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

John McCafferty
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

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

SARS-CoV-2: The spike protein

NTD | RBD | FP | HR1 | HR2 | TM | CT |
--- | --- | --- | --- | --- | --- | --- |
319 | 541 | | | | | 1273 |

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

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)

Phage 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
Phage display libraries (either donor VLs or naïve VLs)

123 unique VHs

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

89 RBD binding clusters identified in patients

Illumina sequencing (Alchemab)

Galson JD, et al. (2020)
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

<table>
<thead>
<tr>
<th>Cluster ID</th>
<th>Representative CDRH3</th>
<th>V gene</th>
<th>J gene</th>
<th>Cluster size</th>
<th>Mean mutation</th>
<th>Convergence</th>
<th>Number of CoV-AbDab hits</th>
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<td>VH1-58</td>
<td>J3</td>
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<td>164</td>
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<td>VH3-53</td>
<td>J6</td>
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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
Identification of neutralising antibodies in pseudovirus assay

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?


Potential for inter and intra-binding to spike trimer
Valency enhances viral neutralisation

<table>
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<th>IgG IC$_{50}$ (nM)</th>
<th>Fab IC$_{50}$ (nM)</th>
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<td>N.D</td>
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<td>ION_351</td>
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<td>ION_363</td>
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% Neutralisation

Antibody (nM)
SARS-Cov-2 neutralising antibodies bind multiple epitopes
ION-300 binds distinct, unique epitope at (conserved) N terminus of SARS-CoV-2 RBD
Evolution in real time!
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
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