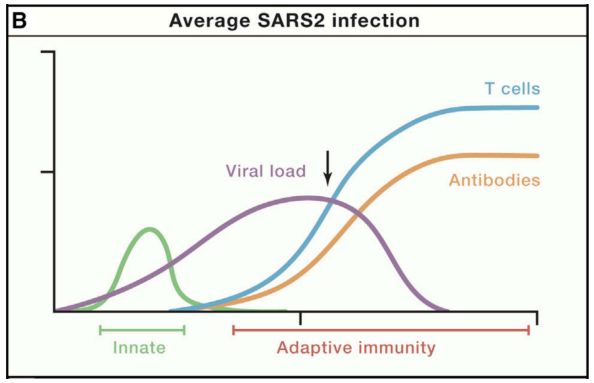
open

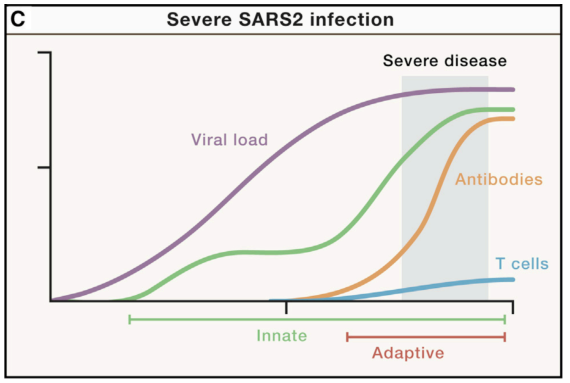
Immune response to coronavirus



From:
Sette A, Crotty S
Adaptive immunity to SARS-CoV-2 and COVID-19
Cell *184*, February 18, (2021) 1-20







Potential of neutralising antibodies

March 10, 2021 20:22 ET | **Source:** Vir Biotechnology, Inc.

Vir Biotechnology and GSK Announce VIR-7831 Reduces Hospitalization and Risk of Death in Early Treatment of Adults with COVID-19

– Independent Data Monitoring Committee recommends stopping Phase 3 COMET-ICE trial early given an 85% reduction in hospitalization or death –

– Vir and GSK plan to immediately seek Emergency Use Authorization in the U.S. and authorizations in other countries –

– Additional new in vitro studies indicate VIR-7831 maintains activity against major circulating COVID-19 variants –

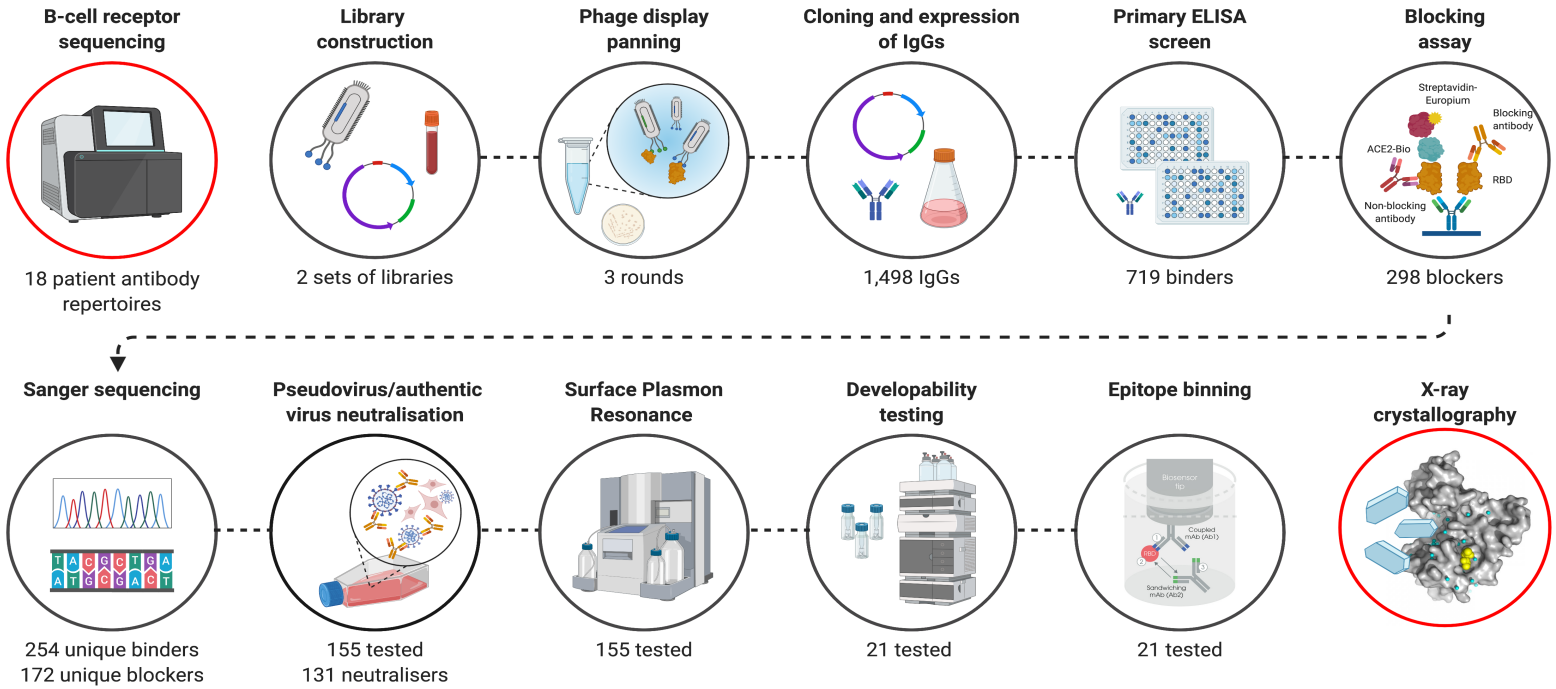
Based on **S309** antibody

-originates from SARS-CoV-1 patient

-cross-reacts with SARS-CoV-2

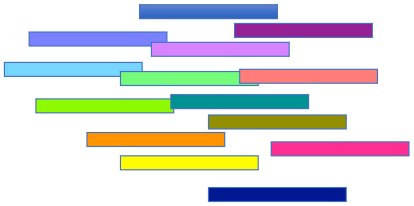
Overview of IONTAS process

Collaborators: Kymab, Alchemab, LifeArc, Abcam
National Institute of Biological Standards and Control (NIBSC)

