Broadly neutralizing SARS-CoV-2 antibodies discovered in multiple patients target the receptor binding domain

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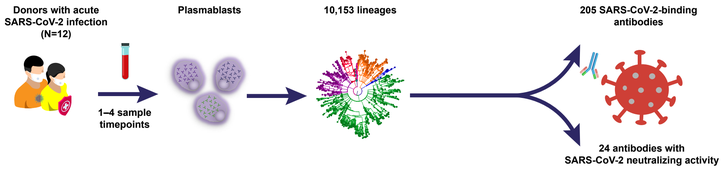
Background

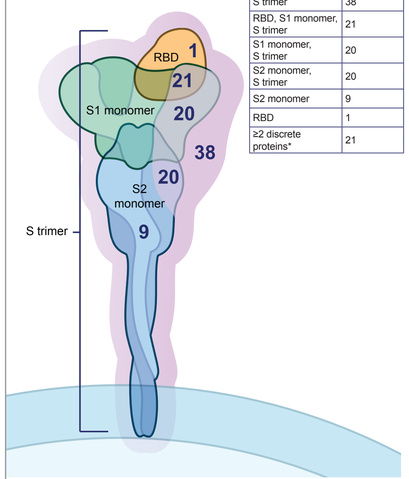
- Emerging SARS-CoV-2 variants have demonstrated reduced susceptibility to currently available antibody treatments for patients with COVID-19; therefore additional anti-SARS-CoV-2 antibodies are needed[1]
- Most antibody therapies currently being developed to treat COVID-19 target the spike protein and prevent viral infection by inhibiting mechanisms including host cell binding, host–viral cell membrane fusion, and virus internalization[1,2]
- The Atreca discovery platform used a target-agnostic approach to identify antibodies produced by plasmablast B cells targeting unique viral epitopes in donors with SARS-CoV-2
- Here we present data describing potently pan-neutralizing antibodies from two distinct B cell lineages that target discrete epitopes on the receptor-binding domain of the spike protein

Methods

- SARS-CoV-2 antibodies were discovered via a target-agnostic process
- Natively paired heavy- and light-chain IgG variable region sequences were generated from plasmablasts obtained from donors with acute SARS-CoV-2 infection
- Antibodies were selected based on abundance, persistence, convergence, and levels of somatic hypermutation
- Binding specificity was analyzed using ELISA, BLI, and SPR



Results



Epitope	Number of Antibodies
Nucleocapsid	54
Envelope	21
S trimer	38
RBD, S1 monomer, S trimer	21
S1 monomer, S trimer	20
S2 monomer, S trimer	20
S2 monomer	9
RBD	1
≥2 discrete proteins*	21

Binding specificity

- Of the 479 antibodies tested, 205 antibodies bind to either the nucleocapsid, envelope, or spike protein of SARS-CoV-2

*Includes antibodies that bind to ≥2 of the following sites: nucleocapsid, envelope, S trimer, or S2 monomer.

Antibody	Epitope	Replication-competent Neutralization (μg/mL)
APN-172182	S Trimer	0.075
APN-016322	RBD, S1, S Trimer	0.181
APN-660065	RBD, S1, S Trimer	1.37
APN-139727	RBD, S1, S Trimer	2.14
APN-327017	RBD, S1, S Trimer	3.4
APN-506382	Unknown	3.92
APN-385033	S1, S Trimer	4.44
APN-932876	RBD, S1, S Trimer	6.25
APN-379397	RBD, S1, S Trimer	12.3
APN-850271	RBD, S1, S Trimer	13.3
APN-105383	S1, S Trimer	20.6
APN-882284	S2, S Trimer, Nucleocapsid	21.43
APN-839539	S1, S Trimer	24.47
APN-261030	RBD, S1, S Trimer	32.76
APN-193247	RBD, S1, S Trimer	33.7
Etesevimab *Positive control*	RBD, S1, S Trimer	0.13
Anti-nucleocapsid *Negative control*	Nucleocapsid	NT