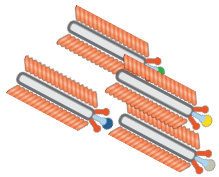


Identifying ACE2 blocking antibodies

PAXgene blood
RNA system

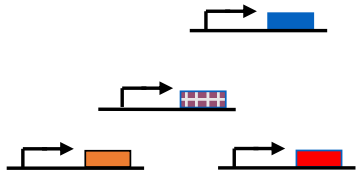


Phage display libraries
(either donor VLs or naïve VLs)



Phase selection
(160 selections)

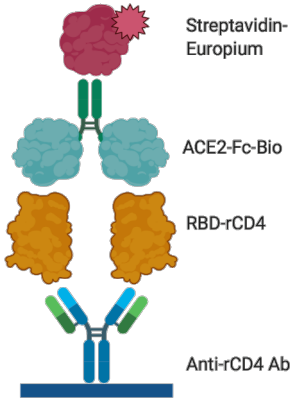
1500 IgGs



254 unique antibodies
-123 unique VHs

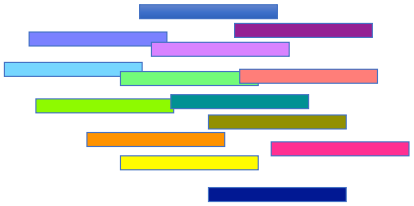
67% ACE2 blockers
(172/254)

33% ACE2 non-blockers
(84/254)

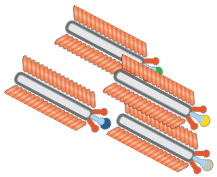


Define epitope
-ACE2 blockers
-ACE2 non-blockers

Analysis of patient antibody response to SARS-CoV-2 RBD domain



Phage display libraries
(either donor VLs or naïve VLs)



123 unique VHs

Illumina sequencing (Alchemab)

3.5 million VH sequences
839,000 clusters
(from 19 donors)

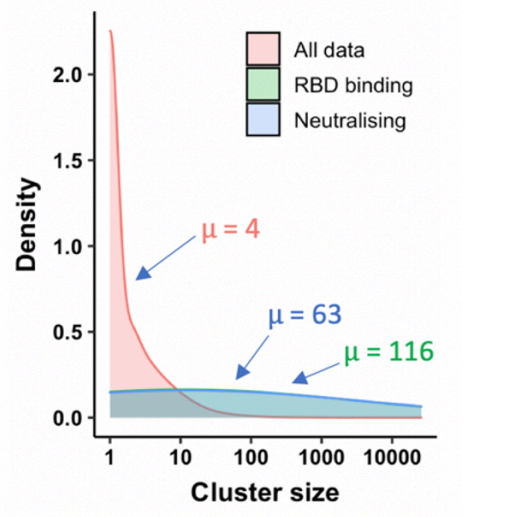
89 RBD binding clusters identified in patients

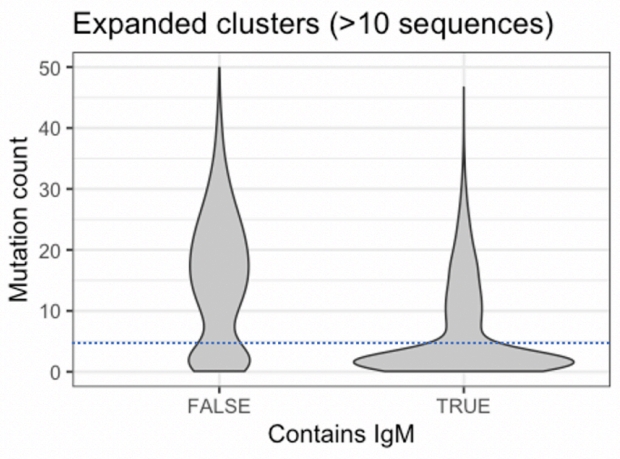
Galson JD, et al. (2020)
Deep Sequencing of B Cell Receptor Repertoires From COVID-19 Patients Reveals Strong Convergent Immune Signatures.
Frontiers in Immunology 1–11.

Antibody response driven by naïve B cell activation

- **Total population (839,000 clusters)**
  - mean 4 sequences/cluster
  - Mean mutations from germline
    - 7.6 mutations/sequence

- **RBD binders (89 clusters)**
  - mean 116 sequences/cluster (clonal expansion)
  - Mean mutations from germline
    - 2.6 mutations/sequence
  - 70% found in IgM population (recent B cell activation)
    - 88% < 5 mutation/sequence





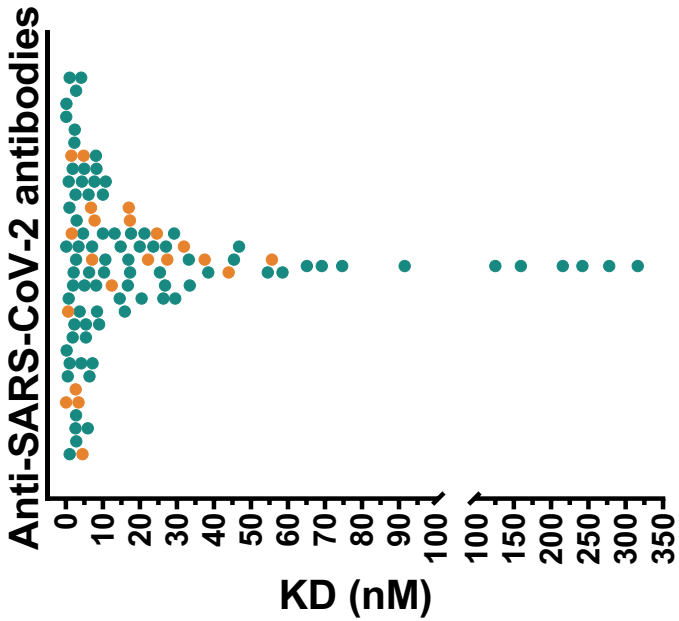
Different patients, same solution!