Potency: <0.001 | 0.001 to <0.1 | 0.1 to <1.0 | ≥1.0

Neutralizing activity of SARS-CoV-2 binding antibodies

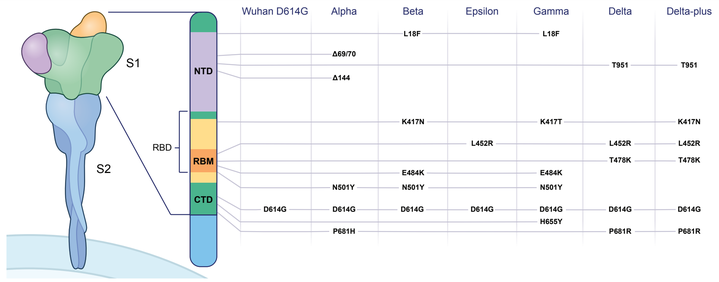
- Eight antibodies had an IC_{50} of ≤10 μg/mL

Antibody lineages, convergent sequences, and unique donors

- For the antibodies that demonstrated IC_{50} values ≤50 μg/mL, we next determined whether any additional sequences in our COVID dataset were either 1) sibling sequences (clonally related sequences originating from the same donor), or 2) convergent sequences (clonally similar sequences but originating from different donors)
- Three sequences related to the most potently neutralizing antibodies were also expressed and tested against a panel of pseudoviral variants along with the 15 antibodies that neutralized replication-competent SARS-CoV-2

Antibody	Number of Sibling* Sequences	Number of Convergent† Sequences	Number of Unique Donors
APN-016322	0	1	2
APN-660065	0	2	2
APN-139727	16	0	1
APN-506382	6	1	2
APN-385033	8	0	1
APN-932876	0	1	2
APN-850271	0	4	4
APN-105383	1	1	2
APN-882284	10	0	1
APN-839539	7	0	1
APN-261030	5	0	1
APN-193247	0	3	3

*Sibling sequences likely originated from the same B cell progenitor and are defined as having the same predicted V germline genes and 75% identity between the H-CDR3 and L-CDR3 nucleotide sequences with those sequences being of the same length.
†Convergent sequences originated from different donors but are similar to sibling sequences in that they have highly similar predicted V germline genes and 85% similarity between the H-CDR3 and L-CDR3 amino acid sequences with at most one gap in the alignment between them.



SARS-CoV-2 variant mutations[3,4]

- Neutralizing activity was assessed against a panel of seven pseudovirus variants, including the delta variant with and without the *K417N* mutation