- 26% (23/89) RBD binding clusters were convergent across at least 2 individuals
- Analysis extended to 1051 sequences of CoV-Ab database
  - Matches identified from other studies

Cluster ID	Representative CDRH3	V gene	J gene	Cluster size	Mean mutation	Convergence	Number of CoV-AbDab hits
1	AAPDCSSTSCYDAFDI	VH1-58	J3	1680	1.7	9	2 mAbs
2	ARDLAVYGMDV	VH3-66	J6	164	2	9	5 mAbs
3	ARDLMVYGMDV	VH3-53	J6	1202	2.1	14	6 mAbs
4	ARDAMSYGMDV	VH3-53	J6	71	0.9	4	2 mAbs
5	ASSLWLRGSFDY	VH3-7	J4	45	1.1	3	1 mAbs
6	AGGPNLNNWFDP	VH5-51	J5	72	1.6	3	1 mAbs

Analysis of patient antibody response Summary

- Early antibody response to SARS-Cov-2 is largely driven by naïve B cell activation
  - Not re-activation of memory B cells
- Convergent antibody response between patients
  - Within this study
  - Across other studies
- 155 antibodies selected for further study
    - 121 ACE2 blocking
    - 34 non ACE2 blocking
- Affinity range 70pM-216nM



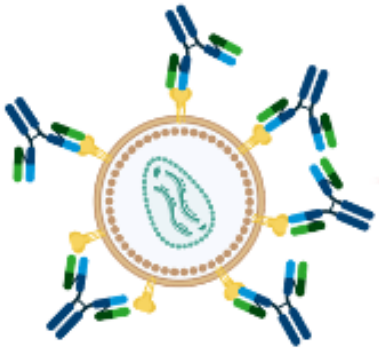
Pseudovirus neutralisation assay

Lentivirus incorporating SARS-CoV2 spike + luciferase gene

HEK293T/17 cells Expressing ACE2 + TMPRSS2

Neutralising

Non-neutralising



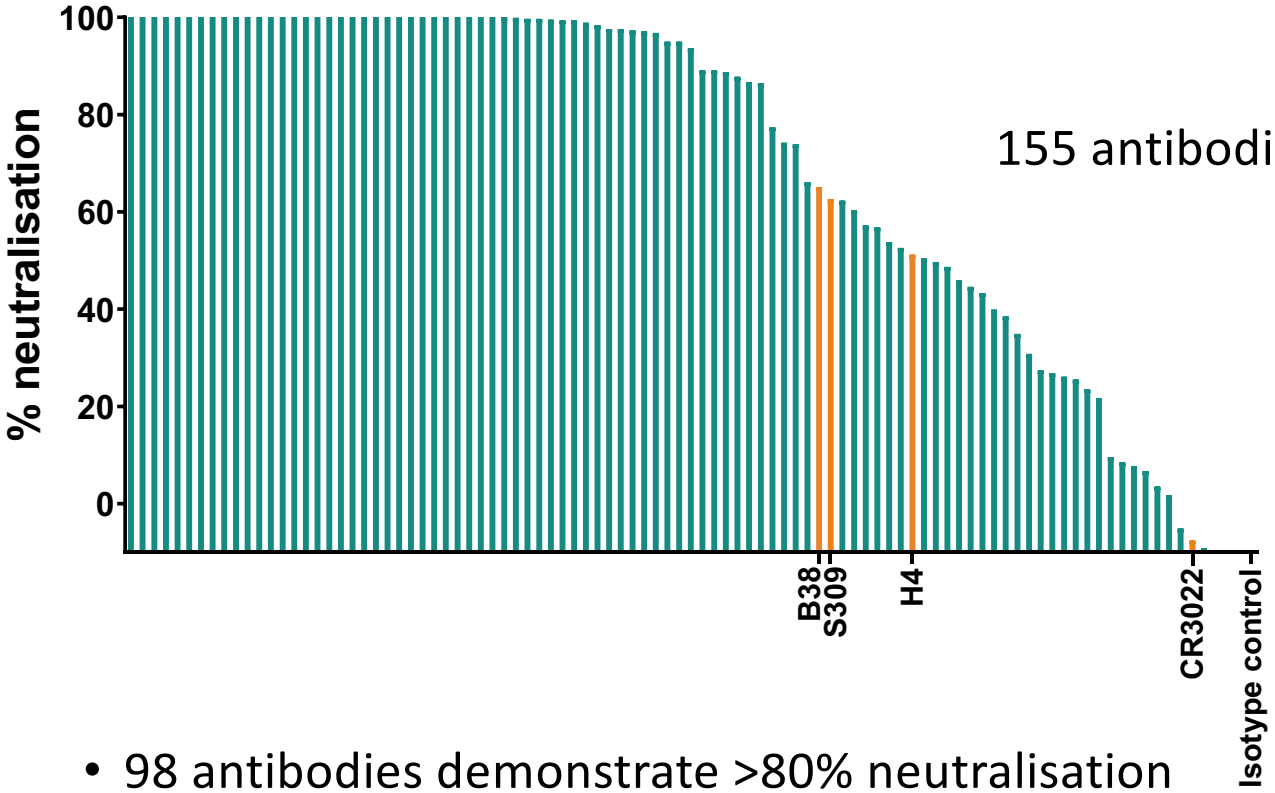
No infection

infection

No light

light

Identification of neutralising antibodies in pseudovirus assay



155 antibodies selected for further study
- 121 ACE2 blocking
-34 non blocking

- 98 antibodies demonstrate >80% neutralisation

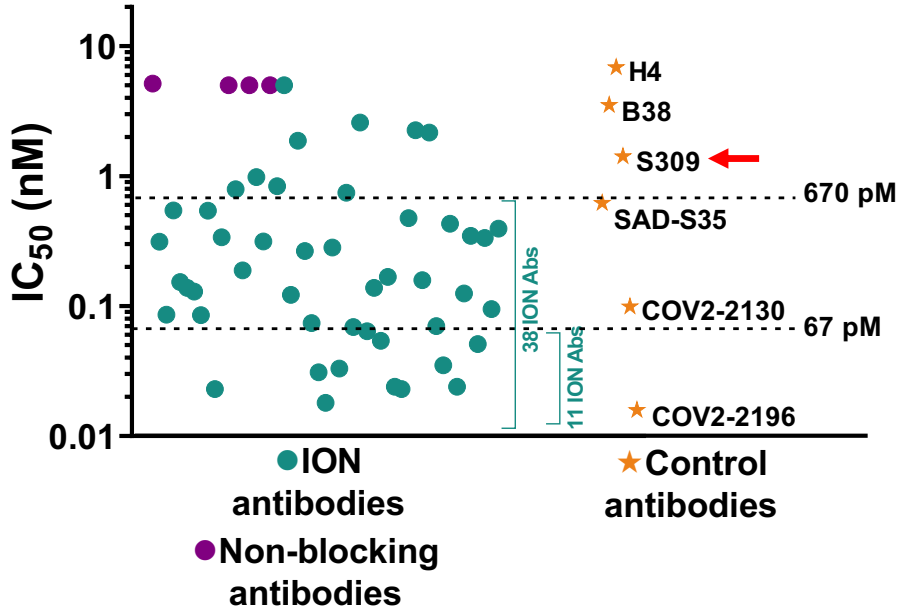
NIBSC

Giada Mattiuzzo
Emma Bentley

Identification of neutralising antibodies in pseudovirus assay

IONTas

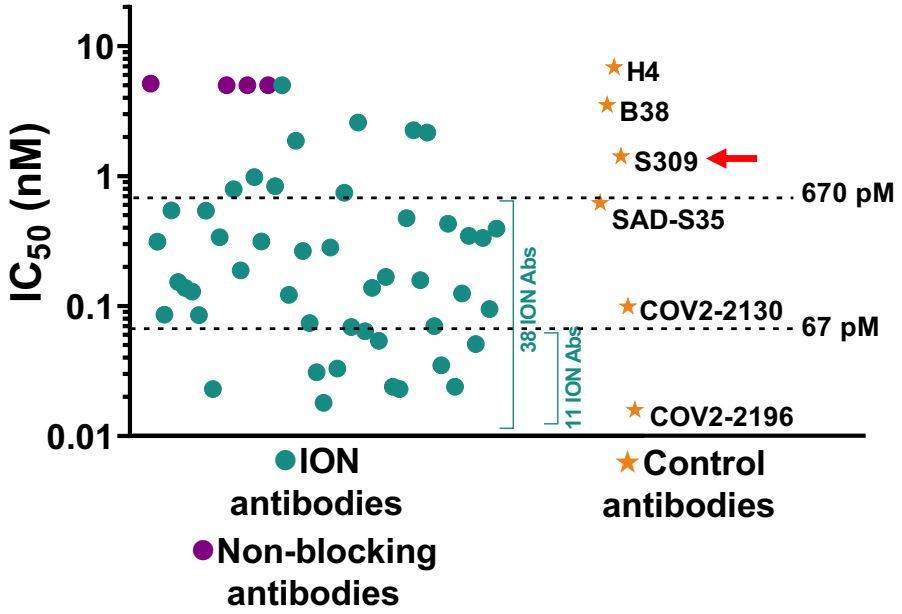
Pseudovirus (51 Abs)



Many potent neutralisers identified!

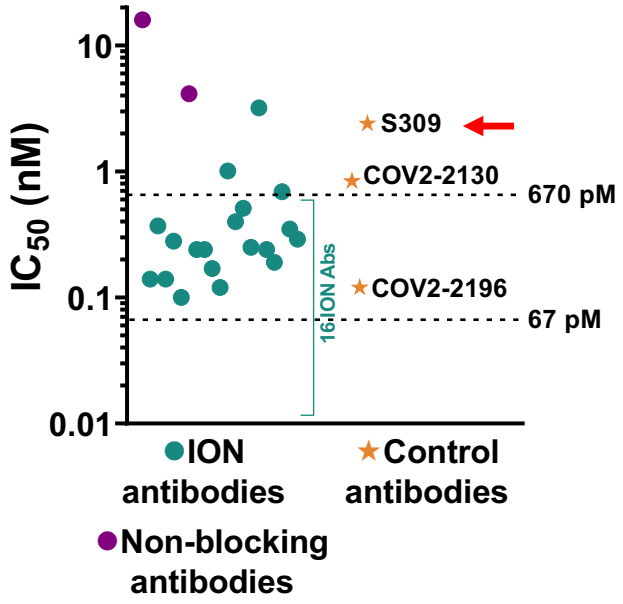
Identification of neutralizing antibodies in live virus assay

Pseudovirus (51 Abs)



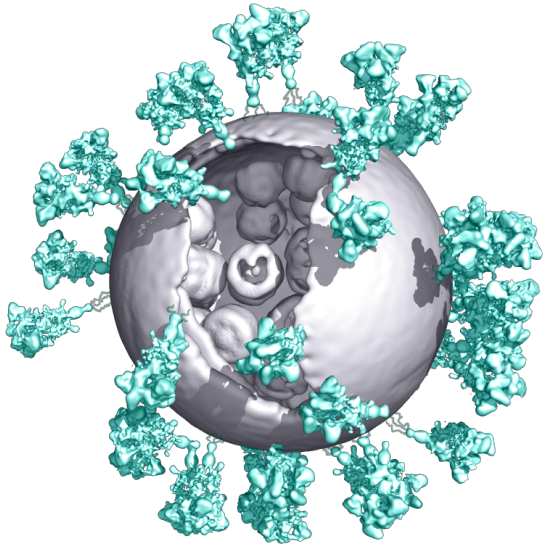
Live virus assay (21 abs)

(Australian isolate VIC01/2020)

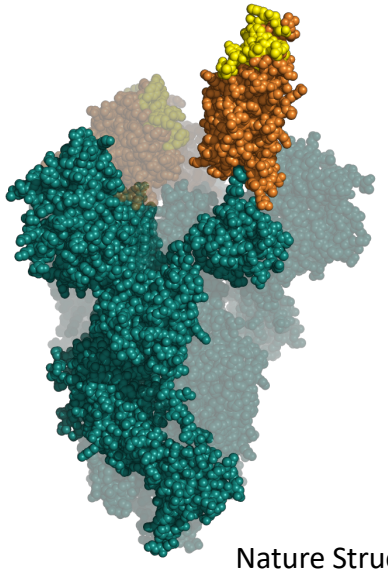


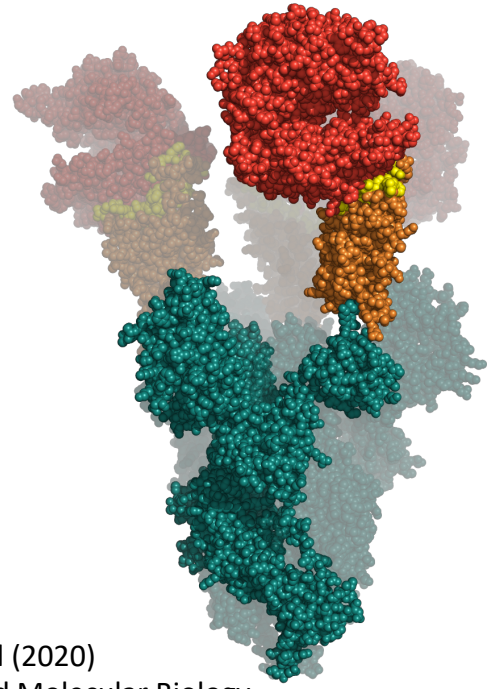
Good correlation between pseudovirus and live virus

Does valency enhance viral neutralization?



Yao et al (2020) Cell *183*, 730–738

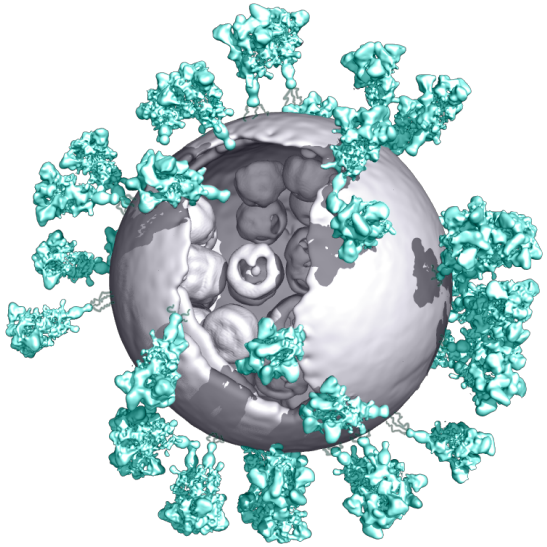


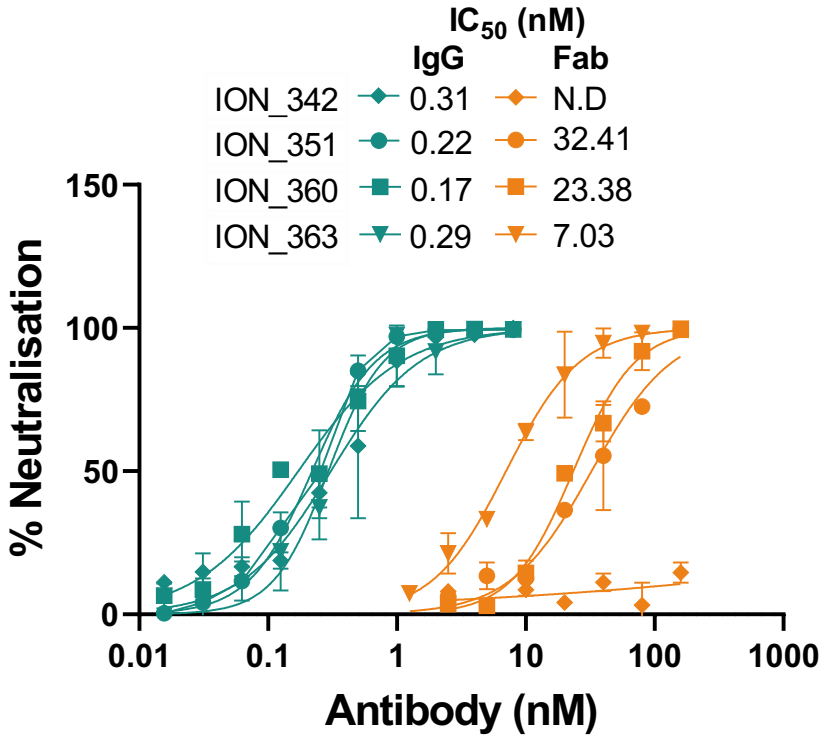


Xiao et al (2020)
Nature Structural and Molecular Biology
28 p202-209

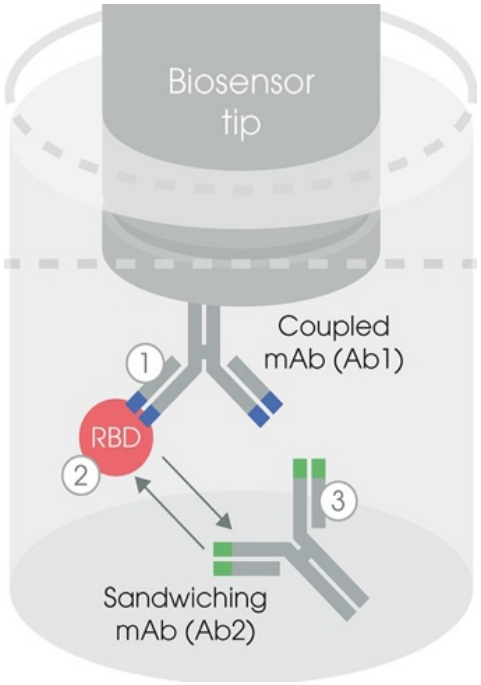
Potential for inter and intra-binding to spike trimer