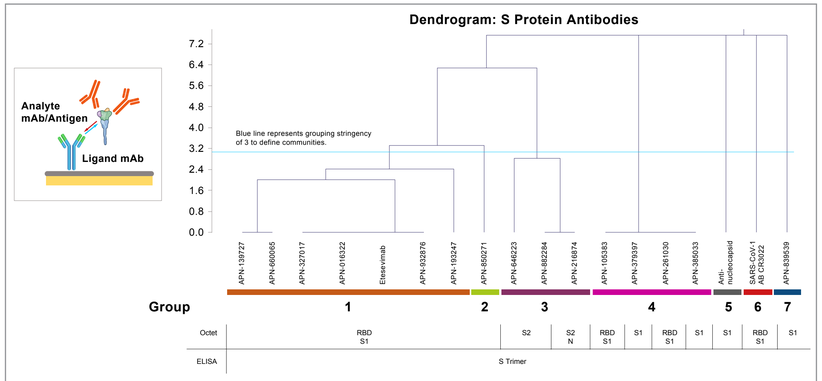
Antibody	Replication-competent Neutralization (μg/mL)	SARS-CoV-2 Pseudovirus Neutralization (μg/mL)							
		Wuhan	Wuhan D614G	Alpha B.1.1.7	Beta B.1.351	Gamma P.1.B.1.1.28	Delta B.1.617.2	Delta-plus B.1.617.2.1	Epsilon B.1.427/9
APN-172182	0.075	0.142	0.040	>50	>50	>50	31.28	>50	>50
APN-016322*	0.181	0.058	0.036	0.275	>50	>50	0.012	>50	0.028
APN-887054*	NT	3.050	1.040	3.402	>50	>50	0.158	>50	1.27
APN-660065†	1.37	0.065	0.058	0.117	0.169	0.158	0.030	0.012	0.032
APN-906072†	NT	0.004	0.003	0.013	0.0180	0.0114	0.003	0.001	0.003
APN-719695†	NT	1.560	0.700	2.686	8.2705	5.3877	0.574	0.078	0.629
APN-139727	2.14	0.774	0.177	0.114	>50	>50	>50	>50	33.65
APN-327017	3.4	1.53	0.830	7.234	>50	>50	0.101	>50	1.16
APN-506382	3.92	>50	5.514	>50	>50	>50	>50	>50	>50
APN-385033	4.44	6.53	21.68	6.07	>50	13.9	>50	39.22	17.13
APN-932876	6.25	6.77	2.21	2.84	>50	>50	>50	>50	>50
APN-379397	12.3	27.70	>50	>50	>50	>50	>50	>50	38.35
APN-850271	13.3	1.04	1.06	0.257	0.563	0.327	0.519	0.809	0.974
APN-105383	20.6	>50	>50	>50	>50	>50	>50	>50	>50
APN-882284	21.43	42.38	>50	>50	>50	40.6	>50	>50	>50
APN-839539	24.47	>50	>50	>50	>50	>50	>50	>50	>50
APN-261030	32.76	4.10	39.1	17.7	>50	19.2	>50	46.2	14.1
APN-193247	33.7	0.215	0.105	>50	>50	>50	0.032	2.29	0.041
Etesevimab *Positive control*	0.13	0.012	0.014	0.417	>50	>50	0.004	>50	0.009
Anti-nucleocapsid *Negative control*	NT	>50	>50	>50	>50	>50	>50	>50	>50

Potency: <0.001 | 0.001 to <0.1 | 0.1 to <1.0 | ≥1.0

Neutralizing activity of SARS-CoV-2 binding antibodies

- Four antibodies demonstrated broad neutralizing activity against all of the variants tested to date
- Three antibodies, APN-660065, APN-906072 and APN-719695, demonstrated 97% to 98% similarity at the amino acid level
  - APN-906072 and APN-719695 originated from a different donor than APN-660065

*Antibodies part of the same convergent sequence space.
†Antibodies part of the same convergent sequence space.



Epitope binning experiments

- Epitope binning experiments suggested limited competition between APN-660065 and APN-850271 for binding to the spike trimer

Antibody	Average T_{m1} (°C)	Average T_{m2} (°C)	Average T_{agg} 266 nm (°C)	Polyspecificity (BVP Binding)
APN-660065	69.2	77.3	79	Not detected
APN-906072	69.7	75.8	78	Not detected
APN-719695	69.9	75.1	76.8	Not detected
APN-850271	69.2	74.1	76.2	Not detected

Developability properties

- All four pan-neutralizing antibodies exhibited favorable developability properties, including high melting temperatures and low polyreactivity

Conclusions

- Diverse broadly neutralizing antibodies will be critical to preventing resistance against emerging SARS-CoV-2 variants
- Using a target-agnostic approach, four pan-neutralizing antibodies that target the RBD and S1 subunit of SARS-CoV-2 were identified
- Favorable developability properties, including high melting temperatures and low polyreactivity, were demonstrated by all four pan-neutralizing antibodies
- Evaluation against the omicron variant is ongoing

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Abbreviations

AB, antibody; BLI, bio-layer interferometry; BVP, baculovirus particles; COVID-19, coronavirus disease 2019; CTD, C-terminal domain; ELISA, enzyme-linked immunoassay; H-CDR3, heavy-chain complementarity-determining region 3; IC_{50}, half-maximal inhibitory concentration; IgG, immunoglobulin G; L-CDR3, light-chain complementarity-determining region 3; mAb, monoclonal antibody; NA, not applicable; NT, not tested; NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SPR, surface plasmon resonance; T_{agg}, aggregation temperature; T_m, melting temperature; V, variable.

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