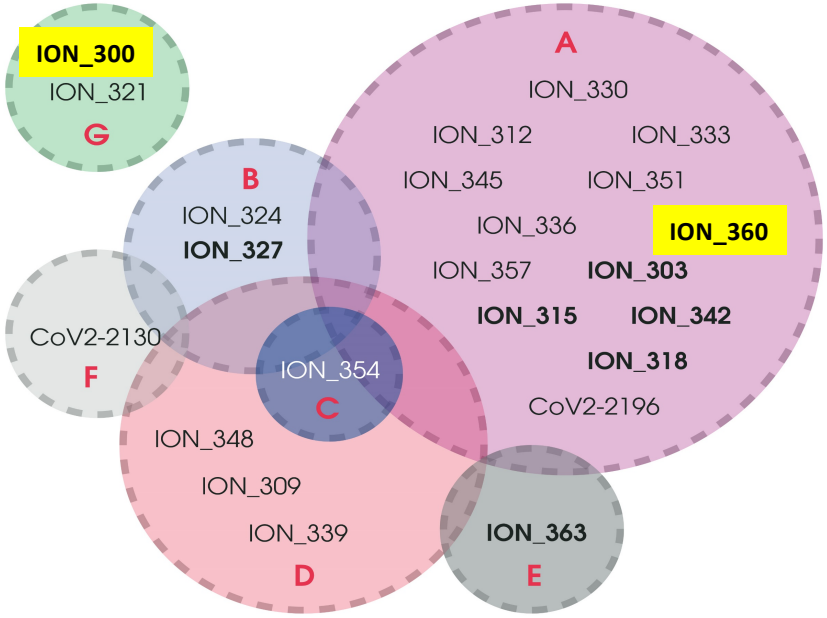
Valency enhances viral neutralisation



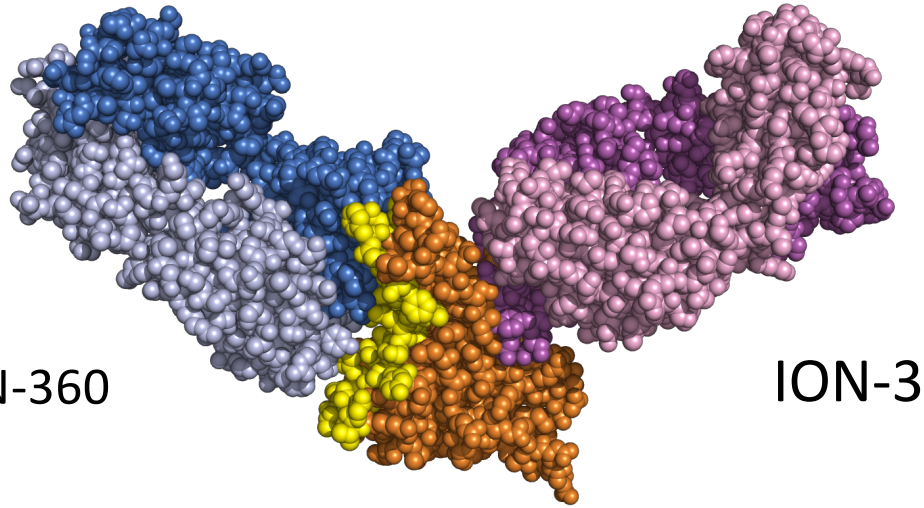


SARS-Cov-2 neutralising antibodies bind multiple epitopes



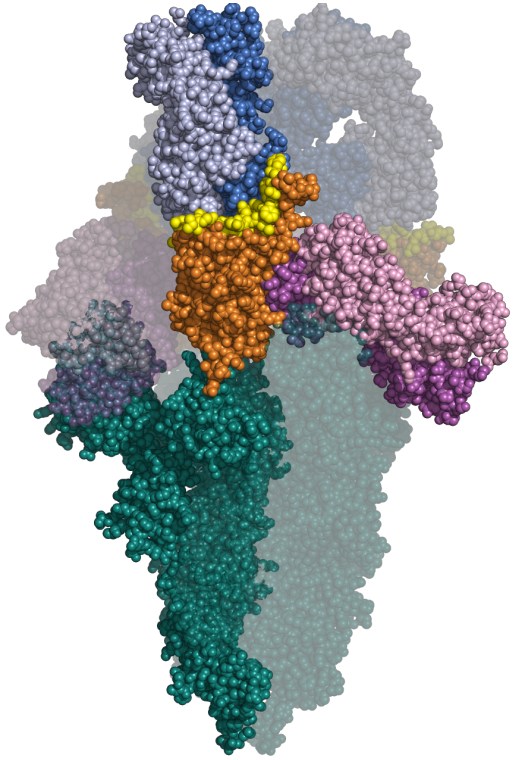


Structure of RBD in complex with ION-300 and ION-360



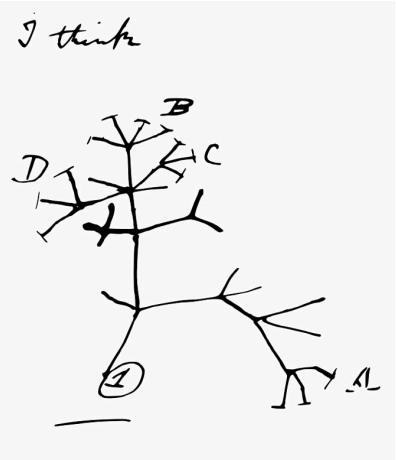
ION-360

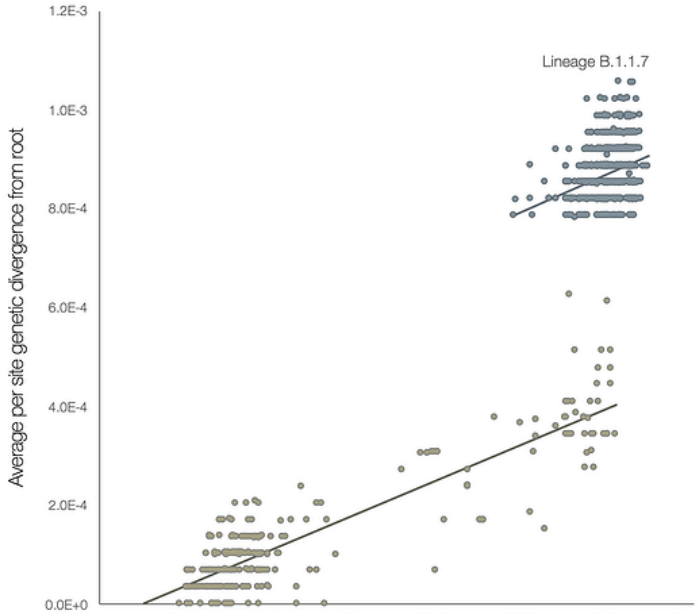
ION-300

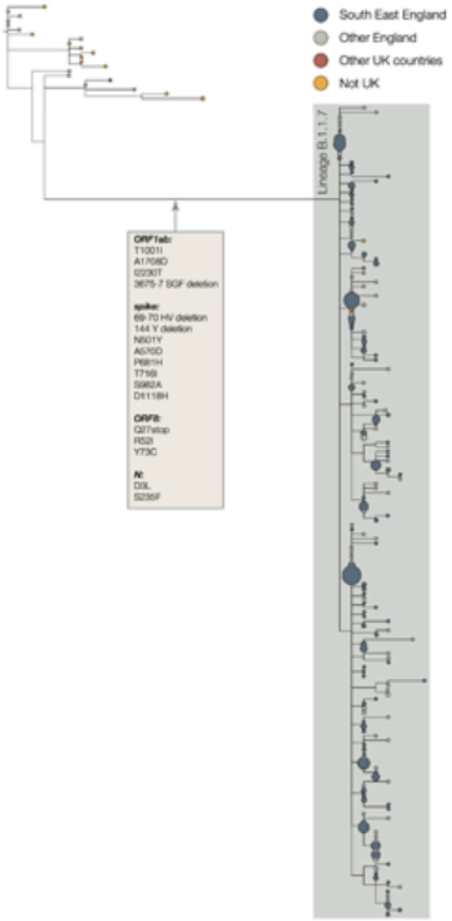


ION-300 binds distinct, unique epitope at (conserved) N terminus of SARS-CoV-2 RBD

Evolution in real time!







arambaut ARTIC Network 9 Dec '20

Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations

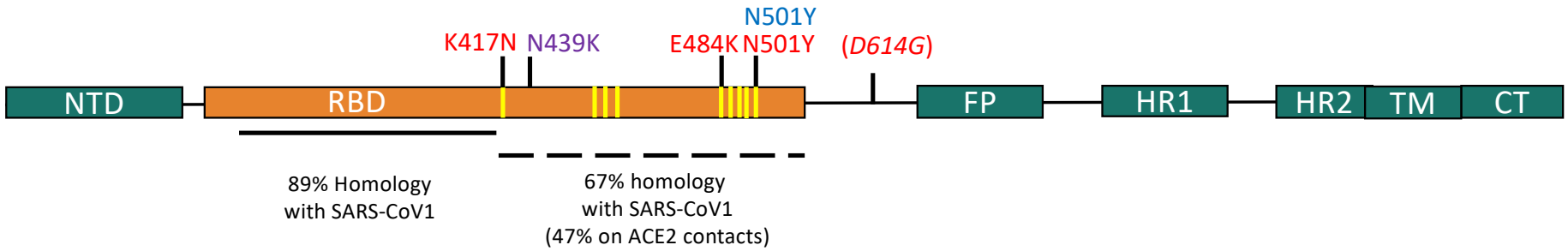
Report written by: Andrew Rambaut[1], Nick Loman[2], Oliver Pybus[3], Wendy Barclay[4], Jeff Barrett[5], Alesandro Carabelli[6], Tom Connor[7], Tom Peacock[4], David L Robertson[8], Erik Volz[4], on behalf of COVID-19 Genomics Consortium UK (CoG-UK)[9].

Dec 2020

1 / 5

Dec 20

Emergence of spike protein variants



"Kent strain" (a.k.a. B.1.1.7/N501Y.V1, multiple changes, 1 change in RBD)

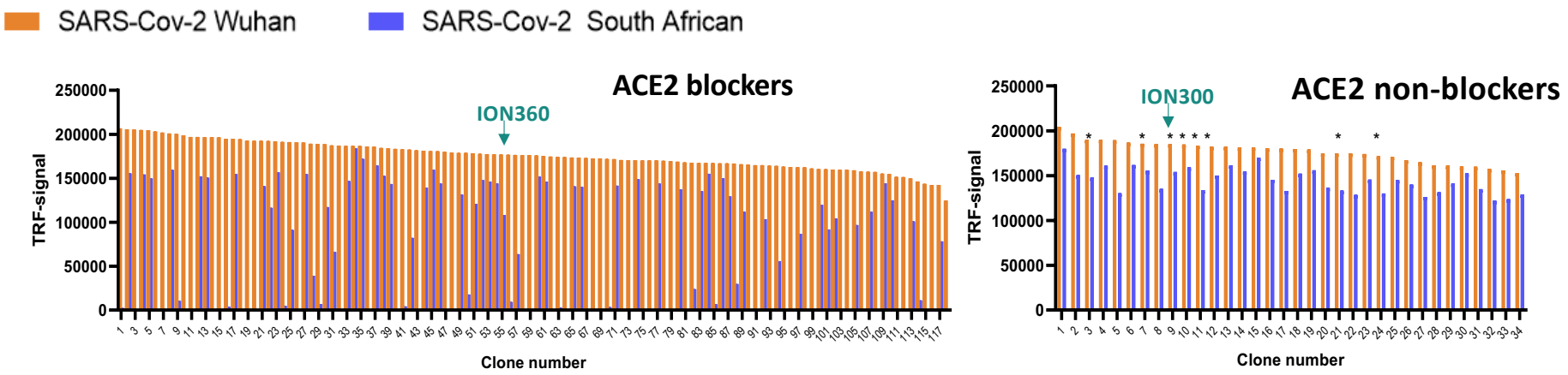
"South African"(a.k.a. B.1.351, N501Y.V2)

N439K-Immune escape variant?

ACE2 blockers give more potent neutralisation…but

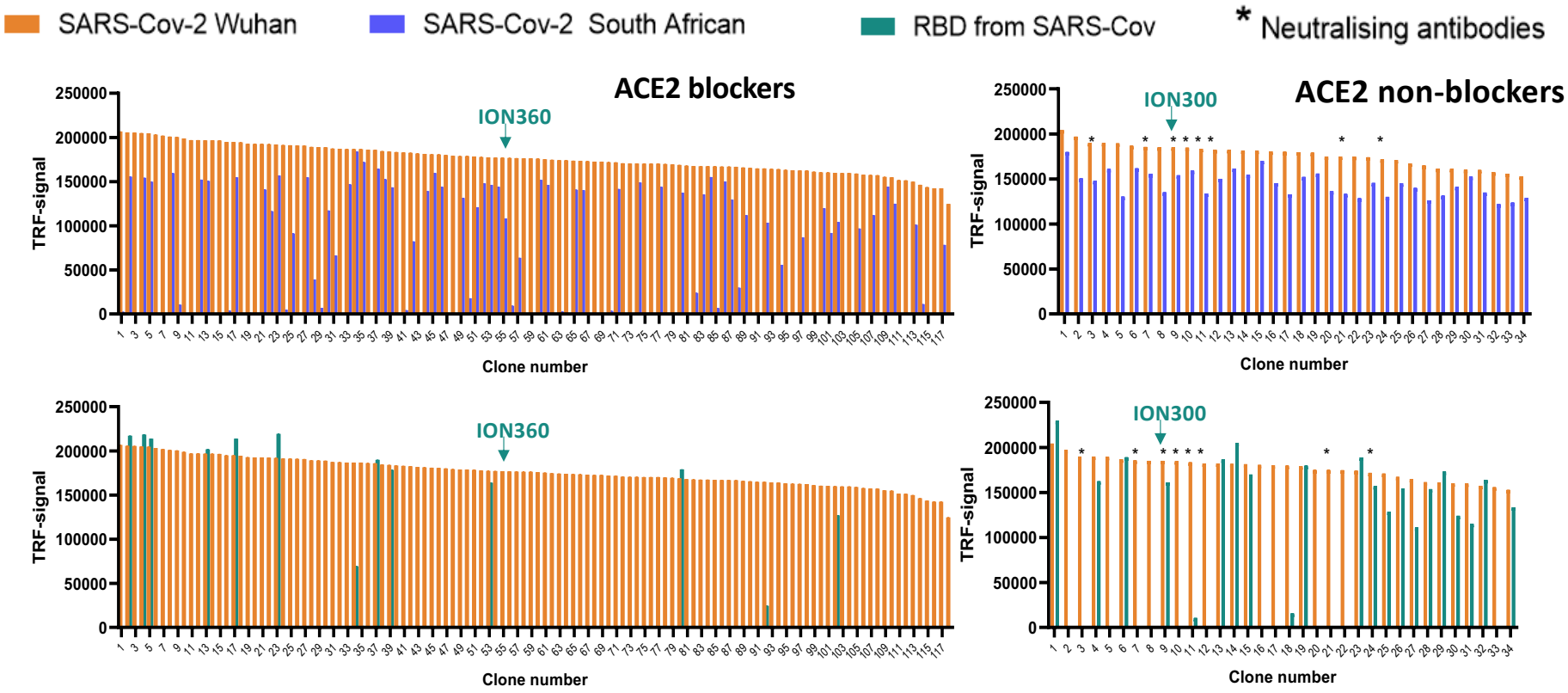
ACE2 binding site (C terminus) shows greatest variability

Cross-Reactive antibodies to South African Variant



ALL ACE2 non-blockers retain binding to South African variant

High proportion retain binding to SARS-CoV-1



Greater cross-reactivity to SARS-CoV-1 from ACE2 non-blockers

Summary

- **Binders derived from phage display libraries from 18 patients**
  - Identified predominantly naïve B cell activation in early response
    - IgM origin and few mutations
    - Convergent response within study and beyond

- **Identification/characterisation of panel of 155 RBD-binding antibodies**
  - Epitope mapping and structural determination reveal multiple epitopes
  - Majority of binders competed with ACE2 (67%)
    - High hit rate and highest potency for viral neutralisation
    - Some susceptibility to viral variants
  - Non-ACE2 binders → lower potency neutralisation
    - With retained binding to major variants
    - Including residues conserved with SARS-CoV-1

Acknowledgments

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- David Matthews

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- Mark D Carr

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- **Jason Lee**
- Paul Pfeffer

Abcam
- **Deidre Flaherty**
- **Omodele Ashiru**
- **Lucia Crippa**
- Jamie Campbell

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